Pertanika J. Trop. Agric. Sci. 48 (3): 815 - 836 (2025)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Effects of Different Pasteurisation Temperatures and Time on Microbiological Quality, Physicochemical Properties, and Vitamin C Content of Red Dragon Fruit (*Hylocereus costaricensis*) Juice

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ABSTRACT

This study examines the effects of different pasteurisation temperatures and times on red dragon fruit juice's microbiological quality, physicochemical properties, and vitamin C content. Microbial analysis used the spread plate technique to determine total plate count (TPC) and yeast and mould count (YMC). Physicochemical properties were assessed through pH, titratable acidity (TA), colour, and total soluble solids (TSS). Vitamin C content was measured using iodometric titration. Pasteurisation at 80°C for 60 s achieved the lowest TPC (3.56 log CFU/mL) compared to 6 log CFU/mL in unpasteurised juice. All pasteurised samples showed YMC below 2 log CFU/mL, demonstrating effective microbial control. However, no significant differences ($p > 0.05$) were observed in TPC between 60°C and 80°C. Pasteurisation caused only slight changes in pH and TSS, with higher temperature (80°C) resulting in lighter juice colour, indicating potential pigment breakdown. Vitamin C, being heat-sensitive, decreased significantly as temperature and time increased, with a maximum reduction of 30.82 mg/100 mL at 80°C for 60 s. During 28 days of storage at 4°C, juice pasteurised at 60°C for 60 s maintained microbial loads within acceptable limits. TA increased slightly, and pH levels shifted from 5.05 to 4.98. TSS remained stable, while colour difference (ΔE) increased, indicating noticeable changes. Low-temperature pasteurisation (60°C for 60 s) resulted in minimal vitamin C loss (9.36%) compared to 80°C (17.59%). In conclusion, pasteurising at 60°C for 60 s effectively balances microbial quality, physicochemical properties, and vitamin C preservation.

Keywords: Microbiological quality, pasteurisation, physicochemical properties, red dragon fruit, vitamin C

ARTICLE INFO

Article history:
Received: 24 September 2024
Accepted: 11 November 2024
Published: 16 May 2025

DOI: https://doi.org/10.47836/pjtas.48.3.09

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e-ISSN: 2231-8542

INTRODUCTION

Red dragon fruit (*Hylocereus costaricensis*) is a tropical fruit endemic to Central and South America that belongs to the Cactaceae family (Harivainda et al., 2008). Red dragon fruit is rich in vitamins, minerals and antioxidants, with vitamin C being a key component contributing to its nutritional value (Susilo et al., 2021). Due to its health benefits and diverse applications in the food industry, red dragon fruit has gained global market appeal (Nguyen, 2020). However, the perishable nature poses significant challenges in postharvest handling, processing, and distribution. Factors such as microbial contamination, enzymatic activity, and oxidation can quickly degrade its quality and safety (Jalgaonkar et al., 2022).

Pasteurisation is a critical step in fruit juice processing to ensure microbiological safety and the overall quality of the product. In this context, fruit juices are pasteurised at different combinations of temperature (more or less than 80°C) and time (more or less than 30 s) (Petruzzi et al., 2017). High temperatures could create structural changes and impair sensory attributes, emphasising the need to retain the original characteristics of the fruit juice. Despite extensive studies on other fruit juices, limited research exists on the effects of pasteurisation specific to dragon fruit juice. Liaotrakoon (2013) investigated the impact of heat treatment on the physicochemical properties, microbial destruction, antioxidative properties and rheological parameters. This study applied thermal treatments to dragon fruit purees at temperatures ranging from 50 to 90°C for 0 to 60 min. In comparison to the initial value, heated dragon fruit purees retained a comparatively high level of vitamin C. During thermal treatment, the betacyanin pigment of the red-flesh dragon fruit puree degraded from betanin to neobetanin.

