

Shelf life and quality assessment of pasteurised red dragon fruit (*Hylocereus polyrhizus* L.) purée: Comparative study of high-pressure and thermal processing

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Abstract

Red dragon fruit (RDF; *Hylocereus polyrhizus* L.) contains various polyphenols with potent antioxidant properties. Purée extracted from RDF has a vibrant red hue, making it a valuable natural food colouring agent suitable for a wide range of culinary applications. To preserve this valuable purée, non-thermal pasteurisation methods, such as high-pressure processing (HPP), have emerged as an alternative to thermal pasteurisation (TP), as they do not impart the adverse effects associated with heat treatment. Therefore, the primary objective of the present work was to compare the impact of HPP and TP on several key attributes of RDF purée during 60-d storage. These attributes included the total betacyanin content (TBC), total phenolic content, total flavonoid content (TFC), antioxidant activities, enzyme activities, microbial growth, and colour stability. The RDF purée samples were divided into three groups: TP-treated (65°C/20 min), HPP-treated (350 MPa/5 min), and an untreated control group. All samples were stored at a controlled temperature of 4 ± 1°C, and analysed at 15-d intervals. Results revealed that preservation method, storage duration, and their interactions, significantly influenced the various parameters studied in RDF purée. Notably, HPP demonstrated superior efficacy in extending the shelf life of RDF purée well beyond 60 d, outperforming both TP and Control. Specifically, TP was proven effective in maintaining the phenolic content, antioxidant activities, and colour stability of the purée. On the other hand, HPP was particularly efficient in suppressing microbial growth and reducing enzyme activities in RDF purée. The findings can potentially transform the way RDF purée is preserved and utilised in the food industry, benefiting both producers and consumers, and contributing to more sustainable and health-conscious food practices.

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Introduction

Dragon fruit, or pitaya, is a tropical fruit that comes from several species of cactus plants belonging to the genera *Hylocereus* and *Selenicereus*. It is native to the regions of Central America, but also cultivated in other parts of the world, including Southeast Asia and Australia. Its striking, scale-like skin gives it a dragon-like appearance, and it comes in vibrant pink, yellow, red, or white varieties. The flesh of the fruit

is pink (*Hylocereus polyrhizus* L.), white (*H. undatus* L.), or yellow (*H. megalanthus* L.), dotted with small black seeds (Ariffin *et al.*, 2009), and has a texture similar to kiwi fruit.

The flesh of red dragon fruit (RDF) is a good source of organic acid, protein, water, and other minerals like vitamin C, iron, and fibre. It also contains antioxidants, which help protect the body against harmful free radicals. Some studies suggest that dragon fruit may have potential health benefits,

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such as supporting digestion, boosting immune system, and promoting heart health (Enaru *et al.*, 2021). Dragon fruit can be enjoyed in various ways. The flesh can be eaten fresh, blended into smoothies, used in salads or fruit bowls, or made into juices, jams, and desserts (Huang *et al.*, 2021).

There is an increasing demand for food industry applications that are environmentally friendly, clean-label ingredients, and plant-based (Huang *et al.*, 2021). The utilisation of RDF has been well-documented owing to its particular hue from the presence of betacyanins (red-purple pigments) and its antioxidant properties. The most abundant betacyanins in RDF are betanin (~36.1%), phyllocactin (~15.9%), and hydrocochenone (~11.7%) (Naderi *et al.*, 2012). These compounds operate as free radical scavengers, preventing biological molecules from being oxidised by both free radical-mediated oxidation and active oxygen-induced (Choo *et al.*, 2018). Nevertheless, the shelf life of betacyanins is influenced by temperature, pH, light, oxygen, and their chemical structures (Enaru *et al.*, 2021).

RDF purée is a smooth and creamy mixture made from the flesh of RDF. It is commonly used as an ingredient in various culinary creations, including beverages, desserts, and sauces. The natural pigments and preservatives in dragon fruit purée are useful for food preservation and packaging, and provide a healthier alternative to synthetic options. Also, the health benefits of dragon fruit, like boosting immunity and aiding digestion, make it a valuable ingredient in health supplements. Its purée can be incorporated into dietary supplements to increase dietary diversity, adding exotic and tropical flavours to everyday meals.

Cross-contamination during packaging and uncontrolled storage conditions can cause microbial spoilage. The behaviours and deterioration induced by microorganisms or naturally occurring enzymes such as polyphenol oxidase (PPO) and peroxidase (POD) may alter the physicochemical properties (*e.g.*, appearance, flavour, and colour) of RDF purée. Similarly, the stability and sensitivity of most polyphenols, bioactive compounds, and chemical compositions of RDF are temperature-, light-, pH-, and oxygen-dependent. Lipid oxidation, particularly the seeping of linoleic and linolenic acid from the seeds (Ariffin *et al.*, 2009), could also affect the quality of RDF products.

Several proactive approaches have been geared towards improving the quality of RDF purée, and extending its shelf life *via* thermal and non-thermal pasteurisation methods (Ijod *et al.*, 2022; Salazar-Orbea *et al.*, 2023). Thermal pasteurisation (TP) is one of the safe and cost-effective methods to prolong shelf life by killing or inhibiting spoilage microorganisms. However, heat treatment may negatively affect the quality and stability of RDF purée, resulting in undesirable changes in physicochemical, nutritional, and sensorial properties. Most importantly, thermal treatment may degrade the bioactive compounds in RDF purée, particularly betacyanins, compared to unheated ones (Liaotrakoon *et al.*, 2013).

Non-thermal pasteurisation methods like high-pressure processing (HPP) have emerged as an alternative approach to conventional TP (Nawawi *et al.*, 2023b). The utilisation of HPP can inhibit enzyme activities and microbial growth without impacting sensory attributes (Li and Padilla-Zakour, 2021). Numerous studies have reported the HPP application on anthocyanins purée in the range of 300 and 600 MPa, rendering the food safe as it applies uniformly to product size and shape (Patras *et al.*, 2009).

The comparison between pasteurisation and other stability improvement methods, like freeze-drying, for dragon fruit purée is indeed a critical aspect to consider for its application value. While highly effective, freeze-drying may not always be practical for large-scale commercial operations due to its higher costs. Therefore, HPP and TP are widely used in the food industry due to their scalability.

Despite the extensive literature on thermally treated RDF purée, to the best of our knowledge, no study has explored the effect of HPP on the physicochemical properties and shelf life of RDF purée. Therefore, the present work aimed to investigate the effect of HPP and TP on the physicochemical properties and shelf life of RDF purée during storage. Pearson correlation, general linear model (GLM), and principal component analysis (PCA) were conducted to further understand the correlations between the tested parameters.

Materials and methods

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), potassium chloride, Trolox, and sodium acetate

trihydrate were purchased from Sigma-Aldrich (St. Louis, USA). Folin-Ciocalteu reagent, 2,4,6-tripyridyl-s-triazine (TPTZ), sodium carbonate, and gallic acid were purchased from Merck (Darmstadt, Germany). Peptone water, potato dextrose agar (PDA), and plate count agar (PCA) were purchased from HiMedia Laboratories (Mumbai, India). Other chemicals and solvents used were of analytical grade, and purchased from Fisher Scientific (Leicestershire, UK).

