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Quality Changes during Storage of Red Dragon Fruit Juice after High-Intensity Pulsed Electric Field (HIPEF) Pasteurization

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ABSTRACT

This study evaluated the quality and safety of red dragon fruit (*Hylocereus polyrhizus*) juice following pasteurization using high-intensity pulsed electric fields (HIPEF), subsequent to ultrasound-assisted extraction. Ultrasound treatment was performed at 40% amplitude for 10 minutes, followed by cold pressing. HIPEF pasteurization was applied at 40 kV cm^{-1}, with a pulse width of 21 µs and a total of 600 pulses. The treated juice was compared with an untreated control, focusing on betacyanin content, total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity, total soluble solids, pH, enzymatic activities (polyphenol oxidase [PPO] and peroxidase [POD]), and microbial counts. During refrigerated storage at 4 ± 1 °C over a period of two months, juice subjected to HIPEF treatment exhibited significantly higher retention of betacyanin compounds compared to the control. TPC initially declined, followed by a notable increase, peaking on day 30. TFC remained consistently higher than the control throughout the first month, after which a gradual decrease was observed. Antioxidant activity was well maintained, with 82% retention after two months. HIPEF treatment significantly reduced enzymatic activities, with PPO and POD levels decreasing by 86.3% and 81.4%, respectively, despite minor fluctuations during storage. Microbiological analysis revealed an immediate 5-log reduction in total plate count and minimal initial yeast and mold presence. Over the storage period, yeast and mold counts increased slowly, while bacterial counts declined, likely due to the inherent antibacterial properties of dragon fruit. No psychrophilic bacterial growth was detected. Overall, HIPEF pasteurization effectively preserved the quality attributes and microbial safety of red dragon fruit juice for up to two months under refrigerated conditions.

Introduction

Pitaya, commonly known as dragon fruit, is highly valued for its potent antioxidant capacity and documented anticancer, antilipidemic, and antidiabetic properties. Among its various cultivars, red dragon fruit (*Hylocereus polyrhizus*) is particularly noted for its high phenolic content and antioxidant activity, whereas white dragon fruit (*Hylocereus undatus*) is comparatively richer in sugars (Arivalagan et al., 2021). However, dragon fruit has a limited shelf life and is susceptible to fungal contamination by species such as *Rhizopus*, *Fusarium*, *Botryosphaeria*, and *Colletotrichum* (Chaemsanit et al., 2018). At ambient temperature, the fruit remains viable for up to 10 days, whereas under refrigeration, its shelf life extends to approximately 25–30 days (Tarte et al., 2023).

Juice production from dragon fruit represents a viable method for developing value-added products

such as ready-to-serve (RTS) beverages, squash, jelly, syrup, candy, and juice powder (Jalgaonkar et al., 2018). Conventionally, fruit juices are preserved using chemical additives to prevent microbial and enzymatic spoilage. Common preservatives include sodium benzoate, potassium metabisulfite, sorbic acid, and parabens, with benzoates being widely employed in soft drinks (Mandal, 2019). The Codex Alimentarius, an international food safety standard, permits benzoic acid or sodium benzoate at concentrations ranging from 0.05% to 0.1% by volume, with a maximum permissible limit of 1000 mg kg^{-1} in food products (Islam et al., 2019). Exceeding these limits poses significant health risks, including asthma, headaches, and skin rashes; therefore, soft drinks containing benzoates are contraindicated for individuals with asthma (Mandal, 2019).

As an alternative, non-thermal pasteurization methods—combined with refrigerated storage—offer a safer and more nutritionally favorable approach. Unlike thermal pasteurization, which involves elevated temperatures that can degrade sensory and nutritional quality, non-thermal techniques preserve the delicate aroma, flavor, and bioactive compounds of fresh juice. Traditional thermal treatments inactivate pathogenic microorganisms and spoilage enzymes such as peroxidase, polyphenol oxidase, and pectin methylesterase, but often at the cost of deteriorating the juice's functional and organoleptic properties (Aghajanzadeh & Ziaiifar, 2021; Roobab et al., 2022).

High-intensity pulsed electric field (HIPEF) processing is an emerging non-thermal technology that has shown promise as a viable alternative to thermal pasteurization. The application of pulsed electric field (PEF) technology in juice processing has been demonstrated to enhance microbial safety and product quality, particularly when combined with aseptic packaging to extend refrigerated shelf life. Several studies have explored PEF pasteurization across various fruit juices (Brito & Silva, 2024; Wibowo et al., 2022). For example, PEF treatment of strawberry juice at 35 kV cm^{-1} and 155 Hz for 27 µs extended its shelf life by at least 28 days (Yildiz et al., 2021). In another study, PEF treatment of orange juice at electric field strengths ranging from 13.82 to 25.6 kV cm^{-1} and treatment durations between 1033 and 1206 µs produced superior juice quality compared to thermal pasteurization (Agcam et al., 2016). Salinas-Roca et al. (2017) evaluated the effects of HIPEF (35 kV cm^{-1}, 1800 µs) on *Listeria innocua* inhibition, enzyme activity, and the overall quality of mango juice. Their findings confirmed that HIPEF ensured microbiological stability, significantly reduced enzymatic activity, improved retention of bioactive compounds, and preserved sensory and color attributes over 75 days of refrigerated storage—further establishing HIPEF as a credible non-thermal alternative.

Despite growing evidence supporting HIPEF's efficiency, a clear research gap exists regarding its application to red dragon fruit juice, particularly when combined with ultrasound-assisted extraction followed by cold pressing. There is limited information on the comparative quality and safety of HIPEF-treated versus untreated red dragon fruit juice during refrigerated storage. Accordingly, this study aims to address this gap by evaluating the impact of HIPEF pasteurization on the physicochemical properties, enzymatic activity, antioxidant stability, and microbial safety of red dragon fruit juice throughout cold storage.