Total plate count (TPC) and yeast and mold count (YMC) provide insight into the microbial load in juice but do not differentiate between beneficial, pathogenic, or spoilage microorganisms. Therefore, high TPC and YMC values indicate potential spoilage and are useful for evaluating shelf life and guiding quality control measures. Research by Mandha et al. (2023) found that the physicochemical, microbial and qualitative parameters of watermelon, pineapple, and mango juices differed significantly. For example, pasteurisation negatively affects watermelon juice colour and significantly reduces vitamin C content after 10 min ($p < 0.05$). Thus, analysing different pasteurisation times is crucial to optimise the quality of dragon fruit juice. In addition, evaluating the physicochemical properties after pasteurisation and the shelf life of the thermally treated juice is important to prove that pasteurisation can maintain the juice's valuable properties. A study by Rabie et al. (2014) concluded that pasteurised juice stored at 4°C had a longer storage life of at least 21 days than fresh juice and better-preserved physicochemical properties. Storage studies are significant for estimating fruit juice's shelf life and pasteurised fruit juices' marketability over extended periods such as 90 and 180 days of storage (Wurlitzer et al., 2019).

High-temperature pasteurisation ranges from 72°C to 90°C, while low-temperature pasteurisation is about 63°C (Petruzzi et al., 2017). Low-temperature long-time (LTLT)

pasteurisation, at about 63°C, effectively removes microorganisms using less energy, thus supporting sustainable juice production (Myer et al., 2016). On the other hand, high-temperature pasteurisation requires more energy due to the rapid heating and sustained high temperatures (Ağçam et al., 2018). Besides, studies suggested that pasteurisation conditions differ based on juice characteristics (Petruzzi et al., 2017). For instance, Saikia et al. (2016) found that watermelon juice pasteurised at 60°C for 30 s had low total soluble solids after refrigeration, while Ahmad et al. (2015) noted a significant decrease in vitamin C content in guava juice at 85°C for 60 s. In mango and watermelon juices, pasteurisation at 80°C for 60 s reduced TPC and YMC to below 1 log CFU/mL (Mandha et al., 2023).

Despite extensive studies analysing the pasteurisation of other juices, limited research exists on how temperature and time variations during pasteurisation affect red dragon fruit juice's quality and shelf life. The energy efficiency and sustainability of different pasteurisation conditions for red dragon fruit juice require further investigation. Hence, this study aims to evaluate the effects of both low (60°C) and high (80°C) pasteurisation temperatures for 30 s and 60 s on the microbiological and physicochemical quality as well as the vitamin C content of dragon fruit juice.

MATERIALS AND METHODS

Materials and Reagents

Fully ripened red dragon fruits from Vietnam and aluminium foil were purchased from Lotus, Sungai Dua (Penang, Malaysia). Buffered peptone water (BPW), plate count agar (PCA), and Dichloran Rose-Bengal Chloramphenicol Agar (DRBC) were obtained from HiMedia (Mumbai, India). Sodium hydroxide (NaOH) and ethanol were sourced from Classic Chemical Sdn. Bhd. (Malaysia) and phenolphthalein powder from HmbG Chemical (Malaysia). Potassium iodide, iodine, and soluble starch were acquired from R&M Chemical (London, UK) and ascorbic acid from HiMedia (Mumbai, India).

Preparation of Red Dragon Fruit Juice

Red dragon fruits were cleaned, peeled, and sliced before being juiced using a fruit juice extractor (Hanabishi, Malaysia). The juice was then sieved to achieve a uniform puree. In the first phase, 400 mL of juice samples were divided into five beakers, with one serving as the control while the others were prepared for pasteurisation.

Pasteurisation of Red Dragon Fruit Juice

Pasteurisation was conducted at 60°C and 80°C for 30 s and 60 s, following Valliere & Harkins (2020) with minor modifications. Four hundred millilitres of red dragon fruit juice were placed in a pre-sterilised 500 mL beaker covered with aluminium foil. The juice

sample was heated to the desired temperature in the water bath (Memmert, New York, US) and rapidly cooled in an ice-water bath before storage at 4°C.

Microbiological Analysis

Total Plate Count

The amount of 25 mL of red dragon fruit juice was added to 225 mL of BPW, and this dilution was referred to as 10^{-1}. Serial dilutions were performed until 10^{-5}. Spread plates were prepared using 0.1 mL aliquots of these dilutions on PCA and incubated at 37°C in an incubator for 24 h. The colonies that had grown on the plate were counted, and CFU per mL were calculated. The average mean number of CFU per mL was calculated using Equation 1.