Purée preparation

Ripen RDFs at commercial maturity (35 d after anthesis) with each fruit weighing between 250 - 300 g were purchased from a local market in Perak, Malaysia. The fruits were cleaned under running tap water, peeled by hand, and weighed. The pulp was cut

into four pieces, and the seeds were mechanically removed using a depulper machine at 1,460 rpm with a 1.5 mm sieve for 10 min (Bonina 0.5DF, Itabuna, Brazil). The RDF pulp was filtered using a spin filter machine through a 0.25 mm sieve at 2,800 rpm for 20 min (MH-819AD 6Kgs Detachable Liquid Extracting Machine, Hsin Chu City, Taiwan) to remove the remaining seeds. The obtained RDF purée was then separated into three treatment methods: Control (untreated), TP, and HPP (Figure 1).

Purée pasteurisation method

Thermal pasteurisation (TP)

RDF purée (1 L) was subjected to thermal treatment using a double boiler procedure at $65 \pm 2^\circ\text{C}$ for 20 min (Liaotrakoon *et al.*, 2013). The purée was then aseptically transferred into a sterile jar, and

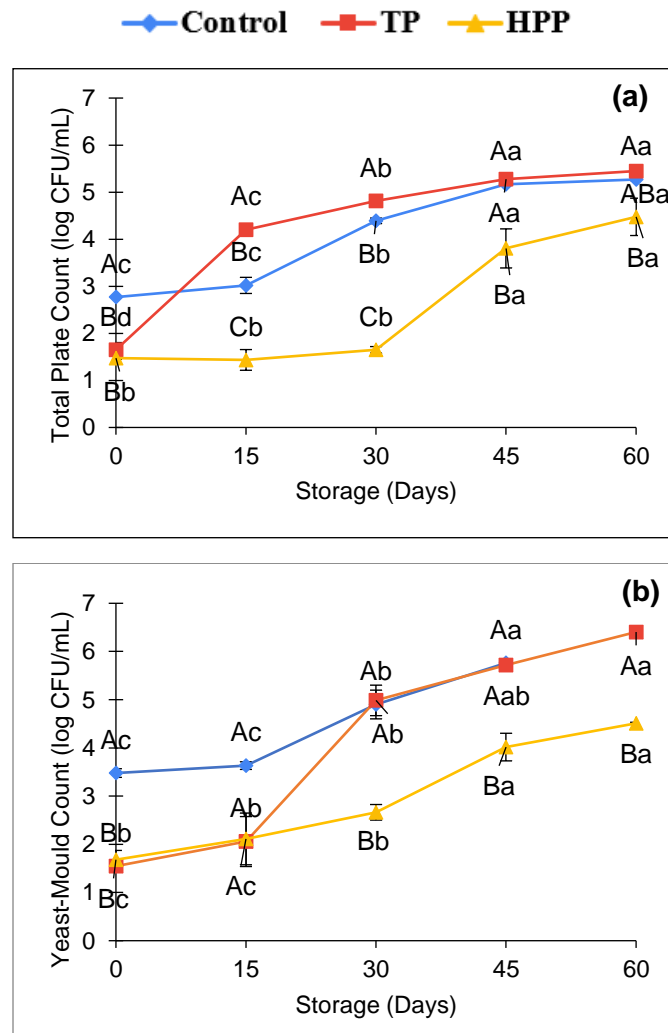


Figure 1. Changes in (a) total plate count (TPC), and (b) yeast and mould count (YMC) of RDF purée during storage at 4°C for 60 days, as subjected to thermal pasteurisation (TP) and high-pressure processing (HPP) treatments, and untreated (Control). Values with similar uppercase letter in each storage day are not significantly different ($p > 0.05$). Values with similar lowercase letter in each treatment are not significantly different ($p > 0.05$).

immediately cooled in ice water. Around 50 mL of RDF purée was filled at $45 \pm 1^\circ\text{C}$ into 50 mL sterile PET bottles, leaving 1.0 ± 0.2 cm of headspace. The samples were instantly cooled in ice water to reach room temperature ($25 \pm 1^\circ\text{C}$) within 30 min.

High-pressure processing (HPP)

Approximately, 50 mL of RDF purée was filled into 50 mL sterile PET bottles, leaving 1.0 ± 0.2 cm of headspace. Twenty bottles of RDF purée were treated for a non-thermal pasteurisation process in a 55 L capacity HPP unit (Hiperbaric, Burgos, Spain) by adapting the procedures described by Bodelón *et al.* (2013) with slight modifications ($350 \text{ MPa}/25 \pm 1^\circ\text{C}/5 \text{ min}$).

Storage study

TP-treated, HPP-treated, and untreated (Control) RDF purées were stored at $4 \pm 1^\circ\text{C}$ for 60 d. The storage condition of 4°C refers to a temperature range of 2 to 8°C , which is known as cold storage conditions. This temperature range is commonly used for storing heat-sensitive products that must not be frozen. The storage period for pasteurised juice or purée at 4°C can vary based on the specific product and processing method. According to Choo *et al.* (2018), pasteurised RDF juice can be stored at 4°C for up to 8 w. In the present work, the physicochemical and microbiological analyses were evaluated in triplicate at 15-d intervals.

Determination of microbial loads

The pour-plate technique was used for microbiological analysis. Briefly, 1 mL of RDF purée was serially diluted in 0.1% peptone water (O'Toole, 2016) using appropriate dilution factors. Next, 1 mL from each dilution was mixed with either PCA (for Total Plate Count; TPC) or PDA (for Yeast and Mould Count; YMC) that was maintained at 60°C before pouring the mixture into Petri plates. The inoculated PCA and PDA plates were allowed to solidify before being placed in the incubator (IN30plus Memmert, Schwabach, Germany) for 24 to 48 h at 37°C for PCA, and 30°C for 5 d for PDA. Microbial counts were performed exclusively on plates containing 30 to 300 colonies on PCA, and 10 to 100 colonies on PDA (O'Toole, 2016). The results were represented as log colony-forming units (CFU) per millilitre of RDF purée (log CFU/mL).

Determination of pH value and total soluble solid

The pH value of the RDF purée was determined at room temperature ($25 \pm 1^\circ\text{C}$) using a calibrated pH meter (Mettler Toledo, Schwerzenbach, Switzerland). The TSS of the RDF purée was determined using an ATAGO digital handheld pocket refractometer (Model PAL-08S, Tokyo, Japan), and expressed in Brix degree ($^\circ\text{Brix}$). All measurements were performed in triplicate.

Determination of residual enzyme activity

Polyphenol oxidase

The PPO activity was determined according to Zhu *et al.* (2021). For enzymatic reaction solution, each 50 μL RDF purée was mixed with 2.95 mL of substrate comprising 1 mL of catechol and 1.95 mL of 0.2 M phosphate buffer (pH 7.5). The mixture was vortexed vigorously for 10 s, followed by measuring the absorbance at 410 nm. This was performed in triplicate at 2-s intervals for 4 min. The residual enzyme activities were estimated using Eq. 1:

$$\text{Residual activity of enzymes (\%)} = 100 \times \frac{A_t}{A_0} \quad (\text{Eq. 1})$$

where, A_t and A_0 = specific activities of treated and untreated samples, respectively.

