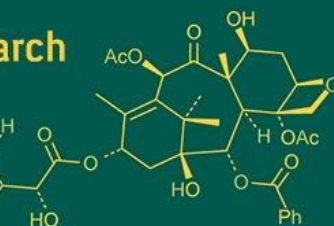
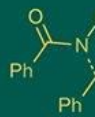


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Impact of thermal pasteurization by retort technology on the quality and safety of red dragon fruit (*Hylocereus polyrhizus*) juice during refrigerated storage

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Abstract

This study evaluates the quality and safety of red dragon fruit (*Hylocereus polyrhizus*) juice processed using retort pasteurization (90 °C for 5 minutes) and stored under refrigeration for two months. Quality parameters, including betacyanin content, total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, total soluble solids (TSS), and pH, were analyzed alongside microbial safety assessments. Retort pasteurization effectively preserved juice quality, retaining 91.9% of betacyanins, 90.82% of TPC, and 95.19% of DPPH radical scavenging activity immediately after pasteurization. During storage, 81.9% of betacyanins, 98.61% of TPC, and 80.19% of DPPH activity were maintained after two months. Although pasteurization caused a slight reduction in betacyanin content and a significant decrease in TPC due to heat-induced oxidation, TFC increased post-treatment. TSS levels declined due to sugar depletion and Maillard reactions, while pH remained stable. Microbial analysis revealed no mesophilic bacterial growth for 20 days, no psychophilic growth throughout storage, and minimal yeast and mold growth by the end of the storage period. These findings demonstrate that retort pasteurization effectively extends the shelf life of dragon fruit juice while preserving its quality and antioxidant properties. However, additional control measures are recommended to further prevent yeast and mold growth during extended storage.

Keywords: Bioactive compounds, dragon fruit juice, pitaya, preservation, retort technology, storage study, thermal pasteurization

1. Introduction

Consumption of soft drinks is ever-increasing among the population across the world. Sugar-sweetened beverages are part of a broader category of unhealthy foods that are high in calories but low in nutrients. It leads to obesity among children and adolescents (Pettigrew *et al.*, 2015) [24]. A survey among adolescents showed that more than half of the boys and over a third of the girls drink soft drinks daily. A quarter of the students prefer soft drinks over water or milk, and about 40% noted that soft drinks are commonly available at home (Denney-Wilson *et al.*, 2009) [6]. Children who consume more soft drinks, relative to milk and 100 percent fruit juice, were found to be at a greater risk of developing dental caries (Lim *et al.*, 2008) [17]. These studies point out to the need for the consumption of fresh fruit juices over sugar-sweetened beverages.

Red dragon fruit (*Hylocereus polyrhizus*) or pitaya is an auspicious fruit enriched with medicinal properties due to its high bioactive potential. It is rich in antioxidant pigments betalains, which have anti-cancerous properties. The fruit is very low in sugar and is very good for diabetic patients. Moreover, the fruit lowers the bad LDL cholesterol, aids in weight loss, and prevents heart diseases (Nishikito *et al.*, 2023; Hossain *et al.*, 2021) [20, 10]. Studies have shown that the consumption of fruit can reduce the risk of human breast cancer, liver cancer, prostate cancer, and colon cancer (Bhadoria *et al.*, 2024) [3]. The peel extract has been proven to be effective against the proliferation of cancer melanoma cells (Wu *et al.*, 2006) [29]. Fresh dragon fruit juice has a very short shelf life. They are easily spoiled by yeast molds. It is prone to spoilage by species like *Rhizopus*, *Fusarium*, *Botryosphaeria*, and *Colletotrichum* (Chaemsanit *et al.*, 2018) [5].

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Due to this reason, juices are preserved conventionally by thermal pasteurization followed by the addition of preservatives for longer shelf life in ambient temperature. However, thermal pasteurization may significantly reduce the heat-sensitive bioactive compounds in the juice. The excessive addition of sugars and preservatives further affects the quality of juice and health of consumers harmfully. Hence, alternative technologies are required for fresh pitaya juice preservation.

Retort technology is an advanced thermal processing technology that can be used for sterilization and pasteurization of various kinds of foods. The literature review showed a research gap on the scope of retort pasteurization of red dragon fruit juice and evaluation of its quality and safety during refrigerated storage. Retort pouch processing of the juice may enhance the economic potential of the dragon fruit industry by promoting the production of value-added products such as squash, jelly, syrup, powder, and many more from the juice during off-seasons. Hence, this study was undertaken on this background to assess the changes in quality and safety of the retort pasteurized red dragon fruit juice in comparison to unpasteurized juice over two months of refrigerated storage.

2. Materials and Methods

2.1 Raw material

Fully matured red dragon fruits (Malaysian red variety) were collected from Brahma's red dragon fruit farm, located in Chirakkekcode, Thrissur, Kerala, India. The fruits were thoroughly washed, peeled, and cut into pieces under hygienic conditions, then packed into standing pouches and sealed. These pouches were initially placed in a blast freezer for rapid freezing for 1 hour, followed by storage in a deep freezer at -18 ± 1 °C.

2.2 Chemicals

The Folin & Ciocalteu's reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium hydroxide, sodium carbonate, sodium nitrate, aluminium chloride, guaiacol, catechol, and buffer solution (pH 7), plate count agar, potato dextrose agar were sourced from Sisco Research Laboratories Pvt. Ltd, Maharashtra, India. Sodium hydroxide and hydrogen peroxide were procured from Merck Life Science Pvt. Ltd, Mumbai, India. Ethanol was supplied by Gemini Associates, Goa, India. All chemicals and reagents used were of analytical grade.

2.3 Juice extraction

Frozen fruits were left to thaw in the refrigerator at 4 °C overnight, while fresh fruits were washed, peeled, and cut into pieces before being subjected to ultrasonic pre-treatment at 40% amplitude for a duration of 10 minutes. To ensure surface sterilization, the ultrasonic probe was wiped with a tissue soaked in 70% alcohol one day prior to treatment. The ultrasonic pre-treatment was applied to increase the concentration of bioactive compounds in the juice, based on preliminary studies. The ultrasonic device used (Shalom Ultrasonics, India) operated at a frequency of 20 kHz, with a maximum power output of 750 W and a horn diameter of 25 mm. Following the pre-treatment, juice extraction was carried out using a cold press juicer (Model: 19003594, Pigeon, India), which has a power rating of 150 W and a speed of 75 RPM. The experiments were conducted at the Agribusiness Incubator, Department of Agricultural Engineering, College of Agriculture, Vellanikkara, Thrissur.