Materials and Methods

Raw materials

Approximately 5 kg of fully ripe red dragon fruits (*Hylocereus polyrhizus*, Malaysian red variety) were harvested in the morning from Brahma's Red Dragon Fruit Farm, located in Chirakkekode, Thrissur, Kerala, India (10.5570° N, 76.2914° E). The fruits were collected on the same day for further processing. After harvest, the fruits were hygienically washed, peeled, and cut into pieces. They were then packed in stand-up pouches, sealed, and subjected to blast freezing for 1 hour, followed by storage in a deep freezer at −18 ± 1 °C.

Chemicals

The following analytical-grade chemicals and reagents were used: Folin–Ciocalteu's reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium hydroxide, sodium carbonate, sodium nitrate, aluminium chloride, guaiacol, catechol, buffer solution (pH 7), plate count agar, potato dextrose agar, hydrogen peroxide, and ethanol.

Ultrasound-assisted juice extraction

Frozen fruits were thawed overnight in a refrigerator at 4 °C. Fresh fruits were washed, peeled, and chopped before undergoing ultrasonic pre-treatment. Ultrasound treatment was conducted at 40% amplitude for 10 minutes to enhance the extraction of bioactive compounds, as determined by preliminary studies. Prior to use, the ultrasonic probe was surface-sterilized by wiping with tissue soaked in 70% ethanol.

The ultrasound device (Shalom Ultrasonics, India) operated at 20 kHz frequency, with a maximum power output of 750 W and a horn diameter of 25 mm. Following ultrasonic treatment, the juice was extracted using a cold press juicer (Model: 19003594, Pigeon, India), operating at a power of 150 W and a speed of 75 RPM. All experiments were conducted at the Agribusiness Incubator,

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PEF pasteurization

After ultrasound-assisted extraction, non-thermal pasteurization of the juice was carried out using a batch-type pulsed electric field (PEF) system (Sureview Instruments LLP, India). The system was operated at an input of 220 V AC and 60 Hz, delivering a maximum output voltage of 40 kV. The treatment chamber was equipped with two coaxial stainless steel electrodes housed in PTFE, connected to a high-voltage pulse generator. The chamber was sterilized by wiping with alcohol at least two hours prior to treatment.

PEF pasteurization was performed under previously optimized conditions: electric field strength of 40 kV cm^{-1}, pulse width of 21 µs, and 600 pulses. Post-treatment, 50 mL of juice was filled into 14 sterilized PET bottles and stored at 4 ± 1 °C for further analysis. An equal number of untreated control samples were also stored under identical conditions. Samples were analyzed at 10-day intervals throughout the storage period, from 26 October 2024 to 28 December 2024. Two bottles were withdrawn at each interval—one for chemical analysis and the other for microbial evaluation.

Quality and safety evaluation

Betacyanin content

Betacyanin content was determined using a UV-Visible spectrophotometric method as described by Nurul and Asmah (2014), with slight modifications. One milliliter of juice was diluted in 10 mL of distilled water and centrifuged at 4500 rpm for 10 minutes. The absorbance of the supernatant was measured at 538 nm using a UV-Vis spectrophotometer (UV-1800, SHIMADZU, Japan). Results were expressed as betacyanin equivalents (mg 100 mL^{-1}), calculated using Equation 4. All measurements were performed in triplicate, and the average values were reported.

$$Concentration\ of\ Betacyanins\ (mg\ 100\ mL^{-1}) = \frac{A \times MW \times 1000}{\varepsilon \times L} \quad (1)$$

where, A = absorbance at 538 nm, MW= molecular weight of Betacyanin (550 g mol^{-1}), $\boldsymbol{\varepsilon}$ = Molar extinction coefficient of Betacyanin (60,000), L= path length (1.0 cm).

Total phenolic content

Total phenolic content in the fruit juice was determined using the Folin–Ciocalteu method, with slight modifications as described by Palety et al. (2020). The TPC was calculated using a standard calibration curve prepared from gallic acid solutions. Briefly, 0.2 mL of fruit juice was mixed with 1.8 mL of distilled water, followed by the addition of 0.2 mL of Folin–Ciocalteu reagent (previously diluted tenfold with distilled water). The mixture was allowed to stand at room temperature for 5 minutes. Subsequently, 0.8 mL of 20% sodium bicarbonate (Na_2CO_3) was added, and the solution was vortexed and kept at room temperature for 30 minutes. Absorbance was measured at 720 nm using a UV-Vis spectrophotometer (Model: UV-1800, SHIMADZU, Japan). TPC was expressed as milligrams of gallic acid equivalents (mg GAE 100 mL^{-1}). Each measurement was conducted in triplicate, and the average value was recorded.

Total flavonoid content

Total flavonoid content was determined using the aluminium chloride colorimetric assay, following the method of Zhishen et al. (1999). A 0.5 mL aliquot of the juice extract was diluted with distilled water to a final volume of 5 mL. To this, 0.3 mL of 5% sodium nitrite ($NaNO_2$) was added and allowed to react for 5 minutes. Then, 0.3 mL of 10% aluminium chloride ($AlCl_3$) solution was added, followed by 2 mL of 1 M sodium hydroxide (NaOH) after one minute. The volume was made up to 10 mL with distilled water, mixed thoroughly, and the absorbance was recorded at 510 nm using a UV-Vis spectrophotometer (Model: UV-1800, SHIMADZU, Japan). TFC was calculated using a standard curve of quercetin and expressed as milligrams of quercetin equivalents (mg QE 100 mL^{-1}). All measurements were repeated three times, and the average value was reported.

DPPH radical scavenging activity

The antioxidant activity of dragon fruit juice was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, with slight modifications as described by Singh et al. (2022). A 0.1 mM DPPH solution was prepared by dissolving 4 mg of DPPH in 100 mL of ethanol. Juice samples were initially centrifuged at 4500 rpm for 10 minutes. One milliliter of the supernatant was then diluted to 30 mL with distilled water and centrifuged again under the same conditions. Subsequently, 2 mL of the diluted juice was mixed with 2 mL of DPPH solution and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Model: UV-1800, SHIMADZU, Japan). The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$Antioxidant\ activity\ (\%) = \frac{(Ac-As)}{AC} \times 100 \quad (2)$$

Where, Ac and As indicated the absorbance of control and absorbance of the sample respectively.