Yeast and Mold Count

A volume of 25 mL of red dragon fruit juice was mixed with 225 mL of BPW, constituting an initial 10^{-1} dilution. Serial dilutions up to 10^{-5} were performed, and 0.1 mL aliquots of these dilutions were spread on DRBC agar. Next, the plates were incubated at 25°C for 5 days in an incubator, and the number of CFU per mL was calculated using Equation 1.

$$\frac{CFU}{mL} = \frac{Number\ of\ Colonies\ Counted}{Dilution\ \times\ Volume\ of\ sample\ plated\ in\ mL} \quad [1]$$

Physicochemical Analysis

Colour

A colourimeter (Konica Minolta CM-5, Singapore) was calibrated with white and black standards to analyse the juice sample's colour using CIELAB colour values: L* (brightness to darkness), a* (green to red), and b* (blue to yellow). Measurements were taken in triplicate, and equations were applied to determine chroma (C*), hue angle (h), and colour difference (Kong et al., 2020).

$$\text{Chroma, } C^* = \sqrt{a^{*2} + b^{*2}} \quad [2]$$

$$\text{Hue angle, } h^\circ = \tan^{-1}\frac{b^*}{a^*} \quad [3]$$

$$\text{Colour difference, } \Delta E, = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad [4]$$

pH

The pH of the fruit juice samples was determined using a pH meter (Mettler Toledo, Darmstadt, Germany) calibrated using solutions with different pH values of 4.6, 7.2, and 9.2. The pH meter was immersed in the juice sample, and the reading was recorded. Three replicates were taken for each sample.

Total Soluble Solids (TSS)

The TSS in the fruit juice samples was measured using a digital refractometer (Hanna Instruments, Johor Bharu, Malaysia). Before the test, the instrument was calibrated by placing a few drops of distilled water on the detector. Approximately one drop of fruit juice was dripped onto the detector, and the reading was recorded. Three replicates were taken for each sample.

Titratable Acidity (TA)

An amount of 5 mL dragon fruit juice samples was diluted to 50 mL of distilled water and titrated against 0.1 M NaOH to a pink endpoint using phenolphthalein as an indicator. Before that, 0.1 M NaOH was prepared by dissolving 0.4 g in 100 mL water, whereas 1% phenolphthalein was made by diluting 1 g in 100 mL of 95% ethanol. Total TA was calculated using Equation 5, as described by Kong et al. (2020).

$$TA(\%) = \frac{N\ x\ V_1 \times Eq.Wt}{V_2 \times 10} \quad [5]$$

where N is the NaOH normality, V_1 is the NaOH volume (in mL), V_2 is the sample volume (in ml), and Eq. Wt. is the equivalent weight of citric acid (64.04g/mol).

Vitamin C Analysis

The vitamin C content of red dragon fruit juices was analysed using the redox titration method described by Satpathy et al. (2020) with minor modifications, including standardisation using an ascorbic acid solution.

Preparation of Iodine Solution

A solution with a concentration of 0.005 mol/L was prepared. An amount of 2 g of potassium iodide and 1.3 g of iodine were weighed and added to a 100 mL beaker. Distilled water (5 mL) was added, and the mixture was swirled for a few minutes until the iodine was completely dissolved. The iodine solution was then transferred to a 1 L volumetric flask, ensuring all traces of the solution were rinsed into the volumetric flask using distilled water. Then, the solution was made up to the 1 L mark with distilled water.

Preparation of Starch Indicator

A solution with a concentration of 0.5% was prepared. In a 100 mL conical flask, 0.25 g of soluble starch was weighed and added to 50 mL of boiled distilled water. The mixture was stirred to dissolve the starch and then cooled to room temperature before use.