2.4 Retort pasteurization

Around 100 ml of juice was filled into 14 retort pouches, and exhaust processing was carried out using a steam exhausting unit (Model: RSMFLS-1003/92, Revathi Electronics and Controls, Thane, Maharashtra, India). The pouches were then sealed using a pneumatic sealer (Model: QS 300X 10 PNI V2, Sevana Electrical Appliances Pvt. Ltd, Kochi, Kerala, India). After sealing, the pouches were placed in a steam-air retort (Model No: SS304; Make: ITech Equipment, Bengaluru, India) for thermal pasteurization, which was performed at optimized conditions of 90 °C for 5 minutes, as determined from preliminary studies. Following the pasteurization process, the pouches were automatically cooled, unloaded from the retort, and stored under refrigeration at 4 °C. Fresh, unpasteurized juice in PET bottles was used as the control and also stored under refrigerated conditions. Chemical and microbial analyses were conducted on both the control and PEF-pasteurized juice samples at 10-day intervals over a two-month period of refrigerated storage. The storage study was conducted from 26/10/24 to 28/12/24.

2.5 Quality and safety evaluation

2.5.1 Betacyanin content

The Betacyanin content of the juice was determined using the UV visible spectrophotometric method at 538 nm according to Nurul & Asmah. (2014) ^[21] with slight modifications. One ml juice sample was dissolved in 10 ml distilled water and then centrifuged at 4500 rpm for 10 min. The absorbance of the supernatant was measured at 538 nm using a spectrophotometer. Betacyanin content was expressed as betacyanin equivalents (mg/100 ml) using Eqn.4. Each measurement was repeated three times, and the average was recorded.

$$\text{Concentration of Betacyanins (mg/100 ml)} = \frac{A \times MW \times 1000}{\epsilon \times L} \quad (1)$$

where,

A = absorbance at 538 nm,

MW = molecular weight of Betacyanin (550 g/mol),

ϵ = Molar extinction coefficient of Betacyanin (60,000),

L = path length (1.0 cm).

2.5.2 Total Phenolic Content

The TPC in the fruit juice was determined using the Folin-Ciocalteu method as described by Palety *et al.* (2020) ^[23] with slight modifications. The TPC was calculated using regression equations from a standard curve of Gallic acid. About 0.2 ml fruit juice was mixed with 1.8 ml distilled water. 0.2 ml FC reagent (previously diluted tenfold with distilled water) was added and allowed to stand at room temperature for 5 minutes. After that, 0.8 ml of 20% sodium bicarbonate was added to the mixture. The tube was vortexed and allowed to stand at room temperature for 30 minutes. The absorbance at 720 nm was measured and the TPC was expressed as Gallic acid equivalents (GAE) in mg/100 ml of fruit juice. Each measurement was repeated three times, and the average was recorded.

2.5.3 Total flavonoid content

The TFC in the samples was analyzed using the aluminum chloride assay, following the method by Zhishen *et al.* (1999) ^[32]. A 0.5 ml portion of the extract was mixed with distilled water to make a 5 ml solution. Next, 0.3 ml of 5%

sodium nitrite (NaNO_2) was added and left to react for 5 min. Afterward, 0.3 ml of 10% aluminum chloride (AlCl_3) was introduced, followed by 2 ml of 1 M sodium hydroxide (NaOH) after 1 minute. The final volume was adjusted to 10 ml with distilled water, mixed thoroughly, and the absorbance was measured at 510 nm. The TFC was then calculated using a quercetin-based calibration curve and expressed as milligrams of quercetin equivalents (mg QE per 100 ml). Each measurement was repeated three times, and the average was recorded.

2.5.4 DPPH radical scavenging activity

DPPH free radical scavenging activity of dragon fruit juice samples was determined according to the method reported by Singh *et al.* (2022) [26] with slight modification. 0.1mM DPPH solution was prepared by dissolving 4 mg DPPH in 100 ml ethanol. 1 ml of fruit juice was diluted to 30 ml with distilled water and centrifuged at 4500 rpm for 10 min. Then 2 ml of the diluted juice was mixed with 2 ml of DPPH solution. The sample was kept in the dark for 30 min and absorbance was read at 517 nm. The following formula was used for the calculation of antioxidant activity.

$$\text{Antioxidant activity (\%)} = \frac{A_c - A_s}{A_c} \times 100 \quad (2)$$

Where, A_c and A_s indicated the absorbance of control and absorbance of the sample respectively. The control sample contained 2 ml ethanol instead of juice sample.

2.5.5 TSS and pH

Total soluble solids was determined using a digital refractometer (PCE-DR series, PCE Americas Inc, USA). The pH of fruit juice was determined using a digital pH meter (μC pH system 361, Systronics, India).

2.5.6 Residual enzyme activity

PPO activity was calculated according to Liao *et al.* (2020) [16]. Every 1 ml of juice was mixed with 2 ml substrate containing 0.07 M catechol and 0.2 M phosphate buffer (pH 7). An increase in absorbance at 420 nm was recorded at intervals of 2 s for 4 min, using a spectrophotometer. The specific activity of PPO was calculated according to the slope of the linear portion of the reaction curve, and the residual activity of PPO was calculated according to Eqn.5. Each measurement was repeated three times, and the average was recorded.