Peroxidase

Determining POD activity required 40 μL of RDF purée diluted four times (Zhu *et al.*, 2021). The RDF purée was added with 15 μL of 1.0% (v/v) guaiacol, 2.93 mL of 0.2 M pH 7.0 phosphate buffer, and 15 μL of 1.5% hydrogen peroxide as the enzymatic reaction solution. All measurements were triplicated at the absorbance of 470 nm and intervals of 2-s for 4 min. The residual enzyme activities were calculated using Eq. 2:

$$\text{Residual activity of enzymes (\%)} = 100 \times \frac{A_t}{A_0} \quad (\text{Eq. 2})$$

where, A_t and A_0 = specific activities of treated and untreated samples, respectively.

Determination of total betacyanin content

The TBC was determined according to Liaotrakoon *et al.* (2013) with slight modifications.

The RDF purée was diluted 50-fold with distilled water, and kept in the dark for 20 min at ambient temperature for equilibrium. TBC was measured with a spectrophotometer set at 540 nm. All observations were made in triplicate, and calculated using Eq. 3:

$$\text{Total betacyanins content } \left(\frac{\text{mg}}{\text{mL}} \right) = \frac{A \times DF \times MW \times 1000}{\epsilon \times L} \quad (\text{Eq. 3})$$

where, A = absorption at 540 nm, DF = dilution factor, MW = molecular weight (550 g/mol), ϵ = molar extinction coefficient in H₂O (60,000 L/mol cm), and L = path length of the cuvettes (1 cm).

Determination of total phenolic content

The Folin-Ciocalteu method described by Nawawi *et al.* (2023a) was referred to with minor modifications to assess the total phenolic content. Briefly, 200 μ L of RDF purée was mixed with 1.5 mL of Folin-Ciocalteu reagent (previously diluted tenfold with distilled water), and left at ambient temperature for 8 min in the dark. The mixture was then added with 1.2 mL of sodium carbonate solution. The tubes were vortexed for 10 s, and allowed to stand in the dark for 120 min. A spectrophotometer was used to measure the sample absorbance (Thermo Scientific BioMate 3 Spectrophotometer, Wilmington, USA) at 765 nm against a blank sample. Gallic acid (1.0 - 0 mg/mL) was used as the standard in the calibration curve. The absorbance was measured in triplicate, and mean values were determined.

Determination of total flavonoid content

The aluminium chloride colorimetric assay was based on Nawawi *et al.* (2023a) with minor modifications. The sample (1 mL) was diluted in a 1:4 distilled water ratio. Next, 1 mL of catechin solution (300 - 0 mg/L) was mixed with 4.0 mL of distilled water. After 5 min, 0.3 mL of NaNO₂ (5%, w/v) and 0.3 mL of AlCl₃ (10%, w/v) were added and allowed to stand. After that, 2.0 mL of 1 M NaOH was added, and the mixture was diluted to 10.0 mL using distilled water. The absorbance was measured at 510 nm, and TFC was calculated as milligrams of catechin equivalents (mg CE/L) per litre of sample.

Kinetics study

The zero-order reaction rate constants (k) and half-lives ($t_{1/2}$) describing total phenolic content,

TBC, and TFC degradation during storage were calculated using Eqs. 4 and 5:

$$C_t = C_o - k^*t \quad (\text{Eq. 4})$$

$$t_{1/2} = \frac{C_o}{2k} \quad (\text{Eq. 5})$$

where, C_o = initial concentration, and C_t = concentration at time t (Azman *et al.*, 2022b).

Determination of antioxidant activity

DPPH radical scavenging activity

The total antioxidant activity of RDF purée samples was determined using the DPPH assay according to Nawawi *et al.* (2023a) with minor modifications. Briefly, 0.15 mM DPPH solution was prepared in ethanol. Next, 1.75 mL of DPPH solution was added with 0.25 mL of RDF purée. A blank was made with an equal volume of distilled water and DPPH. The test tube mixture was briskly vortexed before being left in the dark for 30 min at ambient temperature for incubation. The absorbance was then measured at 517 nm against blank. The percentage of DPPH inhibition was calculated using Eq. 6:

$$\text{DPPH Inhibition (\%)} = \frac{A_o - A_e}{A_o} \times 100 \quad (\text{Eq. 6})$$

where A_o = absorbance of the Control, and A_e = absorbance of the RDF purée.

Ferric-reducing antioxidant power

The ferric-reducing antioxidant power (FRAP) was measured following a modified method by Nawawi *et al.* (2023a). A fresh FRAP reagent (3 mL) was combined with 100 μ L of RDF purée and 300 μ L of distilled water or Trolox solution (2000 - 0 μ M). The mixture was then vortexed for 10 s, and incubated in the dark for 30 min before the absorbance at 593 nm was recorded. The sample's FRAP content was calculated using the Trolox solution standard curve, and the results were expressed in micromoles of Trolox equivalent per litre of sample (μ mol TE/L).

Determination of colour

Colour measurement was conducted using a colorimeter (CR-410, Minolta, Japan). The RDF purée's colour was expressed using units L* (lightness/darkness), a* (redness/greenness), and b* (yellowness/blueness). White tiles were used to

calibrate the instrument. Chroma (C) is the quantitative characteristic of intensity, and hue angle (h°) is a qualitative characteristic of colour. The chroma, hue angles, and total colour difference (TCD) were calculated using Eq. 7–9 (Azman *et al.*, 2022a):

$$\text{Chroma (C)} = \sqrt{(a^*)^2 + (b^*)^2} \quad (\text{Eq. 7})$$

$$\text{Hue angle (} h^\circ \text{)} = \tan^{-1} \left(\frac{b^*}{a^*} \right) \quad (\text{Eq. 8})$$

$$\text{Total color difference (TCD)} = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (\text{Eq. 9})$$

where, L_0^* , a_0^* , and b_0^* = initial values of the RDF purées, respectively.

Statistical analysis

All data were statistically analysed using One-way analysis of variance (ANOVA) *via* Tukey's multiple range tests ($p < 0.05$). Linear Pearson correlation, GLM, and PCA examined the relationships between the tested parameters. The analyses were done using the Minitab V.19 program (Minitab Inc., State College, PA, USA).

Results and discussion

Dragon fruit seeds contain oils that are susceptible to oxidation when exposed to air. This process can cause the development of off-flavours, loss of nutritional value, and a decrease in the overall quality of dragon fruit products (Ariffin *et al.*, 2009). Therefore, in the present work, it was necessary to remove the seeds during the production of RDF purée. The GLM analysis results showed that pasteurisation method, storage time, and their interactions, significantly influenced the variables studied in RDF purée ($p < 0.05$).