The control sample contained 2 mL ethanol instead of juice sample.

TSS

Total soluble solids was determined using a digital refractometer (PCE-DR series, PCE Americas Inc., USA) (Benjuma et al., 2025).

pH

The pH of fruit juice was determined using a digital pH meter (μC pH system 361, Systronics, India) (Benjuma et al., 2025).

PPO residual activity

Polyphenol oxidase (PPO) residual activity was determined following the method described by Liao et al. (2020). One milliliter of juice was mixed with 2 mL of a substrate solution containing 0.07 M catechol and 0.2 M phosphate buffer (pH 7.0). The enzymatic reaction was monitored by recording the increase in absorbance at 420 nm at 2-second intervals over a 4-minute period using a UV-Vis spectrophotometer (Model: UV-1800, SHIMADZU, Japan). The specific activity of PPO was determined from the slope of the linear portion of the absorbance-time curve. Residual PPO activity was calculated using Equation 3. Each measurement was performed in triplicate, and the mean value was reported.

$$PPO\ residual\ activity\ (\%) = 100 \times \frac{A_t}{A_0} \quad (3)$$

Where At and A0 represent the PPO activity of treated and untreated samples, respectively.

POD residual activity

Peroxidase (POD) activity was evaluated using a modified method based on Zhu et al. (2021). One milliliter of juice sample was mixed with 2 mL of 1% (v/v) guaiacol prepared in 0.2 M phosphate buffer (pH 7.0) and 0.2 mL of 1.5% hydrogen peroxide to initiate the enzymatic reaction. The change in absorbance at 470 nm was recorded at 2-second intervals for a duration of 4 minutes using a UV-Vis spectrophotometer (Model: UV-1800, SHIMADZU, Japan). The specific POD activity (expressed as Abs min^{-1}) was calculated from the slope of the linear portion of the absorbance-time curve. Residual POD activity was determined using Equation 6. All measurements were conducted in triplicate, and the average values were reported.

$$Residual\ POD\ activity\ (\%) = 100 \times \frac{A_t}{A_o} \quad (4)$$

Where: A_t and A_0 represent the PPO activity of treated and untreated samples, respectively.

Total plate count

The total microbial load in fruit juice samples was assessed using the pour plate method, as described by Kumari et al. (2023). Petri plates were sterilized in a hot air oven at 170 °C for 1 h. Water for serial dilutions was prepared in cotton-plugged conical flasks and test tubes. Plate count agar (PCA) was prepared according to the manufacturer's instructions, heated on a magnetic stirring mantle until fully dissolved, and then sterilized along with the flasks and test tubes in an autoclave at 121 °C for 15 min.

An initial 10^{-1} dilution was prepared by mixing 10 mL of juice with 90 mL of sterile distilled water. Serial dilutions were carried out up to 10^{-6} using sterile distilled water. For microbial enumeration, 1 mL aliquots from the 10^{-5} and 10^{-6} dilutions were plated for both control and PEF-treated samples. Approximately 15–20 mL of sterile PCA was poured into each Petri dish. After solidification, the plates were incubated at 37 °C for 24–48 h. Microbial colonies were counted and expressed as colony-forming units per ml (CFU mL^{-1}).

Total psychrophilic count

The total psychrophilic count of aerobic bacteria was determined using the same pour plate procedure described above, with plate count agar as the medium. Incubation was performed at 4 °C for 10 days, following the method of Salvia-Trujillo et al. (2011).

Total yeast and mould count

Yeast and mould counts were evaluated using the pour plate method, following the procedure described by Kumari et al. (2023). Potato dextrose agar (PDA) was prepared according to label instructions, boiled until fully dissolved, and sterilized at 121 °C for 15 min in an autoclave. Juice samples (control, PEF-pasteurized, and retort-pasteurized) were serially diluted up to 10^{-6}. One ml aliquots from the 10^{-3} and 10^{-4} dilutions were plated in triplicate for each treatment. After the agar was poured and solidified, the plates were incubated in an inverted position at 25 °C. Colony counts were recorded between 3 and 5 days and expressed as CFU mL^{-1}.

Statistical analysis

To assess the effects of treatment and storage duration on juice quality and safety parameters, a two-factor completely randomized design (CRD) was employed. Analysis of variance (ANOVA) was performed using GRAPES software (developed by Kerala Agricultural University, KAU). Differences among treatment means were considered statistically significant at $P < 0.05$. All experiments were

conducted in triplicate to ensure the accuracy, reliability, and reproducibility of the results.

Results

Changes in major bioactive compounds and DPPH radical scavenging activity of PEF pasteurized dragon fruit juice appear in Table 1.

Changes in betacyanin content during storage

A significant decrease ($P < 0.05$) in betacyanin content was observed during storage. Compared to the control juice, PEF-pasteurized juice retained significantly higher levels of betacyanin throughout the storage period. After two months of refrigerated storage, the control sample exhibited a 22% reduction in betacyanin content, while the PEF-treated juice showed only an 8.4% reduction. Thus, PEF-treated juice maintained significantly better preservation of betacyanin over time.

Table 1. Changes in betacyanin content, TPC, TFC, and DPPH radical scavenging activity of control and PEF pasteurized juice during 60 d of storage.