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

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

The POD activity was evaluated following a modified protocol from Zhu *et al.* (2021) [33]. One ml juice sample was combined with 2 ml of 1% (v/v) guaiacol (0.2 M, pH 7 phosphate buffer) and 0.2 ml of 1.5% hydrogen peroxide to create the enzymatic reaction solution. Absorbance at 470 nm was measured at 2 s intervals for 4 min. The specific

activities of POD (Abs/min) were determined based on the slopes of the linear portion of the reaction curves, and the residual enzyme activity was calculated according to Eqn.6. Each measurement was repeated three times, and the average was recorded.

$$\text{Residual POD activity (\%)} = 100 \times \frac{A_t}{A_0} \quad (4)$$

Where:

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

2.5.7 Microbial analysis

Petri dishes were sterilized by heating at 170 °C for 1 hour in a hot air oven. Water for serial dilutions was placed in conical flasks and test tubes, which were then sealed with cotton. Plate count agar media was prepared according to the manufacturer's instructions and boiled with magnetic stirring on a heating mantle until the solution became clear. The medium, along with the conical flasks and test tubes for dilution, were sterilized in an autoclave at 121 °C for 15 minutes. To assess the total microbial load in fruit juice, the pour plate method was used. First, 10 ml of fruit juice was mixed with 90 ml of sterile distilled water to achieve a 10^{-1} dilution. Serial dilutions were made up to 10^{-6} using sterile distilled water in test tubes. For microbial count, 1 ml from the 10^{-3} and 10^{-4} dilutions was plated for the retort pasteurized juice, while 1 mL from the 10^{-5} and 10^{-6} dilutions was plated for the control juice, following FSSAI guidelines for microbiological food examination. Approximately 15-20 ml of sterile plate count agar was added to each petri dish. Once the agar solidified, the plates were incubated at 37 °C for microbial growth. After 24-48 hours, the colonies were counted and expressed as CFU/ml. The total psychrophilic count of aerobic bacteria was determined on plate count agar using the same procedure, but incubation was done at 4 °C for 10 days.

For the total yeast and mould count, potato dextrose agar media was used. The media was prepared according to specifications, boiled until clear, and sterilized at 121 °C for 15 minutes. Juice samples, both control and retort pasteurized, were serially diluted up to 10^{-6} . One ml from the 10^{-3} and 10^{-4} dilutions was plated for each sample, with two replicate plates per dilution. The media was poured onto the plates, which were then incubated inverted at 25 °C for 5 days.

2.5.8 Statistical analysis

The changes in variables among treatments and storage days were analyzed by two factorial CRD designs using GRAPES software, KAU.

3. Results and discussion

Table 1 shows the changes in betacyanin content, total phenolic content, total flavonoid content, and DPPH radical scavenging activity in red dragon fruit juice during storage after retort pasteurization.

Table 1: Changes in key bioactive compounds and DPPH radical scavenging activity of red dragon fruit juice

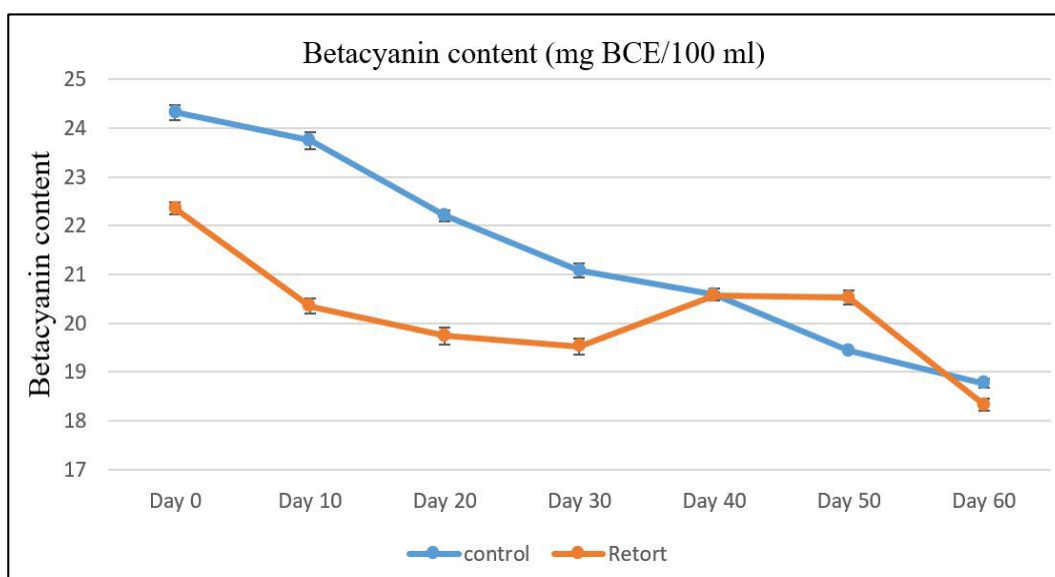
Storage Days	Betacyanin content (mg BCE/ 100 ml)		Total Phenolic Content (mg GAE/100 ml)		Total Flavonoid Content (mg QE/100 ml)		DPPH radical scavenging activity (%)	
	control	Retort	control	Retort	control	retort	control	retort
Day 0	24.32±0.15 ^a	22.36±0.12 ^c	71.06±0.35 ^a	64.54±0.34 ^d	14.16±0.06 ^h	20.16±0.04 ^a	78.6±0.36 ^a	74.82±0.36 ^c
Day 10	23.74±0.17 ^b	20.35±0.16 ^f	51.59±0.46	49.96±0.36 ^k	15.83±0.05 ^f	18.83±0.07 ^b	77.6±0.52 ^b	72.36±0.43 ^d
Day 20	22.20±0.12 ^c	19.74±0.17 ^g	67.96±0.43 ^b	58.48±0.45 ^g	13.96±0.03 ⁱ	17.81±0.06 ^d	70.96±0.43 ^e	71.21±0.31 ^e
Day 30	21.08±0.15 ^d	19.52±0.16 ^{gh}	70.97±0.42 ^a	61.75±0.38 ^f	11.6±0.05 ^k	13.16±0.05 ^j	68.04±0.24 ^h	70.73±0.32 ^{ef}
Day 40	20.59±0.12 ^c	20.56±0.06 ^{ef}	49.43±0.26 ^k	54.27±0.24 ⁱ	18.5±0.02 ^c	20.16±0.03 ^a	70.3±0.23 ^f	64.78±0.24 ⁱ
Day 50	19.43±0.06 ^h	20.53±0.14 ^{ef}	56.38±0.23 ^h	53.75±0.2 ⁱ	15.5±0.03 ^g	15.5±0.07 ^g	75±0.26 ^c	69.64±0.28 ^g
Day 60	18.77±0.09 ⁱ	18.33±0.12 ^j	65.54±0.34 ^c	63.64±0.36 ^e	17.5±0.04 ^e	20.16±0.04 ^a	74.54±0.31 ^c	60±0.27 ^j