Preparation of Ascorbic Acid Solution

An amount of 0.88 g of ascorbic acid was weighed in a 100 mL beaker and added to a 1 L volumetric flask to make up 1 L with the addition of distilled water. The remaining solids in the beaker and funnel were rinsed using small portions of distilled water to ensure all the ascorbic acid was transferred into the volumetric flask.

Titration

A 20 mL sample solution was pipetted into a 250 mL conical flask, followed by 150 mL of distilled water and 1 mL of starch indicator. The sample was titrated with a 0.005 mol/L iodine solution until a lasting purple colour indicated the endpoint. This process was repeated three times with additional sample aliquots. The volume of iodine used was recorded, and the vitamin C content (mg/100 mL) in the juice sample was calculated based on the moles of iodine reacting.

$$\text{Ascorbic acid} + I_2 \rightarrow 2\ I^- + \text{Dehydroascorbic acid}$$

Statistical Analysis

The experimental data was analysed through one-way analysis of variance (ANOVA) utilising Statistical Package for Social Sciences (SPSS) software version 28.0 (IBM Corporation, Endicott, NY, USA). This analysis aims to assess the significance of variations of microbiological, physicochemical, and vitamin C analysis for unpasteurised and pasteurised samples. The significance level for statistical significance was 95% confidence ($p < 0.05$). All experimental data were presented in duplicate or triplicate as mean ± standard deviation. Post hoc comparisons were performed using Tukey's test to identify and compare the statistical differences within the research study.

RESULTS AND DISCUSSION

Microbiological Analysis

Table 1 shows the effect of different pasteurisation temperatures and times on the microbial quality of red dragon fruit juice. TPC decreased significantly ($p < 0.05$) to 3.89 and 3.75 CFU/mL at 60°C for 30 s and 60 s, respectively, indicating that a longer exposure time at 60°C leads to greater microbial reduction. Notably, a significant reduction was achieved

Table 1
Effect of different pasteurisation temperatures and time on the microbiological quality of dragon fruit juice

	Pasteurisation (temperature, time)				
	Unpasteurised	**60 °C, 30 s**	**60 °C, 60 s**	**80 °C, 30 s**	**80 °C, 60 s**
TPC (log CFU/mL)	6.00 ± 0.18^{a}	3.89 ± 0.27^{b}	3.75 ± 0.14^{c}	3.72 ± 0.18^{c}	3.56 ± 0.35^{d}
YMC (log CFU/mL)	3.48 ± 0.23^{a}	$< LOD^{b}$	$< LOD^{b}$	$< LOD^{b}$	$< LOD^{b}$

Note. TPC = Total plate count, YMC = Yeast and mold count. Values expressed as means ± standard deviations, Different letters within a row denote a significant difference ($p < 0.05$), $n = 2$, LOD means the limit of detection where the count of the colonies formed is less than 1

even at the shorter exposure time of 30 s, suggesting that lower temperatures for shorter durations can still effectively reduce microbial load. No significant difference was observed between 60°C (for 30 s and 60 s) and 80°C (30 s). However, pasteurisation at 80°C for 60 s resulted in the lowest microbial load, demonstrating the combined effect of higher temperature and longer exposure. Mandha et al. (2023) state that higher temperatures enhance microbial inactivation due to enzyme denaturation and cell membrane disruption.

Microorganisms in fresh fruit juice can originate from environmental sources like contaminated water, soil, or animals during harvesting and cross-contamination during processing. Soil bacteria and fungi may adhere to fruit surfaces, transferring into the juice during extraction (Alegbeleye et al., 2022). The Food and Drug Administration (FDA) sets limits of 10^5 CFU/mL for TPC and 50 CFU/mL for YMC in fruit juice, and all pasteurised samples in this study met these standards, demonstrating effective microbial reduction regardless of temperature and time. These results align with previous research by Ferreira et al. (2022), where pasteurisation at 71°C for 30 s significantly reduced microbial populations in *Opuntia Ficus-Indica*. This research demonstrated that shorter pasteurisation is able to significantly extend the juices' shelf life by 22 days compared to non-pasteurised samples, highlighting the effectiveness of shorter pasteurisation times in microbial control. After pasteurisation, TPC for red, orange and white prickly pear juices was reduced to approximately 3.06, 3.02, and 3.04 log CFU/mL, respectively, with populations of *Enterobacteriaceae*, yeasts and moulds below detection limits, indicating high microbial safety. Unpasteurised samples showed 3.48 log CFU/mL of YMC, while all pasteurised samples had undetectable YMC. The fruit juice's high carbohydrate and nitrogen content supports yeast growth, but pasteurisation effectively kills these microorganisms. Yeast species such as *Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii* are particularly vulnerable to heat pasteurisation, which reduces their populations below the detection limit of 2 log CFU/mL (Mandha et al., 2023).