Microbial growth

The mean initial counts of TPC and YMC in untreated RDF purée were 2.77 and 3.48 log₁₀ CFU/mL, respectively (Figure 1). Following TP and HPP, both TPC and YMC significantly decreased ($p < 0.05$). This agreed with the findings concerning HPP on carambola juice (HPP: 600 MPa/2.5 min and TP: 110°C/8.6 s) (Huang *et al.*, 2018), and pineapple juice (HPP: 500 MPa/10 min and TP: 95°C/3 min)

(Wu *et al.*, 2021). The cell wall can be severely impaired upon HPP treatment, which leads to microbial lethality or sub-lethal injury (Mor-Mur *et al.*, 2014). After pasteurisation, injured microbial cells can recover and proliferate, highlighting the pertinence of refrigeration to delay microbial growth.

At the end of the storage period, TP-RDF purée exhibited a mean population of 5.45 log CFU/mL for TPC, and 6.40 log CFU/mL for YMC. This is because the growth of YMC is more favourable in a high-sugar environment as water activity (a_w) growth requirement is much lower than those of bacterial growth (Hingle *et al.*, 2019). For HPP-RDF purée, the YMC growth did not exceed the TPC counts due to the longer lag phase observed for YMC in HPP-RDF as compared to those in TP-RDF. The extended lag phase might be attributed to the sugar hydrolysis (Shinwari and Rao, 2020) in HPP-RDF as demonstrated by the sudden drop of the Brix value from day 0 to 15 (Figure 2b).

To our knowledge, there is no regulatory standard of TPC and YMC levels for the shelf-life determination of foods and beverages since it heavily depends on internal manufacturers' guidelines and juice formulation. Manufacturers may conduct in-house evaluation or risk assessment to determine the shelf life of juices, which does not only rely on the microbial quality, but rather is influenced by the physical, chemical, and sensorial quality of the juices. Nevertheless, a microbiological limit of 6.00 log CFU/mL is typically used in many shelf-life studies of juices as spoilage may occur at this level (Patrignani *et al.*, 2009; Alam *et al.*, 2023).

In the present work, the microbiological quality of HPP-RDF purée was within acceptable limits as both TPC and YMC were below 6.00 log for the entire storage period. The shelf life of TP-RDF purée was compromised because YMC exceeded 6.00 log CFU/mL even before the storage period ended. These findings suggested that HPP application can prolong the shelf life of RDF purée beyond 60 d as compared to conventional TP. Aside from shelf-life determination, it is also worth highlighting that HPP pasteurisation can reach 5-log pathogen reduction to evaluate the safety of RDF purée (FDA, 2021).

RDF contains betacyanin and betaxanthin compounds, which are known for their antibacterial and antifungal properties. These compounds inhibit the growth of bacteria like *Staphylococcus aureus* and *Escherichia coli* by disrupting cell membranes, and

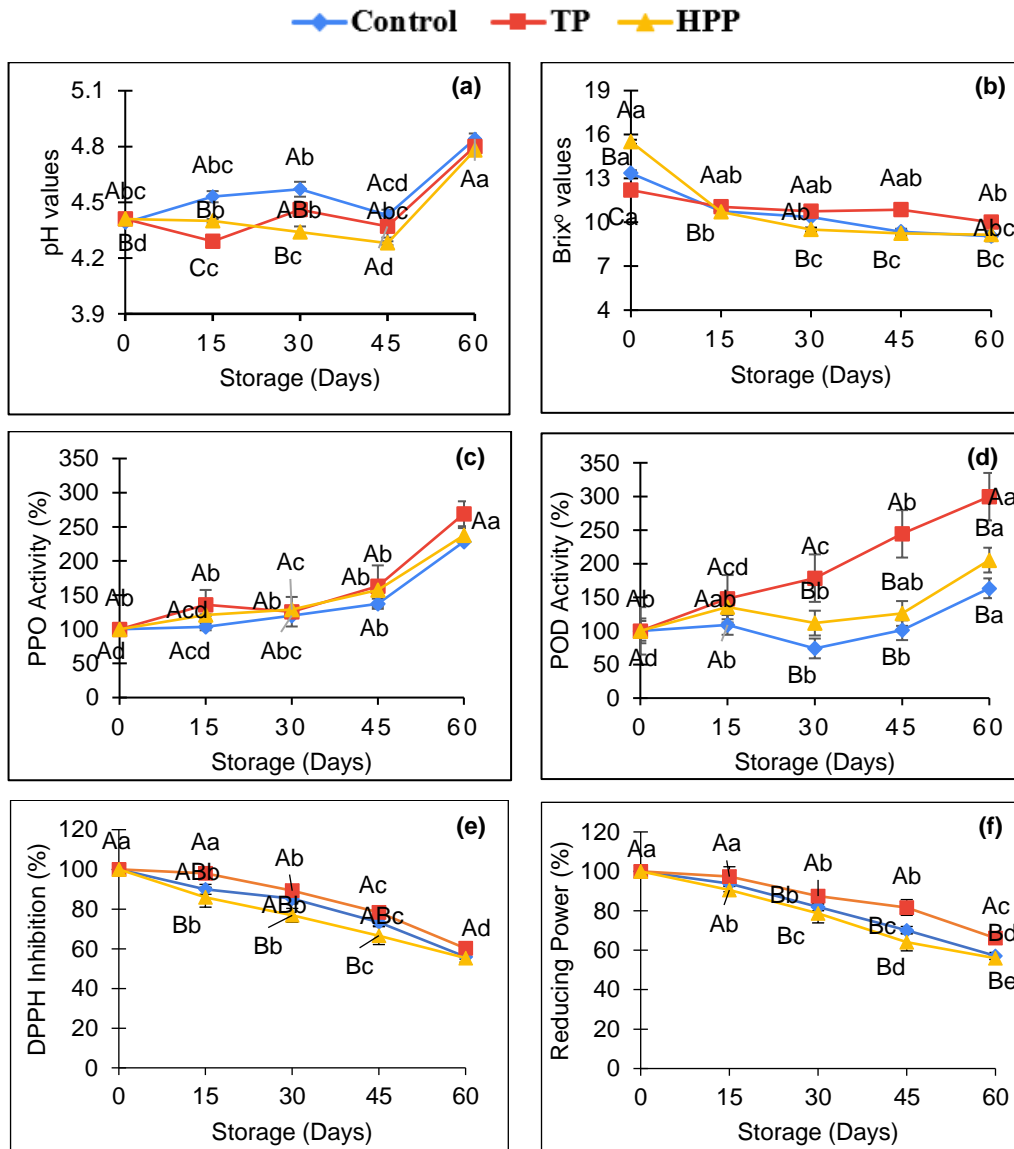


Figure 2. (a) pH values, (b) total soluble solids (TSS), residual of (c) PPO and (d) POD activities, and percentage of (e) DPPH inhibition and (f) reducing power of RDF purée during storage at 4°C for 60 days, as subjected to thermal pasteurisation (TP) and high-pressure processing (HPP) treatments, and untreated (Control). Values with similar uppercase letter in each storage day are not significantly different ($p > 0.05$). Values with similar lowercase letter in each treatment are not significantly different ($p > 0.05$).

interfering with bacterial growth. Studies have shown that adding RDF into yogurt can boost its ability to inhibit *E. coli* growth (Wijesinghe and Choo, 2022).