Different superscript letters indicate significant differences among days and treatments

3.1 Changes in betacyanin content during storage

Retort pasteurization at 90 °C for 5 min resulted in only an 8% reduction in betacyanin content compared to fresh control juice. The reduction is due to the thermal degradation of betacyanins. When heated, the major component of betacyanin called betanin breaks down into cyclo-dopa 5-O-β-glucoside and betalamic acid, causing a reduction in red coloration (Herbach *et al.*, 2007) [9]. In the present study, the betacyanin content fluctuated during storage. It decreased to 19.52 mg/100 ml on day 30, then gradually increased to 20.53 mg/100 ml by day 50. On day 40, no significant difference was observed between control and retort pasteurized juice. However, it dropped again to 18.33 mg/100 ml on day 60. The increase in betacyanin during storage might be because betalains have a unique

property of regeneration after thermal treatment (Khan & Giridhar, 2014) [14]. Yong *et al.* (2018) [31] also observed an increase in betacyanin during storage of pitaya fruit at 4 °C. A study on the heat stability of betacyanins has shown that thermal pasteurization at 65 °C for 30 min was the best treatment for the retention of betacyanins in red dragon fruit juice (Wong & Siow, 2015) [28]. They observed a significant reduction (59%) in betacyanin content at 85 °C for 30 minutes of thermal treatment. On the other hand, canning of beetroot slices at 66 °C showed a 23% loss of betacyanins (Von Elbe *et al.*, 1981) [27]. As compared to these previous thermal treatment studies, the present study showed only a minor reduction in betacyanin content after thermal pasteurization using retort technology.

**Fig 1:** Changes in betacyanin content of during storage

3.2 Changes in Total Phenolic Content during storage

Fig. 2 shows changes in the phenolic content of fresh control juice and retort pasteurized juice during storage. A significant reduction ($p<0.05$) in total phenolic content was observed after retort pasteurization. The total phenolic content of retort pasteurized juice showed a sharp decline from 64.54±0.34 mg GAE/100 ml to 49.96±0.36 mg GAE/100 ml on day 10. After that, it gradually increased and showed 61.75±0.38 mg GAE/100 ml on day 30. After that, again a gradual decline was observed up to day 50. After that, phenolic content again increased to 63.64 mg GAE/100 ml. The control sample also showed a similar pattern. The initial decrease in phenolic compounds in retort pasteurized juice can be attributed to the heat-induced

oxidation of certain phenolic compounds present in the juice. Oliveira *et al.* (2014) [22] reported a significant decrease in total phenolic content due to a reduction in quercetin-3-rutinoside and kaempferol after thermal pasteurization of strawberries. Previous studies have also reported fluctuations in total phenolic content by showing decrease and increase during refrigerated storage of fruit juices. Studies by Esteve & Frigola, (2008) [7] have shown a non-significant increase of phenolic compounds in orange juice during refrigerated storage. This fluctuation can be attributed to the fact that some compounds are formed during storage which react with the Folin-Ciocalteu reagent and significantly enhance the phenolic content.

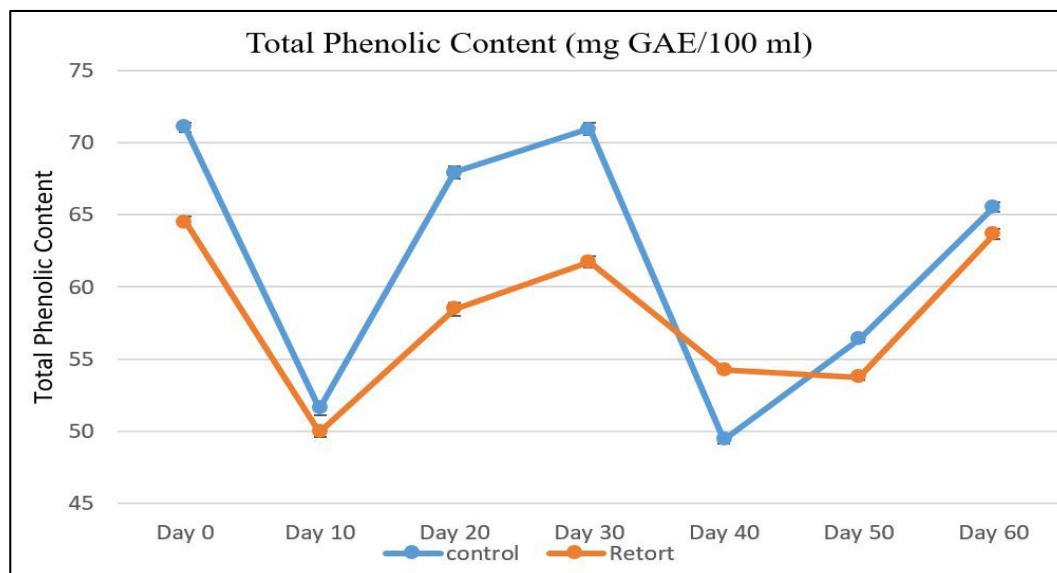


Fig 2: Changes in total phenolic content during storage

3.3 Changes in Total Flavonoid Content during storage

Fig. 3. shows the changes in flavonoid content in fresh and after retort pasteurized. Pitaya juice Retort pasteurization of juice sample significantly enhanced ($p < 0.05$) the total flavonoid content compared to the fresh juice. During storage, the retort pasteurized juice sample showed significantly higher flavonoid content ($p < 0.05$) throughout storage except on day 50. Similar observations were made by Igual *et al.* (2011) [12] where pasteurized grape juice samples lost fewer flavonoids during storage than fresh

juice. The retention of flavonoid compounds might be due to the heat induced inactivation of oxidative enzymes PPO and POD responsible for degradation of flavonoids (Morales-de La Peña *et al.*, 2010) [19]. The HPLC analysis of thermally pasteurized jambolan fruit pulp by Branco *et al.* (2016) [4] revealed that pasteurization led to a 57% increase in gallic acid levels and a 14% rise in quercetin levels. The rise in gallic acid following pasteurization could be attributed to the breaking of covalent bonds, resulting in the release of this compound into the medium.