Storage days	Betacyanin content (mg BCE 100 mL^{-1})		TPC (mg GAE 100 mL^{-1})		TFC (mg QE 100 mL^{-1})		DPPH radical scavenging activity (%)	
	Control	PEF	Control	PEF	Control	PEF	Control	PEF
D 0	24.32 ± 0.15^{c}	24.95 ± 0.14^{b}	71.06 ± 0.35^{a}	62.7 ± 0.32^{e}	14.16 ± 0.06^{k}	17.83 ± 0.05^{d}	78.6 ± 0.36^{b}	79.56 ± 0.36^{a}
D 10	23.74±0.17^{d}	26.95 ± 0.21^{a}	51.59 ± 0.46^{i}	50.17 ± 0.42^{j}	15.83 ± 0.05^{g}	22.23 ± 0.07^{a}	77.6 ± 0.52^{c}	78.68 ± 0.24^{b}
D 20	22.20 ± 0.12^{g}	23.05 ± 0.14^{e}	67.96 ± 0.43^{bc}	67.43 ± 0.46^{c}	13.96 ± 0.03^{l}	21.23 ± 0.02^{b}	70.96 ± 0.43^{f}	77.15 ± 0.42^{c}
D 30	21.08 ± 0.15^{j}	22.82 ± 0.11^{f}	70.97 ± 0.42^{a}	68.38 ± 0.32^{b}	11.6 ± 0.05^{m}	16 ± 0.04^{f}	68.04 ± 0.24^{h}	76.3 ± 0.18^{d}
D 40	20.59 ± 0.12^{k}	21.59 ± 0.05^{i}	49.43 ± 0.26	59.01 ± 0.35^{f}	18.5±0.02^{c}	15.16±0.05^{i}	70.3 ± 0.23^{g}	67.35 ± 0.27^{i}
D 50	19.43 ± 0.06^{i}	21.82 ± 0.12^{h}	56.38 ± 0.23^{h}	57.96 ± 0.25^{g}	15.5± 0.03^{h}	14.5± 0.06^{j}	75 ± 0.26^{e}	71.42 ± 0.32^{f}
D 60	18.77 ± 0.09^{m}	22.85 ± 0.08^{ef}	65.54 ± 0.34^{d}	65.12 ± 0.42^{d}	17.5± 0.04^{e}	18.5±0.03^{c}	74.54 ± 0.31^{e}	65.45 ± 0.25^{j}

Different superscript letters indicate significant differences at $P < 0.05$.

Changes in total phenolic content during storage

Initially, the control juice exhibited a higher total phenolic content (TPC) than the PEF-treated juice. However, by day 10, both samples experienced a significant reduction in TPC. Between days 10 and 30, an increase in TPC was recorded in both control and PEF-treated samples. After 30 days, TPC gradually declined in both treatments. By the end of the storage period, the TPC levels of both the control and PEF-treated juices were nearly identical.

Changes in total flavonoid content during storage

The PEF-treated juice showed significantly higher total flavonoid content (TFC), measured at 17.83 mg QE 100 mL^{-1}, compared to the control immediately after treatment and up to day 30 of storage. The highest TFC was observed on day 10. From day 20 to day 50, a decline in TFC was observed in the PEF-treated juice relative to the control. However, at the end of the storage period, the PEF-treated sample exhibited an increase, reaching 18.5 mg QE 100 mL^{-1}. Statistical analysis revealed significant differences ($P < 0.05$) in TFC across storage days and treatments.

Changes in DPPH radical scavenging activity during storage

Initially, the PEF-treated juice exhibited significantly higher DPPH radical scavenging activity compared to the control. Over time, a gradual decline in activity was observed for both samples. Up to one month of storage, the PEF-treated sample maintained higher DPPH activity. After this point, the control sample exhibited greater antioxidant activity. By the end of the storage period,

the control retained 94.84% of its initial activity, whereas the PEF-treated sample retained 82.7%. These changes were statistically significant ($P < 0.05$) across storage intervals and treatments.

Changes in total soluble solids and pH during storage

Changes in TSS and pH during storage are presented in Table 2. On the day of treatment, no significant difference in TSS was observed between control and PEF-treated samples. However, statistical analysis revealed significant changes ($P < 0.05$) in TSS values between the treatments over the storage period. At the end of storage, PEF-treated juice exhibited a lower TSS value compared to the control.

A significant decrease in pH was observed after two months of storage in both control and PEF-treated juice. Statistical analysis showed significant differences ($P < 0.05$) in pH between the control and PEF as well as between storage days. The highest pH was observed on d 20 in the control and PEF-treated juice.

Table 2. Changes in TSS and pH of control juice and PEF pasteurized juice during storage.

Days of storage	TSS (^{0}brix)		pH	
	control	PEF	Control	PEF
0	12.18 ± 0.02^{c}	12.2 ± 0.03^{c}	4.62 ± 0.01^{f}	4.68 ± 0.01^{e}
10	12.23 ± 0.03^{c}	10.96 ± 0.02^{c}	4.72 ± 0.02^{d}	4.78 ± 0.02^{c}
20	12.60 ± 0.01^{a}	12.3 ± 0.02^{b}	4.92 ± 0.01^{b}	5.15 ± 0.04^{a}
30	11.5 ± 0.005^{e}	10.4 ± 0.005^{i}	4.34 ± 0.03^{h}	4.43 ± 0.02^{g}
40	11.40 ± 0.005^{f}	9.9 ± 0.06^{k}	4.37 ± 0.005^{h}	3.62 ± 0.05^{k}
50	11.4 ± 0.06^{f}	11.1 ± 0.04^{g}	4.23 ± 0.001^{i}	4.22 ± 0.015^{i}
60	11.9 ± 0.04^{d}	10.15 ± 0.03^{j}	4.06 ± 0.005^{j}	4.22 ± 0.01^{i}

Different superscript letters indicate significant differences ($P < 0.05$).

Changes in residual enzyme activity during storage

Table 3 shows the residual enzyme activities in PEF pasteurized juice during storage. The PEF treatment led to an 86.3% reduction in PPO activity and an 81.4% reduction in POD activity. One-way ANOVA showed that significant differences ($P < 0.05$) in residual activity exist between storage days. However, during storage, enzyme activity fluctuated, showing both increases and decreases. Nonetheless, the PEF conditions used in the current study (40 kV cm^{-1}, 21 μs, and 600 pulses) were effective in reducing initial residual activity. The results suggest that more intense PEF conditions are required for complete enzyme inactivation during storage.