Additionally, these compounds can impede the growth of specific fungi, including *Candida albicans*. A study using RDF peel extract demonstrated its potential as an antifungal agent against *C. albicans*. These compounds may work by damaging fungal cell walls, or interfering with fungal metabolism. As a result, the antifungal properties of RDF's colour compounds have attracted interest for potential therapeutic applications (Hendra *et al.*, 2020).

pH and total soluble solids

The physicochemical properties of RDF purée are typically dictated by the proportion of tartness and sweetness, with acids and sugars as the predominant flavouring agents. TSS (expressed as °Brix) and the acidity or pH of RDF purée may vary with different maturity stages (Huang *et al.*, 2021). Figure 2a shows the fluctuation of the RDF purée's pH value which varied between pH 4.2 and 4.8.

Initially, the pH value for both HPP and TP (~pH 4.41) increased significantly ($p < 0.05$) than Control (~pH 4.39). However, after 15 d, there was a

2.7% decrease in TP-RDF purée (~pH 4.29) compared to HPP-RDF (~pH 4.40). This could have been due to the mechanism of texture evolution during heat processing (Renard and Maingonnat, 2012). The applied heat can alter the pH value through the degradation of pectin, which is transformed into pectin cleavage *via* hydrolysis. Furthermore, the pH decrease in TP-RDF could also be linked to the Maillard reaction triggered by heating (Wu *et al.*, 2021).

After 60 d, the pH values of RDF purée drastically increased ($p < 0.05$) by 9%. This contradicted Choo *et al.* (2018) who investigated the stability of fermented red dragon fruit drink (FRDFD), and found that the pH value of FRDFD stored at 4°C increased drastically from week 0 to 2, but remained unchanged until the end of the storage time. Past evidence suggested that pH is likely to fluctuate due to the reversible esterification process of three carboxylic groups in the betanin structure, with higher pH will result in a greater degree of esterification in RDF purée. Also, the increased pH value could be attributed to microbial reproduction (Hu *et al.*, 2020). Overall, the increased pH of RDF purée during storage might have occurred due to several factors, such as microbial activities, enzymatic reactions, chemical reactions, and degradation of acidic compounds. Determining the exact cause of pH increase in RDF purée requires a detailed analysis and monitoring of the storage conditions and the fruit's chemical composition.

Figure 2b shows a gradual decrease in the TSS of HPP-RDF purée from ~15.50 to ~9.15 °Brix. At day 0, TSS increased by 16% after HPP but decreased by 8% after TP. Hingle *et al.* (2019) reported that higher TSS means lower water activity, which is caused by the tremendous mechanical energy delivered to the cell during HPP. TP-treated also exhibited relatively stable TSS value throughout 60-d storage at $4 \pm 1^\circ\text{C}$. This agreed with Li and Padilla-Zakour (2021) who found that heat treatment had no effect on the °Brix value of grape purée which remained constant during storage. Overall, the application of HPP and TP did not significantly affect the pH and TSS values of RDF purées. However, it is worth noting that products produced using both HPP and TP methods require refrigeration, $\text{pH} < 4.5$, or the reduction of water activity to prevent the germination of bacterial spores (Mújica-Paz *et al.*, 2011).

Residual enzymes activities (REA)

Enzyme inactivation is a prerequisite for producing high-quality products (Terefe *et al.*, 2014). Enzymes such as PPO and POD cause undesirable alterations in colour and sensory quality aspects when exposed to ambient oxygen. In the present work, both enzyme activities were equivalent to 100% relative enzyme activity (REA).

After 15-d storage, the residual PPO activity in HPP and TP significantly increased ($p < 0.05$) by ~17% and ~32% compared to Control, respectively (Figure 2c). However, there was a progressive degradation in TP-RDF purée up to ~10% after 30 d. This might have been due to temperature-time interactions that occurred due to enzyme conformational changes and protein-enzyme interaction (Toro-Urbe *et al.*, 2020). Also, the fluctuation trend of PPO might have been influenced by the pH value in TP-RDF purée. According to de Oliveira Carvalho and Orlanda (2017), PPO often remains inactive at pH values lower than 4.0 but increases at higher pH due to the decrease in H^+ ionic strength and the increased availability of the active centre of the enzyme. Also, PPO shows heat resistance at a temperature below 80°C. A study by Terefe *et al.* (2014) reported that a temperature of 80°C and above is required to ensure PPO deactivation in fruit purée.

The present work also found no significant difference in PPO and POD activities between Control (Figure 2c) and HPP (Figure 2d). Similar inhibitory effects of HPP on endogenous enzymes have been reported in previous research. According to Toro-Urbe *et al.* (2020), PPO activity was reduced only minimally for 30 min, and the enzyme became gradually dormant beyond that time. This could be attributed to the interactions between enzymes and other components, such as phenolic compounds. Additionally, the pressure-activated latent form might be associated with the release of membrane-bound enzymes.

Furthermore, a significant increase ($p < 0.05$) in activity for POD in TP-RDF purée was observed (Figure 2d). These enzymes catalysed the oxidation of phenolic compounds using hydrogen peroxide, as supported by a negative correlation between POD and total phenolic content ($r = -0.986$, $p < 0.05$). In contrast, HPP-RDF appeared to effectively suppress POD activities. This suggested that the REA in both

treatments could play a role in the enzymatic browning of RDF purée, particularly as it significantly impacted the retention of betacyanins.

Both PPO and POD have been demonstrated to induce the formation of dark brown polymers with quinone characteristics. This is likely attributed to the destabilisation of betalains, leading to observable colour changes (Terefe *et al.*, 2014). However, this finding was contradicted by Marszałek *et al.* (2015) who reported that the efficiency of TP completely inactivated the PPO and POD activities in strawberry purée compared to HPP. Nevertheless, these findings implied that the catalytic activities of PPO and POD noticeably increased ($p < 0.05$) with prolonged storage time. The overall quality of HPP-RDF purée remained parallel to Control even after 60-d storage. Similarly, Chang *et al.* (2017) reported that HPP-RDF white grape juice exhibited a better inhibitory action on enzyme activities in storage time. Overall, HPP was capable of inhibiting PPO and POD activities, and preventing damage to biochemical quality of the purée.

Total betacyanin content

Betanin, phyllocactin, and hycocerenin are types of betacyanins that have been identified as the predominant water-soluble pigments responsible for imparting colour to RDF (Choo *et al.*, 2018). In the present work, TBC in all treatments was significantly

degraded ($p < 0.05$) during storage (Table 1a). The decrease in betacyanin content might have been due to exposure to oxygen and light during the preparation of the samples, which potentially triggered a reaction affecting the betacyanin compounds (Enaru *et al.*, 2021). The absorption of light leads to the excitation of electrons within the chromophore of betacyanins, subsequently elevating their energy levels and facilitating the release of additional reactive molecules (Herbach *et al.*, 2006).