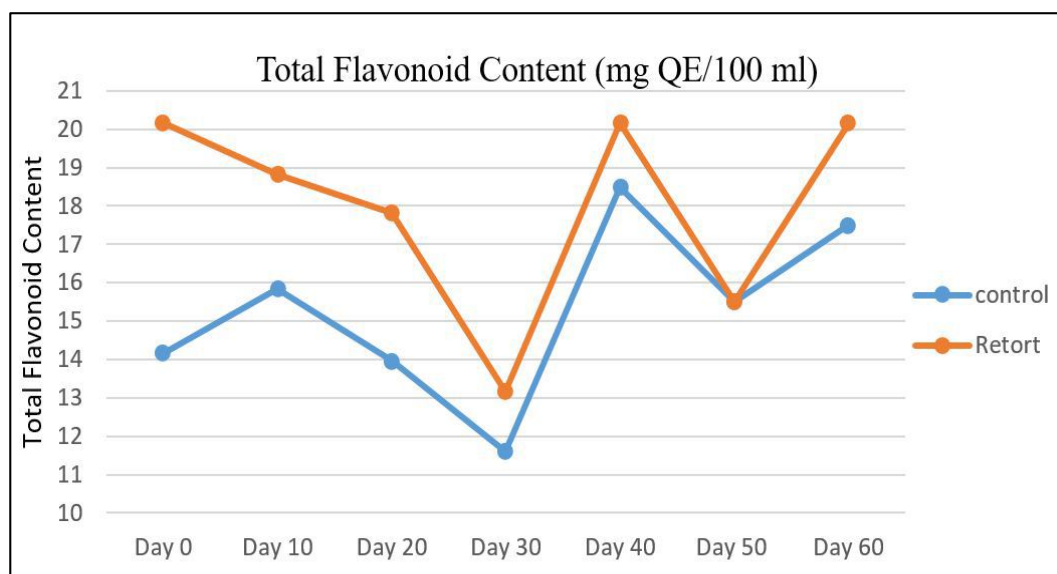


Fig 3: Changes in flavonoid content during storage

3.4 DPPH radical scavenging activity

Fig. 4 illustrates the changes in DPPH radical scavenging activity of retort-pasteurized juice compared to the fresh control during storage. The DPPH radical scavenging activity of the retort-pasteurized juice was significantly ($p < 0.05$) lower immediately after treatment compared to the fresh control. During storage, the radical scavenging activity decreased and on day 20, the activity didn't show a significant difference from the control. The initial reduction is likely due to the heat-induced degradation of betacyanins observed in the study. These findings contrast with those of

Oliveira *et al.* (2014) [22], who reported that thermal pasteurization of peach pieces at 90 °C for 5 minutes did not significantly reduce DPPH radical scavenging activity or total phenolic compounds. However, similar to the present study, they observed a significant reduction in radical scavenging activity during storage. However, compared to the fresh juice, 80.49% of the initial DPPH radical scavenging activity was retained by retort pasteurization after 2 months of refrigerated storage.

The current study also found no correlation between total phenolic content and DPPH radical scavenging activity,

consistent with the findings of Piljac-Žegarac *et al.* (2009)^[35]. Their research on refrigerated storage of several dark-colored fruit juices revealed a similar lack of correlation. This discrepancy arises because the Folin-Ciocalteu reagent reacts with a wide range of reducing compounds, including bound and unbound phenolic compounds, carotenoids, and

vitamin C, which can result in higher values at the end of storage. In contrast, DPPH only reacts with free antioxidants in the juice. The observed reduction in antioxidant pigments, particularly betacyanins, during storage likely contributed to the decline in DPPH radical scavenging activity.

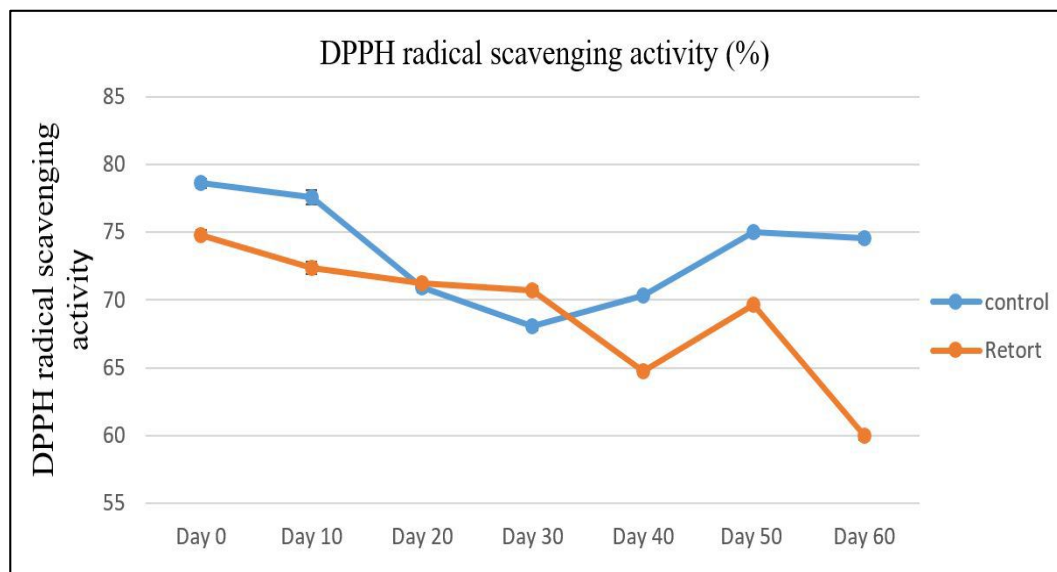


Fig 4: Changes in DPPH radical scavenging activity during storage

3.5 TSS and pH

Table 2 shows the changes in TSS and pH during storage. Fig.5 depicts the changes in TSS during storage. The TSS showed a significant decrease ($p < 0.05$) after retort pasteurization compared to fresh juice. The results were consistent with those of Lamo *et al.* (2019)^[15] who observed a reduction in TSS with higher temperatures used for pasteurizing guava juice and those of Benjamin & Gamrasni, (2020)^[2] who noted a decrease in TSS after thermal pasteurization of pomegranate juice. The reduction in TSS can be attributed to the depletion of reducing sugars and the occurrence of the Maillard reaction at higher temperatures. On the other hand, the results were contradictory to Xu *et al.* (2016)^[30] who observed no change in TSS after thermal pasteurization and during storage of banana puree. The TSS of control and thermally treated juice showed a gradual increase up to day 20 and after that gradually decreased. The increase in TSS of thermally pasteurized juice was also reported by Hameed *et al.* (2019)^[8]. The increase in TSS during storage might be