Table 3. Changes in residual PPO and POD activity of PEF pasteurized juice during storage.

Days of storage	Residual PPO activity (%)	Residual POD activity (%)
D 0	13.66 ± 0.17^{f}	18.5 ± 0.1^{g}
D 10	66 ± 1^{a}	200 ± 0.44^{b}
D 20	4.3 ± 0.21^{g}	84.49 ± 0.16^{f}
D 30	44.27 ± 0.17^{b}	223.93 ± 1^{a}
D 40	19.53 ± 0.10^{e}	89.97 ± 0.08^{e}
D 50	30.7 ± 0.11^{d}	119.5 ± 0.5^{c}
D 60	39.8 ± 0.22^{c}	116.93 ± 0.4^{d}

Different superscript letters indicate significant differences among days of storage ($P < 0.05$).

Changes in total plate count during storage

Table 4 shows the changes in total plate count and total yeast and mould count during refrigerated storage. The initial plate count in the control juice was 18×10^6 CFU mL^{-1}. After PEF pasteurization, the total plate count was reduced to 2×10^1. About 5-log

reduction was achieved after PEF pasteurization. During storage, both the control and treated samples showed an increase in the microbial count for up to 30 d. After that, the count gradually decreased and at the end of 60 d of storage, the control juice exhibited only 20×10^6 CFU mL^{-1} while PEF-treated juice exhibited 15×10^1 CFU mL^{-1}.

Table 4. Changes in total plate count and total yeast and mould count during refrigerated storage.

	Total Plate count (CFU mL^{-1})		Total yeast and mould count (CFU mL^{-1})	
Days	**Control**	**PEF**	**Control**	**PEF**
D 0	$18\times 10^{6\ h}$	$2 \times 10^{1\ k}$	$2 \times 10^{3\ k}$	$3\times10^{1\ k}$
D 10	$50\times10^{6\ c}$	$10 \times 10^{1\ i}$	$60 \times 10^{3\ h}$	$70\times10^{1\ g}$
D 20	$70 \times10^{6\ b}$	$20 \times 10^{1\ g}$	$80 \times 10^{3\ f}$	$50\times10^{1\ j}$
D 30	$80 \times 10^{6\ a}$	$50\times10^{1\ c}$	$90 \times 10^{3\ e}$	$50\times 10^{1\ j}$
D 40	$45 \times 10^{6\ d}$	$35 \times10^{1\ e}$	$110 \times 10^{3\ d}$	$50\times10^{1\ j}$
D 50	$27\times 10^{6\ f}$	$20 \times10^{1\ j}$	TNTC ^{c}	$55\times10^{1\ i}$
D 60	$20 \times10^{6\ i}$	$15\times10^{1\ e}$	TNTC ^{b}	TNTC ^{a}

Different superscript letters indicate significant differences at $P < 0.05$.

Changes in total psychrophilic count during storage

Throughout the storage period, no psychrophilic growth was observed in the control and PEF treated juice. Dragon fruit juice has natural antimicrobial properties and the results of the study showed that both the control and PEF-treated juice were completely safe from psychrophilic bacteria under refrigerated storage at 4 ^{0}C.

Changes in yeast and mould count during storage

On the day of treatment, yeast and mould counts in PEF-treated juice were much less. During storage, the count increased. On d 60, the count in PEF-treated juice was 55×10^1 while the population in the control was too many to count. The increase in counts after 10 d may be due to outgrowth of moulds compared to yeasts. The results revealed that PEF pasteurization can be less effective in controlling yeast and mould growth during refrigerated storage.

Discussion

The decrease in betacyanin content during storage can be attributed to the C15-isomerisation of betanins, the major constituent of betacyanin. Previous studies have also reported substantial degradation of betacyanin during storage. For instance, Herbach et al. (2007) observed greater betacyanin loss under light exposure compared to juice stored in the dark with the addition of 1% ascorbic acid. Conversely, the increase in betacyanin levels reported in some cases is attributed to electroporation, which enhances cell membrane permeability. Several studies have demonstrated the potential of pulsed electric field (PEF) treatment to enhance antioxidant pigments in various agricultural commodities (Zhang et al., 2017; Huang et al., 2019). The initial decline followed by a gradual increase in total phenolic content (TPC) observed in the present study aligns with findings from previous research. Barba et al. (2012) reported a decrease in TPC in blueberry juice after 7 days of storage, followed by an increase over 56 days. The observed rise in phenolic content after PEF treatment has been attributed to the stimulation of the phenylpropanoid biosynthetic pathway, specifically through the activation of the phenylalanine ammonia-lyase (PAL) enzyme. Salinas-Roca et al. (2017) reported the highest phenolic content on day 59 during a 70-day storage period of high-intensity PEF (HIPEF)-treated mango juice. Notably, Lopez-Gámez et al. (2020) provided the first evidence that the increase in phenolic content post-PEF treatment is primarily due to stress induction rather than improved extractability resulting from electroporation. Similarly, Mtaoua et al. (2017) reported an increase in phenolic compounds after one week of storage in HIPEF-treated date juice, followed by a decline after two weeks, consistent with the findings of the present study.

An increase in total flavonoid content (TFC) during storage has also been reported. For example, Soliva-Fortuny et al. (2017) observed increased TFC in PEF-treated apple juice over a 48-hour storage period. However, the degradation of phenolic compounds and flavonoids over time is primarily attributed to the residual activity of polyphenol oxidase (PPO) and peroxidase (POD) enzymes

(Plaza et al., 2011). Previous studies have further indicated that refrigerated storage better preserves the antioxidant activity of juices (Lin et al., 2020). As antioxidant activity in fruit juices is closely associated with the presence of bioactive compounds such as antioxidant pigments, polyphenols, and flavonoids (Morales-de la Peña et al., 2010), the initial increase in DPPH radical scavenging activity observed in the present study may be attributed to the elevated betacyanin and flavonoid content during storage.