The decrease in TBC observed after storage in HPP-RDF purée (~50.64 mg/L) was significantly higher ($p < 0.05$) than TP-RDF (~57.98 mg/L) but lower than Control (~47.03 mg/L). The overall losses of betacyanins in HPP-RDF purée were 49%, while only a 43% decrease was observed in TP-RDF purée after 60-d storage. This finding agreed with Herbach *et al.* (2006) who showed a TBC retention of approximately 40% in purple pitaya juice after six months of dark storage without the addition of ascorbic acid. However, the degradation of RDF betacyanins could not be validated, owing to their varied ripeness and acylated nature.

The present work also determined the zero-order of the reaction to estimate the kinetic parameters of the betacyanins degradation during heat treatment (Table 1a). The result demonstrated that TP-RDF purée seemed to have better TBC retention (~80 d) than HPP. This suggested that HPP

Table 1. Effect of thermal pasteurisation (TP), high-pressure processing (HPP), and untreated (Control) on the estimated half-life value ($t_{1/2}$ days) of total (a) betacyanin, (b) phenolic, and (c) flavonoid contents in RDF purée during storage at 4°C for 60 days, as measured by spectrophotometer.

Sample	k	$t_{1/2}$ (days)	R ²
(a) Total betacyanin content			
Control	0.2366	55.9 ± 1.7 ^b	0.9959
TP	0.1574	80.0 ± 3.9 ^a	0.9726
HPP	0.2658	51.4 ± 1.8 ^b	0.9340
(b) Total phenolic content			
Control	4.8057	46.5 ± 1.2 ^b	0.9814
TP	2.5981	85.9 ± 7.4 ^a	0.9651
HPP	4.9872	47.7 ± 1.8 ^b	0.9882
(c) Total flavonoid content			
Control	7.2431	41.1 ± 0.5 ^b	0.9962
TP	4.8216	56.9 ± 1.4 ^a	0.9633
HPP	7.8334	39.9 ± 2.2 ^b	0.9756

Values are mean ± standard deviation. Means with similar lowercase superscripts in similar row are not significantly different ($p > 0.05$). k: reaction rate constant.

treatment does not improve stability or aid in maintaining TBC levels during storage. The decrease in TBC in HPP-treated purée could potentially be attributed to the elevation in oxygen partial pressure induced by the HPP method (Paciulli *et al.*, 2016). It is important to note that the extent and nature of betacyanins degradation during HPP may vary depending on factors such as pressure level, temperature, time, and the specific food product being processed.

Overall, the processes involving decarboxylation, isomerisation, and cleavage can be associated with the thermal treatment of RDF, leading to the decrease in TBC (Herbach *et al.*, 2006). Heat treatment can indeed cause the degradation of betanin in beetroot into betalamic acid and cyclo-DOPA. This degradation process is often referred to as thermal degradation or thermal decarboxylation (Trych *et al.*, 2022). The temperature during thermal treatment may favour the formation of degradation products that can later revert to betacyanins under certain conditions. According to Wang *et al.* (2020), most of the heat treatment reactions that lead to betanin degradation in beetroot jam are reversible, and that the extent of reversibility depends on the temperature and the acidity level of the environment.

Total phenolic content

Initially, the highest total phenolic content was recorded in HPP-treated RDF purée (~475.78 mg GAE/L) followed by ~446.22 and ~444.17 mg GAE/L for Control and TP, respectively (Table 1b). HPP leads to cellular disruption, which in turn facilitates the extraction of compounds by breaking the bonds between dietary fibre and phenolic content. These interactions, in addition to covalent bonds, are influenced by electrostatic forces and hydrogen bonds (Camiro-Cabrera *et al.*, 2017). Marszałek *et al.* (2015) reported that HPP treatment at 300 MPa/50°C/1 min significantly increased the total phenolic content (free ellagic acid, kaempferol, and quercetin) in HPP-treated strawberry purée compared to TP-treatment at 90°C for 15 min.

The highest total phenolic content was detected in TP-treated purée (~275.45 mg GAE/L) in comparison to HPP (~196.63 mg GAE/L) after 60 d. The decrease in total phenolic content is triggered by the oxidative cleavage of phenolics and their crosslinking with protein (Cao *et al.*, 2012). This result contradicted Škegro *et al.* (2021) who observed

the impact of HPP and TP on smoothies where phenolic content was more stable and less pronounced in the HPP samples compared to TP during cold storage at 4°C for 21 d. Additionally, TP applied to RDF purée can lead to the release and subsequent increase in phenolic content from the fruit tissue. Even though higher total phenolic content was recorded in HPP-RDF purée at day 0, some thermal degradation reactions in TP-RDF may be reversible under certain conditions, allowing for the regeneration of phenolic compounds to some extent.

Furthermore, the degradation kinetics of the total phenolic content were modelled using zero-order kinetics. The estimated half-life value of total phenolic content in the TP-RDF purée (~85.9 d) was more persistent during storage than in HPP-RDF (~47.7 d) and Control (~46.5 d). The decrease in phenolic content in RDF purée can be explained by the high REA, such as PPO and POD, which are responsible for catalysing the oxidation of phenolic compounds. Moreover, there was a strong negative correlation ($p < 0.05$) between the residual enzyme activities of PPO ($r = -0.891$), POD ($r = -0.986$), and total phenolic content in TP-treated purée, suggesting that the phenolic content in RDF purée was significantly influenced by enzyme activities.

Total flavonoid content

The initial TFC in the RDF purée ranged between ~623.75 and ~548.75 mg CE/L, with the highest levels recorded in HPP-treated purée (Table 1c). The increase in TFC after HPP treatment could be attributed to residual PPO and POD activity. This could potentially lead to the breakdown of the bonds in high-molecular-weight phenolic compounds, such as procyanidins, resulting in the release of smaller particle units and monomeric flavanols. Furthermore, this advantage of HPP may promote the release of bioactive compounds from the tissue and improve their extractability (Škegro *et al.*, 2021).

The decrease in TFC during storage followed a pattern similar to the trends observed in TBC and total phenolic content, suggesting a strong correlation between these variables. The TP-RDF purée exhibited a higher estimated half-life time of TFC (~56.9 d) compared to HPP (~39.9 d). Moreover, the pressure greatly affected TFC due to the non-enzymatic oxidative processes. Also, the breakdown of monomers and dimers during heat treatment resulted in the hydrolysis of heat-labile phenolic

compounds, which can be a contributing factor to the enhanced stability of TFC (Alongi *et al.*, 2019). Furthermore, the pattern of TFC change was contingent on various factors, including the intensity of heat treatments, exposure to air or light, and other relevant factors. According to Vieira *et al.* (2018), there is a possibility that certain compounds may be generated during storage, which may subsequently react with the aluminium chloride in the TFC reagent, and elevate the overall flavonoid content. These findings were supported by a strong correlation ($p < 0.05$) between TFC and total phenolic content in HPP-RDF ($r = 0.973$) and TP-RDF purées ($r = 0.997$).