due to the hydrolysis of sugars and degradation of pectic substances present in the fruit juice. On days 30 and 40, there was no significant difference in TSS between control and retort pasteurized juice. The retort pasteurized juice showed more stability in TSS during storage compared to the control.

Fig.6. shows the changes in the pH of control and thermally pasteurized juice during storage. On the day of treatment, the pH of fresh and retort pasteurized juice didn't differ significantly. During storage, retort pasteurized juice showed a stable pH compared to control juice. Only a 2.5% decrease in pH was exhibited by thermally pasteurized juice, while a 12.1% decrease in pH was exhibited by control juice. Similar kind of results were reported by Zulueta *et al.* (2013)^[34] who observed no significant difference in pH after thermal and non-thermal pasteurization but observed a reduction in the pH during refrigerated storage of orange juice-milk beverage at 4 °C. The significant decrease in pH of control juice might be due to bacterial growth and lactic acid fermentation.

Table 2: Changes in TSS and pH during storage

Days of storage	TSS (° brix)		pH	
	Control	Retort	Control	Retort
Day 0	12.18±0.02 ^b	11.46±0.01 ^{ef}	4.62±0.01 ^c	4.65±0.02 ^c
Day 10	12.23±0.03 ^b	11.5±0.02 ^c	4.72±0.02 ^b	4.71±0.01 ^b
Day 20	12.60±0.01 ^a	11.73±0.28 ^d	4.92±0.01 ^a	4.61±0.02 ^d
Day 30	11.5±0.005 ^e	11.5±0.005 ^e	4.34±0.03 ^h	4.60±0.03 ^d
Day 40	11.40±0.005 ^{ef}	11.5±0.04 ^e	4.37±0.005 ^g	4.51±0.01 ^f
Day 50	11.4±0.06 ^{ef}	11.26±0.05 ^g	4.23±0.001 ⁱ	4.54±0.005 ^e
Day 60	11.9±0.04 ^c	11.35±0.06 ^{fg}	4.06±0.005 ^j	4.53±0.005 ^{ef}

Different superscript letters indicate significant differences among days and treatments