Physicochemical Analysis

The effect of different pasteurisation temperatures and holding time on the physicochemical properties of red dragon fruit juice are presented in Table 2. Total acidity (TA) refers to the

amount of acid contained in a substance, and it can be observed that the acidity changes during pasteurisation. The unpasteurised juice maintained a TA of 0.12%, consistent with findings from Alim et al. (2023). TA remained stable for most pasteurised samples compared to unpasteurised ones except for those treated at 80°C for 60 s. Besides, TSS describe the concentration of all soluble compounds in a solution, predominantly sugars. TSS also stayed consistent between 10.4 and 10.6 °Brix across pasteurisation conditions. The pH was unchanged for unpasteurised and 60°C pasteurised samples, but the lowest was recorded at 80°C for 60 s. Pasteurisation did not significantly alter TA and TSS, aligning with previous findings by Mandha et al. (2023), but did significantly affect colour and pH. In addition, pasteurisation increased L*, a*, b*, h°, and C values, indicating brighter and more intense juice colour, especially at 80°C for 60 s. In this case, no ΔE value is provided for the unpasteurised juice sample because it serves as the reference point. The colour differences in the other samples are measured relative to this unprocessed state.

According to Food Regulation 1985 (235), the acidity of fruit juice should not exceed 3.5% w/v. In this study, all fresh and pasteurised samples remain below this regulatory threshold, which confirms their acceptability for consumption. The acidity decreases slightly as the pasteurisation temperature increases, reaching its lowest point (0.08%) at 80°C for 60 s. Generally, fruit juices have TA within the range of 0.3% to 1.5% depending on the fruit type, like ripeness, with lower values typically associated with less acidic fruits, including red dragon fruit (Vern et al., 2023). Pasteurisation reduces acidity by eliminating bacteria that produce acids as byproducts during fermentation. Fruit juices can naturally undergo acetic acid fermentation due to the presence of microorganisms such

Table 2
Effect of different pasteurisation temperature and time on the physicochemical properties of red dragon fruit juice

	Pasteurisation (temperature, time)				
	Unpasteurised	**60 °C, 30 s**	**60 °C, 60 s**	**80 °C, 30 s**	**80 °C, 60 s**
TA (%)	0.12 ± 0.02^{a}	0.11 ± 0.01^{a}	0.10 ± 0.01^{a}	0.11 ± 0.01^{a}	0.08 ± 0.01^{b}
pH	5.05 ± 0.01^{a}	5.03 ± 0.01^{a}	5.01 ± 0.01^{a}	4.70 ± 0.02^{b}	4.50 ± 0.01^{b}
TSS (°Brix)	10.60 ± 0.10^{a}	10.40 ± 0.03^{a}	10.60 ± 0.03^{a}	10.50 ± 0.01^{a}	10.60 ± 0.03^{a}
L*	13.50 ± 0.15^{a}	13.08 ± 0.02^{bc}	13.43 ± 0.03^{a}	15.77 ± 0.02^{bd}	16.02 ± 0.01^{bd}
a*	43.28 ± 0.01^{a}	43.49 ± 0.01^{b}	43.96 ± 0.01^{c}	45.87 ± 0.01^{d}	46.15 ± 0.01^{e}
b*	21.44 ± 0.01^{a}	21.72 ± 0.01^{b}	22.44 ± 0.01^{c}	27.14 ± 0.01^{d}	27.53 ± 0.01^{e}
h°	25.83 ± 0.10^{a}	26.53 ± 0.01^{b}	27.04 ± 0.01^{c}	30.07 ± 0.02^{d}	30.27 ± 0.01^{e}
C	49.20 ± 0.10^{a}	48.61 ± 0.02^{b}	49.36 ± 0.02^{c}	54.16 ± 0.02^{d}	54.60 ± 0.01^{e}
ΔE	-	0.94 ± 0.01^{a}	1.05 ± 0.02^{a}	6.66 ± 0.01^{b}	7.18 ± 0.01^{c}