Antioxidant activity

Various methods can be used to measure antioxidant activity, suggesting that a single assay may not fully capture the complete spectrum of antioxidant activity present in the sample being evaluated. In the present work, the antioxidant activity of RDF purée was assessed using the DPPH and FRAP assays. Both HPP and TP treatments exhibited a similar trend of antioxidant activity in scavenging activity and reducing power (Figures 2e and 2f). However, the TP-RDF purée generally possessed higher antioxidant activities compared to HPP during storage.

Initially, the scavenging activity of RDF purée significantly increased ($p < 0.05$) after being treated with HPP and TP by 7 and 17% DPPH inhibition, respectively. Similar increment of reducing power was observed in HPP- (4%) and TP-treated purées (9%). TP treatment applied to fruits and vegetables has been shown to boost antioxidant activity by modifying the phytochemical composition, and promoting the development of Maillard reactions following heat treatment. Subsequently, the Maillard reaction can lead to the formation of antioxidant compounds (Liaotrakoon *et al.*, 2013). HPP can disrupt the cell structure of the dragon fruit purée, leading to the release of bioactive compounds, including antioxidants like polyphenols and betacyanins, from the cellular matrix. Such release makes these compounds more accessible for antioxidant activity. HPP can improve the solubility of certain antioxidant compounds, making them more available for interaction with free radicals and other reactive species. This enhanced solubility can boost the antioxidant capacity of the purée (Wu *et al.*, 2021).

The enhancement of these antioxidant activities was presumably related to the higher betacyanin, flavonoid, and phenolic contents. This was supported by a strong correlation ($r > 0.9$, $p < 0.05$) between TBC, TFC, and total phenolic content with scavenging activity and reducing power in Control, HPP, and TP-RDF purées. These findings suggested that phenolic compounds, particularly betacyanins, are significant contributing factors to antioxidant activity.

Colour characteristics

The colour of a food product plays a crucial role in consumer acceptability, and alterations in its colour properties over time during storage have garnered considerable attention. In the present work, the colour of RDF purée was significantly affected by the treatment applied, and the changes were assessed by monitoring the L^* , a^* , and b^* values. As depicted in Figure 3a, the lightness (L^*) values exhibited fluctuations during storage. The exposure period during thermal treatment slightly increased the L^* values, indicating higher lightness, as a consequence of the degradation of betacyanin content.

Moreover, the degradation of betacyanins resulted in a rapid decrease in a^* (redness) and chroma values (colour intensity) (Figures 3b and 3e). This indicated a lower purity of the colour due to the formation of various compounds during treatments (Salazar-Orbea *et al.*, 2023). Similar effects were observed by Herbach *et al.* (2006) who reported the decrease in chroma due to the increase in betacyanin degradation in purple pitaya juice without ascorbic acid supplementation. Also, a study by Salazar-Orbea *et al.* (2023) demonstrated a significant decrease in all strawberry purée colour changes from a vibrant to a dull colour.

Furthermore, the total colour difference (TCD) revealed that the HPP-RDF purée had similar changes as Control, while the lowest TCD was observed in TP-RDF (Figure 3d). TCD is an important quantity since it reflects the ability of the individual eye to distinguish between two colours (Salazar-Orbea *et al.*, 2023). Similar findings were reported by Škegro *et al.* (2021) whereby the non-parametric analysis showed that HPP samples had no significant difference with untreated samples for L^* , a^* , and b^* values. However, theoretically, the TCD value of HPP-treated should be lower than TP (Patras *et al.*, 2009). Nevertheless, the effect of HPP on RDF purée depends on the processing conditions, such as

pressure, temperature, and time. The present work also found that the hue angle (h°) of RDF purée fluctuated upon storage (Figure 3f). The h° was noted

to increase during purée preparation due to betacyanin degradation and the formation of yellow colour by the products (Herbach *et al.*, 2006).

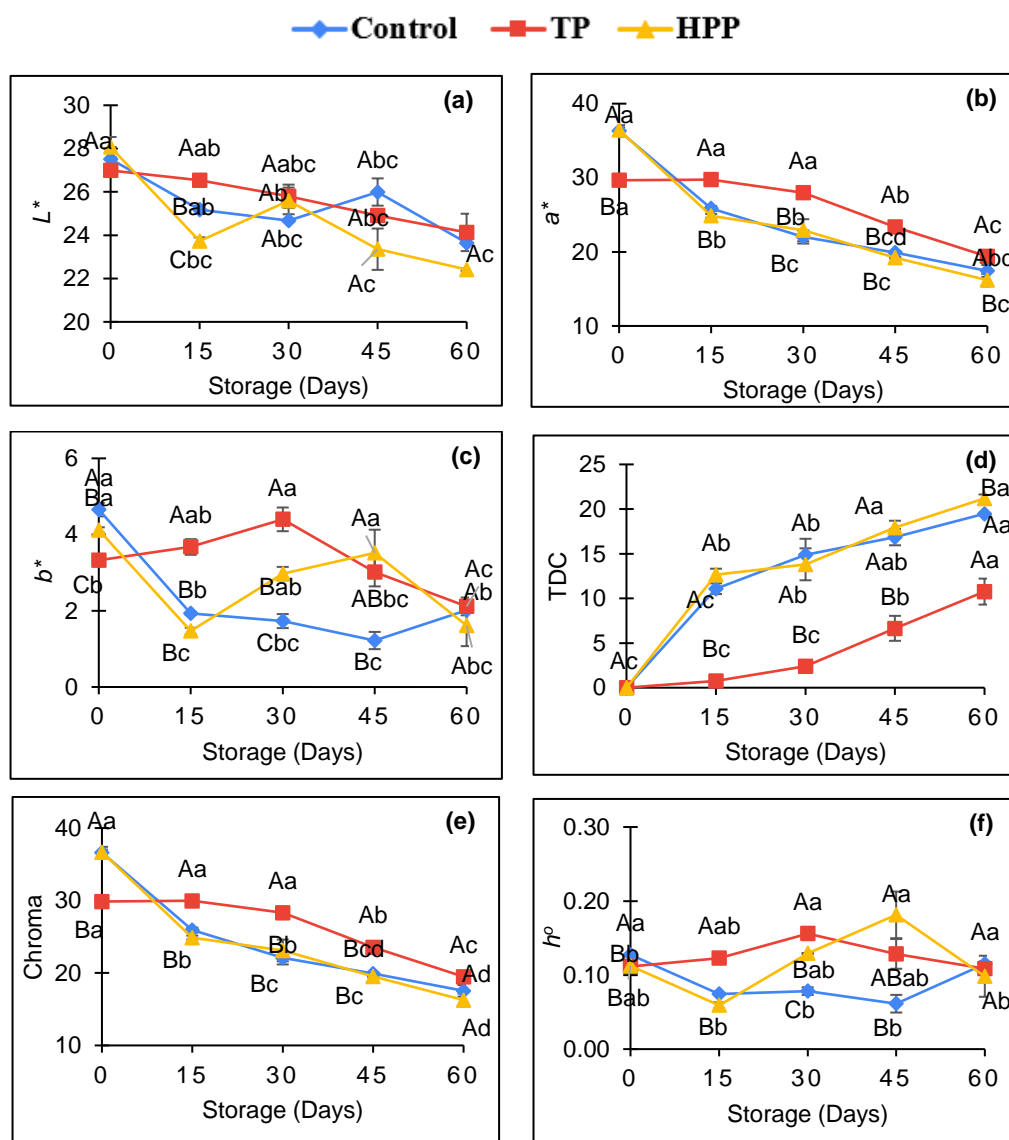


Figure 3. Colour characteristics of RDF purée during storage at 4°C for 60 days, as subjected to thermal pasteurisation (TP) and high-pressure processing (HPP) treatments, and untreated (Control). Values with similar uppercase letter in each storage day are not significantly different ($p > 0.05$). Values with similar lowercase letter in each treatment are not significantly different ($p > 0.05$).