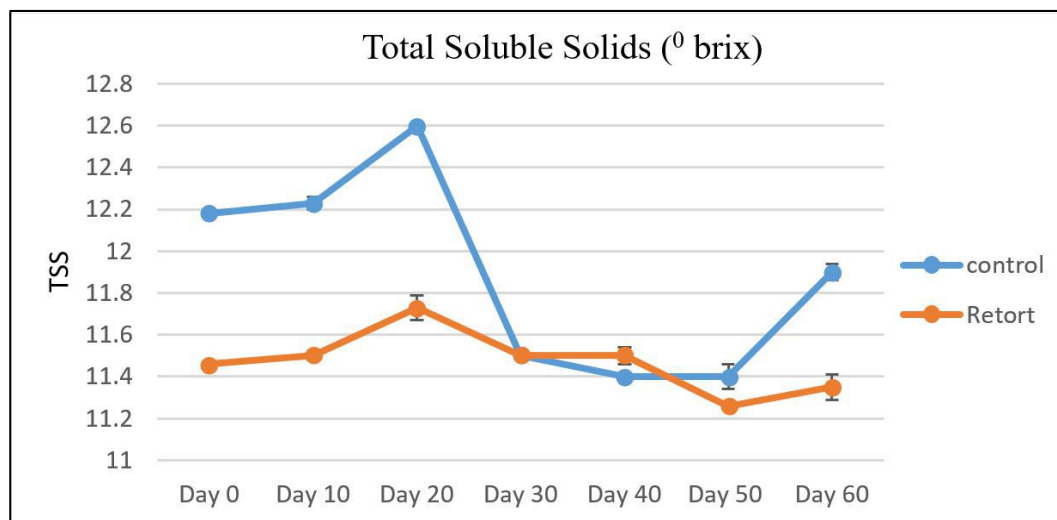


Fig 5: Changes in TSS during storage

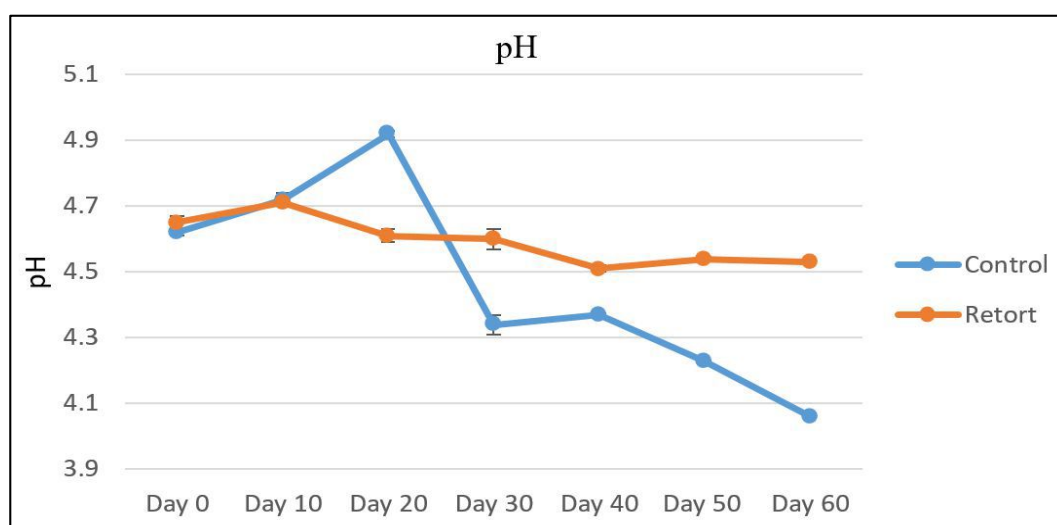


Fig 6: Changes in pH during storage

3.6 Residual Enzyme activity

The PPO and POD are two important spoilage enzymes present in fruit juices. These enzymes cause undesirable color and flavor changes and oxidation of phenolic compounds in fruit juices. The POD uses hydrogen peroxide to catalyze the oxidation of phenolic compounds. (Ağçam *et al.*, 2018) ^[1]. Table 3 shows the changes in residual activities of PPO and POD during storage of red pitaya juice and Fig.7 is the graphical representation of the changes. On the day of treatment at 90 °C for 5 min, retort pasteurized juice showed significantly low residual PPO activity (0.015%) compared to POD (13.9%) activity. Earlier research has shown that thermal pasteurization at elevated temperatures significantly decreases PPO activity. BALTACIOĞLU (2018) ^[36] examined the inactivation of PPO in peach juice at temperatures ranging from 40 to 70 °C for 10 to 40 minutes and found substantial reductions in PPO activity between 50 °C and 70 °C, with a 99% inactivation occurring at 60 °C for 10 minutes. The enzyme inactivation during pasteurization is due to the changes in structure and conformation of enzymes due to heat. Ağçam *et al.*, (2018) ^[1] have reported that compared to PPO, POD shows higher thermal stability. Mandha *et al.* (2023) ^[18] in their studies on thermal pasteurization of mango, pineapple and watermelon juices have shown that POD is more heat resistant than PPO. The results of the present study also

supported these findings as 13.9% residual POD activity was observed after retort pasteurization while PPO showed zero residual activity. During storage, PPO activity increased and reached a maximum (32.65±0.57%) on day 20. On the other hand, maximum residual POD activity (50±0.35%) was observed on day 30. After that, enzyme activities were gradually decreased and at the end of 60 days of storage, both enzyme residual activities were below 15%. Similar kind of fluctuations in enzyme activities during storage after thermal treatment was reported by Salinas-Roca *et al.* (2017) ^[25] in their study on mango juice. This fluctuation is due to incomplete inactivation and presence of isoenzymes.

Table 3: Changes in residual enzyme activity during storage

Days of storage	Residual PPO activity (%)	Residual POD activity (%)
Day 0	0.015±0.002 ^g	13.9±0.13 ^e
Day 10	9.5±0.5 ^d	33.33±0.25 ^b
Day 20	32.65±0.57 ^a	14.28±0.06 ^d
Day 30	5.8±0.06 ^e	50±0.35 ^a
Day 40	10.86±1 ^c	30±0.22 ^c
Day 50	1.53±0.005 ^f	10±0.04 ^g
Day 60	12.3±0.08 ^b	10.81±0.06 ^f

Different superscript letters indicate significant differences among storage days

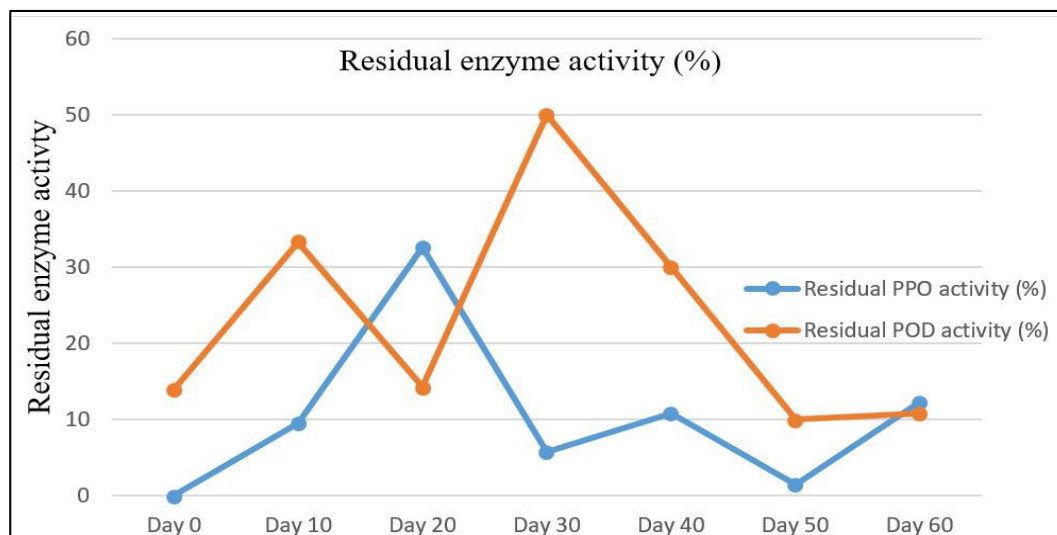


Fig 7: Changes in residual enzyme activity during storage

3.7 Microbial analysis

Table 4 shows the changes in total plate count and total yeast and mould count during storage. Fig.8 shows the total plate count in control and thermally pasteurized juice after 10 days and 60 days of storage. From the day of treatment to 20 days of storage, no bacterial growth was observed, and the juice was completely safe. On day 50, control juice exhibited 28×10^5 colonies while retort pasteurized juice showed only 4×10^3 colonies. Even after 60 days of storage, the bacterial growth in thermally pasteurized juice was much less and was almost safe. After 30 days of storage, no increase in the number of bacterial colonies was observed and surprisingly, after day 40, a reduction in bacterial count was observed in the control juice. It might be due to the natural antibacterial and antioxidant properties exhibited by dragon fruit juice. Yong *et al.* (2018) [31] have found that the refrigerated storage of pitaya fruit for 6 days increased the antibacterial activity against several gram-positive and gram-negative organisms.

No psychrophilic growth was detected during the entire storage period in both control and retort pasteurized juice. The results were in agreement with Hu *et al.*, (2020) [11] who

also observed no psychrophilic growth during refrigerated storage of thermally pasteurized jaboticaba juice.