No significant change in total soluble solids (TSS) following PEF treatment was also reported by Evrendilek and Özkan (2024), who observed that PEF-treated fresh grape, orange, and tomato juices showed no notable alteration in TSS. However, during storage, significant differences ($P < 0.05$) in TSS emerged between control and PEF-treated juices. These results contrast with those of Guo et al. (2014) and Salinas-Roca et al. (2017), who found no significant changes in TSS during the storage of PEF-treated pomegranate and mango juices, respectively. The low TSS observed in the present study may be attributed to incomplete enzyme inactivation.

Similarly, the observed reduction in pH could be explained by the production of organic acids through microbial fermentation or, again, by incomplete enzyme inactivation (Singh and Sharma, 2017). The decrease in residual enzyme activity following PEF treatment is likely due to conformational changes in the enzyme's secondary and tertiary structures (Manzoor et al., 2020). However, fluctuations in this residual activity may be due to incomplete enzyme inactivation and the presence of isoenzymes (Salinas-Roca et al., 2017). The findings of the present study contradict those of Sulaiman et al. (2017), who reported no regeneration of polyphenol oxidase (PPO) during storage. In contrast, Moritz et al. (2012) found no PPO inactivation after PEF treatment at 35 kV cm^{-1}, 1 Hz frequency, 300 pulses, and a specific energy input of 265 kJ kg^{-1}.

The increase in total plate count in PEF-treated juice during storage may be attributed to the recovery and growth of sublethally injured natural microflora following PEF treatment (Li et al., 2021). These findings highlight the need for more rigorous PEF parameters in future studies—such as increasing pulse number or treatment duration—to ensure complete microbial inactivation. Interestingly, the reduction in microbial count observed in the control juice during storage could be due to the natural antimicrobial properties of the fruit. Yong et al. (2018) found that fruits stored for six days at 4 °C exhibited enhanced antimicrobial activity against 10 pathogenic Gram-positive and six Gram-negative bacteria compared to freshly harvested fruits. Similarly, Rahayu et al. (2019) reported that red dragon fruit (*Hylocereus polyrhizus*) extracts exhibited notable antibacterial activity against *Streptococcus mutans*, a bacterium responsible for dental caries in humans. Notably, the peel extract showed greater inhibitory activity than the pulp extract.

Previous studies have also shown that PEF is less effective against yeast and mold than against bacteria. For example, Fabroni et al. (2024) reported a shelf life of only 20 days for PEF-treated orange juice. In untreated smoothies, yeast-induced spoilage occurred within eight days when stored at 4 °C or 7 °C. In contrast, although PEF treatment partially inactivated yeast in smoothies, it did not prevent mold growth, which led to spoilage after 14 days at 7 °C or 18 days at 4 °C (Timmermans et al., 2016).

Conclusion

A storage-related study of red dragon fruit juice obtained through ultrasound-assisted extraction and high-intensity pulsed electric field (HIPEF) pasteurization demonstrated notable retention of bioactive compounds and antioxidant activity. After two months of storage, the juice retained 91.5% of its initial betacyanin content and 82.26% of its initial DPPH radical scavenging activity. Total phenolic content (TPC) and total flavonoid content (TFC) increased over the storage period, with the highest TPC (68.38 ± 0.32 mg GAE 100 mL^{-1}) observed at 30 days and the highest TFC (22.23 ± 0.07 mg QE 100 mL^{-1}) at 10 days. Total soluble solids (TSS) showed no significant difference between the control and PEF-treated juice initially, but both declined over time. A significant decrease in pH was observed in both control and treated samples by the end of the two-month storage period. PEF pasteurization effectively reduced polyphenol oxidase (PPO) activity by 86.3% and peroxidase (POD) activity by 81.4%; however, enzyme activity fluctuated during storage, likely due to incomplete inactivation. A microbial reduction of approximately 5 log CFU mL^{-1} was achieved with PEF pasteurization. The total plate count in both control and treated samples increased until day 30, followed by a gradual decline, possibly due to the juice's inherent antimicrobial properties. Notably, no psychrophilic microbial growth was detected throughout the storage period. After two months, the PEF-treated juice exhibited lower yeast and mould counts compared to the control; however, the persistence of yeast and mould during storage indicated that PEF pasteurization was less effective against these organisms than against bacteria. These findings suggest that incorporating natural antimicrobial agents and optimizing PEF parameters—such as increasing pulse number or treatment duration—may enhance microbial control during extended storage.

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Conflict of Interest
The authors indicate no conflict of interest in this work.

References

Agcam E, Akyildiz A, & Evrendilek GA. 2016. A comparative assessment of long-term storage stability and quality attributes of orange juice in response to pulsed electric fields and heat treatments. Food and Bioproducts Processing 99, 90–98. https://doi.org/10.1016/j.fbp.2016.04.006

Aghajanzadeh S, & Ziaiifar AM. 2021. Pasteurization of juices with non-thermal technologies. In Sustainable Food Processing and Engineering Challenges (pp. 25–73). Elsevier. https://doi.org/10.1016/B978-0-12-822714-5.00002-4

Arivalagan M, Karunakaran G, Roy TK, Dinsha M, Sindhu BC, Shilpashree VM, Satisha GC, & Shivashankara KS. 2021. Biochemical and nutritional characterization of dragon fruit (Hylocereus species). Food Chemistry 353, 129426. https://doi.org/10.1016/j.foodchem.2021.129426

Barba F, Jäger H, Meneses N, Esteve M, Frígola A, & Knorr D. 2012. Evaluation of quality changes of blueberry juice during refrigerated storage after high-pressure and pulsed electric fields processing. Innovative Food Science & Emerging Technologies 14, 18–24. https://doi.org/10.1016/j.ifset.2011.12.004