Note. Values expressed as means ± standard deviations, Different letters within a row denote a significant difference ($p < 0.05$), $n = 3$, TA = Titratable acidity, TSS = Total soluble solids, L* = Lightness, a* = Redness, b* = Yellowness, H° = Hue angle, C = Chroma, ΔE = Colour difference

as *Acetobacter* species. These bacteria can convert the sugars in the juice into acetic acid and other byproducts, increasing acidity. Natural fermentation involves native bacteria fermenting raw materials without artificial inoculation. It is supported by Sourri et al. (2022), who reported that *Acetobacter* species, such as *Acetobacter pasteurianus* and *Acetobacter aceti*, are commonly found on fruit surfaces and are heat sensitive. However, this process can also lead to the growth of spoilage and pathogenic bacteria, making fermentation unpredictable. Consequently, pasteurisation effectively eliminates these acid-producing microbes by applying heat that denatures proteins and disrupts cell membranes, thus preventing spoilage and maintaining the quality of the juice (Saud et al., 2024).

Small changes in °Brix suggest that pasteurisation does not significantly affect the solute as sugar level generally remains stable under typical pasteurisation conditions (Nicklas et al., 2015). The TSS observed (10.6 °Brix) differs from the reported 12 °Brix due to variations in fruit ripeness of dragon fruits used as mature and ripened fruit have higher TSS in the juice, thus not influenced by pasteurisation (Yıkmış, 2020). The starch stored in the fruit is converted into sugars like glucose and fructose upon ripening, increasing the fruit's sweetness and achieving higher TSS in the juice.

The pH of red dragon fruit juice (5.05) aligns with previous findings from Arivalagan et al. (2021) that mentioned the pH of dragon fruit juice is slightly acidic, between 4.8 and 5.40. According to Kong et al. (2020), the significant decrease in pH of the high temperature pasteurised sample might be due to the higher dissociation of citric acid, which is abundant in red dragon fruit. The release of H^+ ions in the dissociation process at higher temperatures contributes to the acidity of the solution, resulting in a lower pH (Angonese et al., 2021).

Higher pasteurisation temperatures and durations increase L* values, indicating a lighter juice colour, likely due to pigment degradation. The highest L* value was observed at 80°C for 60 s, showing a significant change from the unpasteurised sample. The a* value reflects redness, which has increased slightly with temperature but remained minimal compared to unpasteurised samples. Similarly, b* values rise with temperature, indicating yellowing, which may result from chemical changes during pasteurisation. Pasteurisation degrades betanin, leading to a shift in colour from red to yellow.

Dragon fruit's vibrant red colour comes from betalains, particularly betacyanin, which degrades with heat (Liaotrakoon, 2013). Betacyanin content drops significantly with higher temperatures, from 80% at 50°C to 32% at 90°C. Heating above 80°C causes more pronounced colour changes and higher betacyanin loss. Besides, the hue angle (h°), which indicates the dominant colour tone, shifts from red to purple at higher temperatures. The result aligns with Wong and Siow (2015), who found greater betacyanin retention at lower temperatures, at 65°C than 85°C. Chroma values representing colour intensity also increase with higher temperatures. According to Kong et al. (2020), ΔE values can be categorised as not noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3.0),

clearly visible (3.0–6.0) and significant (6.0–12.0). The increase in ΔE values at higher temperatures indicates significant colour changes, while pasteurisation at 60°C results in minimal colour change, not exceeding 1.05. These findings are consistent with those of Moo-Huchin et al. (2017), who reported increased colour degradation in watermelon juice with higher pasteurisation temperatures due to carotenoid oxidation. Dragon fruit, which contains a relatively low concentration of carotenoids (0.86 mg/100 mL), shows similar trends as those obtained in this study. The results are also supported by Kong et al. (2020), who observed a significant decrease in the L* value in pomegranate juice after being heated at 95°C, the 30s.