No yeast and mold growth was observed in retort pasteurized juice for up to 10 days. After that minor growth was observed and at the end of storage the yeast and mold count was 10×10^3 CFU/ml while the colonies in control juice were too numerous to count. The few colonies found might be of heat-stable molds. Kelfkens, (2024) [13] studied the occurrence of fungi in fruit-based baby food puree packed in retort pouches. The study found a concerning occurrence of heat-stable molds in 28 out of 83 pouches and observed a count ranging from 2×10^0 to 9×10^7 CFU/ml. They suggested several possible reasons for the occurrence such as packaging leaks, ascospore activation and germination, and insufficient heat treatment, It might also arise from contaminated water used for steam exhausting and cooling and poor cleaning practices. Even though the the number of yeast and mold colonies observed in the present study was very few, it is a possible concern and additional safety measures have to be taken to avoid yeast and mold growth.

Table 4: Changes in total plate count and total yeast and mold counts during storage

Days of storage	Total plate count (CFU/ml)				Total yeast and mold count (CFU/ml)			
	Control		Retort		Control		Retort	
	10^{-5} dilution	10^{-6} dilution	10^{-3} dilution	10^{-4} dilution	10^{-3} dilution	10^{-4} dilution	10^{-3} dilution	10^{-4} dilution
Day 0	21×10^5 d	8×10^6 e	0 b	0 b	2×10^3 g	0 g	0 b	0 e
Day 10	50×10^5 a	13×10^6 c	0 b	0 b	60×10^3 f	20×10^4 f	0 b	0 e
Day 20	40×10^5 b	12×10^6 cd	0 b	0 b	80×10^3 e	50×10^4 e	10×10^3 a	6×10^4 bc
Day 30	50×10^5 a	20×10^5 a	1×10^3 b	0 b	90×10^3 d	60×10^4 d	10×10^3 a	5×10^4 cd
Day 40	50×10^5 a	15×10^5 b	3×10^3 a	2×10^4 a	110×10^3 c	80×10^4 c	10×10^3 a	8×10^4 a
Day 50	28×10^5 c	11×10^5 d	4×10^3 a	2×10^4 a	TNTC ^b	TNTC ^b	9×10^3 a	7×10^4 ab
Day 60	5×10^5 e	5×10^5 f	3×10^3 a	2×10^4 a	TNTC ^a	TNTC ^a	10×10^3 a	4×10^4 d

Different superscript letters indicate significant differences among days of storage

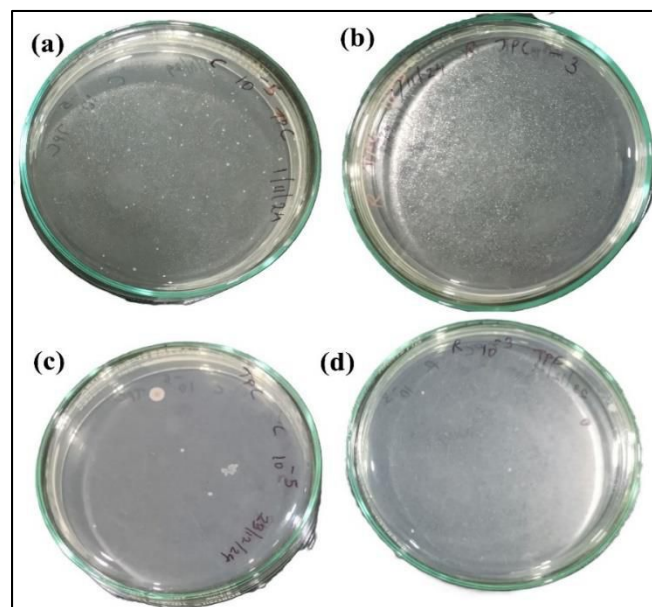


Fig 8: Total plate count in 10^{-5} dilutions of (a) control juice after 10 days of storage, (b) retort pasteurized juice after 10 days of storage, (c) control juice after 60 days of storage, (d) retort pasteurized juice after 60 days of storage.

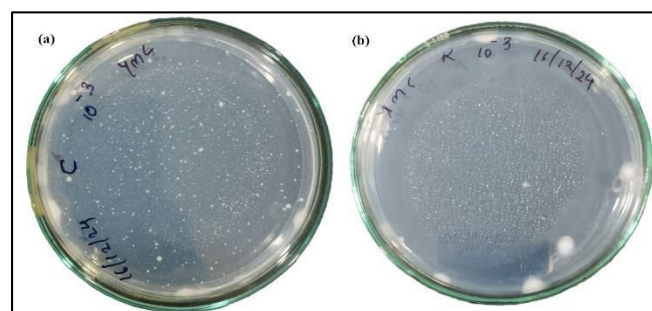


Fig 9: Total yeast and mould count after 50 days of storage in (a) control, (b) retort pasteurized juice

4. Conclusion

The study concluded that retort pasteurization at 90 °C for 5 minutes effectively preserved the quality of juice, maintaining high levels of betacyanins, total phenolics, and flavonoids. Although there was a slight reduction in betacyanin content and a significant reduction in total phenolics due to heat-induced oxidation, flavonoid content increased. The juice retained 91.9% of betacyanins, 90.82% of total phenolic content (TPC), and 95.19% of its DPPH radical scavenging activity immediately after pasteurization. After 2 months of refrigerated storage, 81.9% of the initial betacyanin content, 98.61% of TPC, and 80.19% of DPPH activity were retained. Importantly, retort pasteurization also inhibited microbial growth, with no mesophilic bacterial growth up to 20 days, no psychrophilic growth during entire storage period and minimal yeast and mold growth by the end of the storage period. However, additional control measures are recommended to prevent yeast and mold growth during storage.

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7. Conflicts of Interest

The authors have no conflicts of interest to disclose.

8. Authors' contribution

C Nithya: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing-original draft

K P Sudheer: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Visualization, writing-review & editing

S.Abdullah: Conceptualization, Formal analysis, Supervision, writing-review & editing

Lakshmi E. Jayachandran: Conceptualization, Formal analysis, writing-review & editing

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