Benjuma A Al, Salem BA, Eshkourfu RO, Hamarsheh RO, & Amawi KF. 2025. Study of the effect of storage on the physicochemical characteristics of some industrial juices traded in local markets. In Artificial Intelligence, Sustainable Technologies, and Business Innovation: Opportunities and Challenges of Digital Transformation (pp. 331–340). Springer. https://doi.org/10.1007/978-3-031-77925-1_29

Brito IPC, & Silva EK. 2024. Pulsed electric field technology in vegetable and fruit juice processing: A review. Food Research International 114207. https://doi.org/10.1016/j.foodres.2024.114207

Chaemsanit S, Matan N, & Matan N. 2018. Effect of peppermint oil on the shelf-life of dragon fruit during storage. Food Control 90, 172–179. https://doi.org/10.1016/j.foodcont.2018.03.001

Evrendilek GA, & Özkan BH. 2024. Pulsed electric field processing of fruit juices with inactivation of enzymes with new inactivation kinetic model and determination of changes in quality parameters. Innovative Food Science & Emerging Technologies 94, 103678. https://doi.org/10.1016/j.ifset.2024.103678

Fabroni S, Platania GM, Amenta M, Ballistreri G, Galvano F, Nges IA, & Timpanaro N. 2024. Pulsed electric field as a mild treatment for extended shelf-life and preservation of bioactive compounds in blood orange juice. Applied Sciences 14(16), 7275. https://doi.org/10.3390/app14167275

Guo M, Jin TZ, Geveke DJ, Fan X, Sites JE, & Wang L. 2014. Evaluation of microbial stability, bioactive compounds, physicochemical properties, and consumer acceptance of pomegranate juice processed in a commercial scale pulsed electric field system. Food and Bioprocess Technology 7, 2112–2120. https://doi.org/10.1007/s11947-013-1185-6

Herbach KM, Maier C, Stintzing FC, & Carle R. 2007. Effects of processing and storage on juice colour and betacyanin stability of purple pitaya (Hylocereus polyrhizus) juice. European Food Research and Technology 224, 649–658. https://link.springer.com/article/10.1007/s00217-006-0354-5

Huang W, Feng Z, Aila R, Hou Y, Carne A, & Bekhit AEDA. 2019. Effect of pulsed electric fields (PEF) on physico-chemical properties, β-carotene and antioxidant activity of air-dried apricots. Food Chemistry 291, 253–262. https://doi.org/10.1016/j.foodchem.2019.04.021

Islam MS, Zahan N, Hossain MS, & Rouf ASS. 2019. Determination of preservatives in fruit juice products available in Bangladesh by a validated RP HPLC method. Dhaka University Journal of

Pharmaceutical Sciences 18(2), 195–208. https://doi.org/10.3329/dujps.v18i2.43262

Jalgaonkar K, Mahawar MK, Kale S, Kale PN, Bibwe B, Dukare A, Kannaujia P, & Meena VS. 2018. Response surface optimization for development of dragon fruit based ready to serve drink. Journal of Applied and Natural Science 10(1), 272–278. https://journals.ansfoundation.org/index.php/jans/article/view/1617/1547

Kumari S, Aditya PK, & Mandal R. 2023. Microbial analysis of fresh fruit juice available in the market. Environment and Ecology 41(4), 2342–2347. https://doi.org/10.60151/envec/TCCI5654

Li L, Yang R, & Zhao W. 2021. The effect of pulsed electric fields (PEF) combined with temperature and natural preservatives on the quality and microbiological shelf-life of cantaloupe juice. Foods 10(11), 2606. https://doi.org/10.3390/foods10112606

Liao H, Zhu W, Zhong K, & Liu Y. 2020. Evaluation of colour stability of clear red pitaya juice treated by thermosonication. LWT 121, 108997. https://doi.org/10.1016/j.lwt.2019.108997

Lin YS, Huang WY, Ho PY, Hu SY, Lin YY, Chen CY, Chang MY, & Huang SL. 2020. Effects of storage time and temperature on antioxidants in juice from Momordica charantia L. and Momordica charantia L. var. abbreviata Ser. Molecules 25(16), 3614. https://doi.org/10.3390/molecules25163614

López-Gámez G, Elez-Martínez P, Martín-Belloso O, & Soliva-Fortuny R. 2020. Pulsed electric fields affect endogenous enzyme activities, respiration and biosynthesis of phenolic compounds in carrots. Postharvest Biology and Technology 168, 111284. https://doi.org/10.1016/j.postharvbio.2020.111284

Mandal D. 2019. Food preservative chemistry: Effects and side effects. Journal of the Indian Chemical Society 96(12), 1519–1528.

Manzoor MF, Zeng XA, Ahmad N, Ahmed Z, Rehman A, Aadil RM, Roobab U, Siddique R, & Rahaman A. 2020. Effect of pulsed electric field and thermal treatments on the bioactive compounds, enzymes, microbial, and physical stability of almond milk during storage. Journal of Food Processing and Preservation 44(7), e14541. https://doi.org/10.1111/jfpp.14541

Morales-de La Peña M, Salvia-Trujillo L, Rojas-Graü M, & Martín-Belloso O. 2010. Impact of high intensity pulsed electric field on antioxidant properties and quality parameters of a fruit juice–soymilk beverage in chilled storage. LWT-Food Science and Technology 43(6), 872–881. https://doi.org/10.1016/j.lwt.2010.01.015

Moritz J, Balasa A, Jaeger H, Meneses N, & Knorr D. 2012. Investigating the potential of polyphenol oxidase as a temperature-time-indicator for pulsed electric field treatment. Food Control 26(1), 1–5. https://doi.org/10.1016/j.foodcont.2011.11.016