Principal component analysis

PCA was performed to summarise the relationship between the pasteurisation method and storage time (Figure 4a). As shown in Figure 4b, the first two principle components explained 78.3% of the total variance, where the distribution of the quality attributes was defined by 66.4% of PC1, and 11.9% of PC2. Accordingly, the biplot (Figure 4c) showed storage at day 0 (Control, TP, and HPP), day 15 (TP), and day 30 (TP) located on the positive axis of PC1,

suggesting favourable results for TBC, TSS, TFC, total phenolic content, antioxidant activities, and colour characteristics. In contrast, storage at day 30 (Control and HPP), day 45 (Control, TP, and HPP), and day 60 (Control, TP, and HPP) were placed on the opposite side, and were highly correlated with PPO, POD, YMC, TPC, pH, and TCD. These results showed that the enzyme and microbial activities gradually increased with the increase in storage time, which was related to pH and storage temperature. The

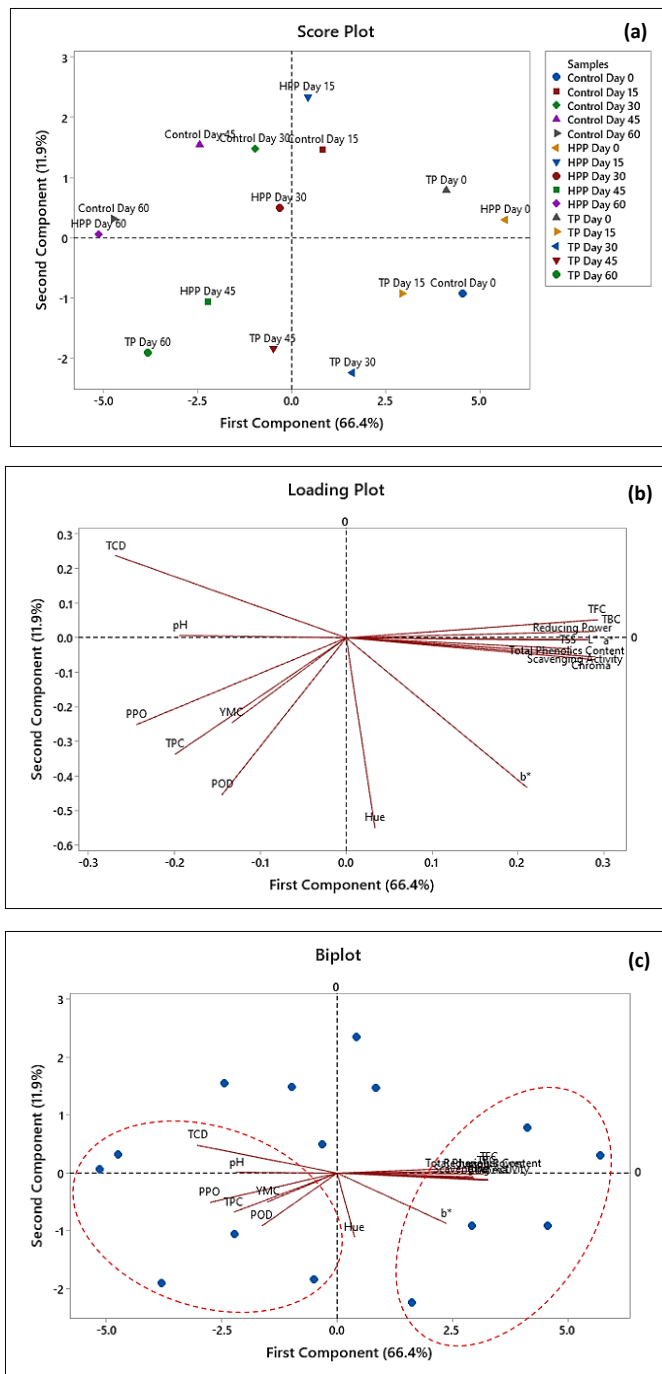


Figure 4. (a) Score plot, (b) loading plot, and (c) biplot of principal component analysis based on the effect of different pasteurisation methods and storage times on RDF purée. HPP: high-pressure processing; TP: thermal pasteurisation; TCD: total colour difference; PPO: polyphenol oxidase; POD: peroxidase; TPC: total plate count; YMC: yeast and mould count; TFC: total flavonoid content; TBC: total betacyanin content; and TSS: total soluble solids. L^* : lightness/darkness, 100 to 0; a^* : positive = redness, negative = greenness; b^* : positive = yellowness, negative = blueness. Results are mean \pm standard deviation of duplicate ($n = 2$).

PCA analysis revealed that TP had strong preservation effect on phenolic content, antioxidant activity, and colour characteristics similar to Control and HPP at day 0. The increase in PPO and POD activities caused a decrease in phenolic content after 30-d storage. Overall, HPP effectively decreased microbial growth and enzyme activity, while TP efficiently preserved the phenolic content, antioxidant activity, and colour characteristics in RDF purée.

Conclusion

In the present work, factors such as pasteurisation method, storage time, and their interactions significantly influenced ($p < 0.05$) the tested parameters. Both HPP and TP treatments significantly reduced ($p < 0.05$) the initial counts of TPC and YMC in the RDF purée to below the detection limit. Furthermore, the microbiological quality of HPP-RDF purée was within acceptable limits as both TPC and YMC were below 6.00 log during the entire storage period, suggesting that HPP application could extend the shelf life of RDF purée beyond 60 d as compared to conventional thermal treatment. Also, HPP treatment was more efficient in inhibiting enzyme activity than TP. On the other hand, the application of TP on RDF purée showed better retention of total phenolic content, TBC, TFC, and antioxidant activities with the lowest TCD compared to HPP. Overall, the present work proved that HPP could indeed be an efficient method to reduce the microbial and enzyme activities in RDF purée during storage. However, TP could be more effective in retaining the total betacyanins, total phenolics, and total flavonoids in RDF purée. In conclusion, the information reported in the present work on RDF purée preservation methods can potentially benefit multiple sectors, including food production, consumer health and nutrition, sustainability, as well as scientific research. It can also guide industrial practices, product development, and regulatory decisions, ultimately benefiting both producers and consumers.

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