Mtaoua H, Sánchez-Vega R, Ferchichi A, & Martín-Belloso O. 2017. Impact of high-intensity pulsed electric fields or thermal treatment on the quality attributes of date juice through storage. Journal of Food Processing and Preservation 41(4), e13052. https://doi.org/10.1111/jfpp.13052

Nurul S, & Asmah R. 2014. Variability in nutritional composition and phytochemical properties of red pitaya (Hylocereus polyrhizus) from Malaysia and Australia. International Food Research Journal 21(4). http://www.ifrj.upm.edu.my

Palety KK, Umakanth A, Narsaiah TB, Addepally U, Sirisha K, & Harini A. 2020. Evaluation of total phenolics and antioxidants of fresh and commercial fruit juices. Journal of Scientific Research 64(3). https://doi.org/10.37398/JSR.2020.640310

Plaza L, Sánchez-Moreno C, De Ancos B, Elez-Martínez P, Martín-Belloso O, & Cano MP. 2011. Carotenoid and flavanone content during refrigerated storage of orange juice processed by high-pressure, pulsed electric fields and low pasteurization. LWT-Food Science and Technology 44(4), 834–839. https://doi.org/10.1016/j.lwt.2010.12.013

Rahayu YC, Sabir A, & Setyorini D. 2019. Antibacterial activity of red dragon fruit extract (Hylocereus polyrhizus) on Streptococcus mutans. International Journal of Applied Pharmaceutics 11(4), 60–63. https://doi.org/10.22159/ijap.2019.v11s4.35293

Roobab U, Abida A, Chacha JS, Athar A, Madni GM, Ranjha MMAN, Rusu AV, Zeng XA, Aadil RM, & Trif M. 2022. Applications of innovative non-thermal pulsed electric field technology in developing safer and healthier fruit juices. Molecules 27(13), 4031. https://doi.org/10.3390/molecules27134031

Salinas-Roca B, Elez-Martínez P, Welti-Chanes J, & Martín-Belloso O. 2017. Quality changes in mango juice treated by high-intensity pulsed electric fields throughout the storage. Food and Bioprocess Technology 10, 1970–1983. https://doi.org/10.1007/s11947-017-1969-1

Salvia-Trujillo L, Morales-de la Peña M, Rojas-Graü MA, & Martín-Belloso O. 2011. Microbial and enzymatic stability of fruit juice-milk beverages treated by high intensity pulsed electric fields or heat

during refrigerated storage. Food Control 22(10), 1639–1646. https://doi.org/10.1016/j.foodcont.2011.03.022

Singh A, Swami S, Panwar NR, Kumar M, Shukla AK, Rouphael Y, Sabatino L, & Kumar P. 2022. Development changes in the physicochemical composition and mineral profile of red-fleshed dragon fruit grown under semi-arid conditions. Agronomy 12(2), 355. https://doi.org/10.3390/agronomy12020355

Singh SK, & Sharma M. 2017. Review on biochemical changes associated with storage of fruit juice. International Journal of Current Microbiology and Applied Sciences 6(8), 236–245. https://doi.org/10.20546/ijcmas.2017.608.032

Soliva-Fortuny R, Vendrell-Pacheco M, Martín-Belloso O, & Elez-Martínez P. 2017. Effect of pulsed electric fields on the antioxidant potential of apples stored at different temperatures. Postharvest Biology and Technology 132, 195–201. https://doi.org/10.1016/j.postharvbio.2017.03.015

Sulaiman A, Farid M, & Silva FV. 2017. Quality stability and sensory attributes of apple juice processed by thermosonication, pulsed electric field and thermal processing. Food Science and Technology International 23(3), 265–276. https://doi.org/10.1177/1082013216685484

Tarte I, Singh A, Dar AH, Sharma A, Altaf A, & Sharma P. 2023. Unfolding the potential of dragon fruit (Hylocereus spp.) for value addition: A review. eFood 4(2), e76. https://doi.org/10.1002/efd2.76

Timmermans RAH, Nederhoff AL, Groot MN, Van Boekel MAJS, & Mastwijk HC. 2016. Effect of electrical field strength applied by PEF processing and storage temperature on the outgrowth of yeasts and moulds naturally present in a fresh fruit smoothie. International Journal of Food Microbiology 230, 21–30. https://doi.org/10.1016/j.ijfoodmicro.2016.04.014

Wibowo M, Bakri A, Hariono B, Wijaya R, & Brilliantina A. 2022. Application of pulsed electric field in pasteurization of orange juice of siam cultivar: Study on nutritional, physical, chemical properties, and total microorganism. IOP Conference Series: Earth and Environmental Science 980(1), 012007. https://doi.org/10.1088/1755-1315/980/1/012007

Yildiz S, Pokhrel PR, Unluturk S, & Barbosa-Canovas GV. 2021. Shelf life extension of strawberry juice by equivalent ultrasound, high pressure, and pulsed electric fields processes. Food Research International 140, 110040. https://doi.org/10.1016/j.foodres.2020.110040

Yong YY, Dykes G, Lee SM, & Choo WS. 2018. Effect of refrigerated storage on betacyanin composition, antibacterial activity of red pitahaya (Hylocereus polyrhizus) and cytotoxicity evaluation of betacyanin rich extract on normal human cell lines. LWT 91, 491–497. https://doi.org/10.1016/j.lwt.2018.01.078

Zhang ZH, Wang LH, Zeng XA, Han Z, & Wang MS. 2017. Effect of pulsed electric fields (PEFs) on the pigments extracted from spinach (Spinacia oleracea L.). Innovative Food Science & Emerging Technologies 43, 26–34. https://doi.org/10.1016/j.ifset.2017.06.014

Zhishen J, Mengcheng T, & Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 64(4), 555–559. https://doi.org/10.1016/S0308-8146(98)00102-2

Zhu W, Ai Y, Fang F, & Liao H. 2021. Application of thermosonication in red pitaya juice processing: Impacts on native microbiota and quality properties during storage. Foods 10(5), 1041. https://doi.org/10.3390/foods10051041