Vitamin C Content Analysis

The effect of temperature and time on vitamin C in pasteurised and unpasteurised red dragon fruit juice is recorded in Table 3. Pasteurisation at 60°C for 30 s results in a significant decrease in vitamin C concentration, followed by an increase in the pasteurisation duration (60 s), which has resulted in a further decline to 33.9 mg/100 mL ($p < 0.05$). It indicates that mild vitamin C degradation. Higher temperatures often accelerate degradation in which pasteurisation at 80°C for a shorter duration of 30 s caused a more significant decrease in vitamin C content extending the time to 60 s at 80°C results in a further decline to 30.82 mg/100 mL, demonstrating more substantial degradation due to the combined effects of higher temperature and longer duration.

Fresh dragon fruit juice contains 37.4 mg/100 mL of vitamin C, higher than the 33 mg/100 mL reported by Liaotrakoon (2013). This variation is attributed to different ripeness levels of the fruit used. Ernest et al. (2017) state that vitamin C concentration decreases as fruit maturity increases. However, pasteurisation reduces vitamin C content because ascorbic acid is heat-sensitive. It is consistent with findings by Tchuenchieu et al. (2018), who observed that pasteurisation adversely affects vitamin C in orange juice. The degradation follows a first-order kinetic pattern, suggesting that temperature influences ascorbic acid breakdown during thermal processing.

Table 3
Effect of different pasteurisation temperature and time on vitamin C concentration

	Pasteurisation (temperature, time)				
	Unpasteurised	**60°C, 30 s**	**60°C, 60 s**	**80°C, 30 s**	**80°C, 60 s**
Vitamin C concentration (mg/100 mL)	37.40 ± 0.10^{a}	34.80 ± 0.01^{b}	33.90 ± 0.01^{c}	31.26 ± 0.05^{d}	30.82 ± 0.02^{e}

Note. Values expressed as means ± standard deviations; Different letters within a row denote a significant difference ($p < 0.05$), $n = 3$

Unpasteurised samples have significantly higher vitamin C than pasteurised ones due to ascorbic acid's susceptibility to oxidation, particularly heat and oxygen (Liaotrakoon, 2013). Oxidation converts ascorbic acid into dehydroascorbic acid and other products, such as furfural, 2-furoic acid, and 3-hydroxy-2-pyrone. Furfural can polymerise with amino acids, leading to browning in ascorbic acid-containing juices (Yin et al., 2022). Mandha et al. (2023) found a rapid decline in vitamin C in watermelon and mango juices with extended pasteurisation at 80°C. Similarly, Klopotek et al. (2005) reported a 35% reduction in vitamin C in strawberry juice after high-temperature pasteurisation at 85°C. Conversely, Giavoni et al. (2008) found that fresh orange pulp juice contains 17.63 mg/100 g of vitamin C, with no significant reduction after pasteurisation, likely due to due to the presence of solid parts where juices with the highest pulp concentration containing the highest level of vitamin C.

The microbiological quality of red dragon fruit juice is effectively enhanced through pasteurisation at 60°C for 60 s, as evidenced by a significant reduction in TPC to 3.75, which is almost the same with a higher pasteurisation temperature. However, extended time is crucial as 60°C for 30 s results in a higher TPC of 3.89 ($p < 0.05$). The physicochemical properties remain stable, with TA at 0.10%, indicating no adverse effect on acidity compared to the unpasteurised sample. Importantly, this pasteurisation condition avoids the significant decrease in pH observed at a higher temperature (80°C), thus maintaining the juice's desirable flavour profile (Ghorai, 2023). Vitamin C slightly degrades to 33.9 mg/100 mL, but colour preservation is effective. Therefore, pasteurisation at 60°C for 60 s ensured microbial quality, preserved physicochemical properties and minimised vitamin C degradation.

Effect of Storage on Microbial Quality

Table 4 shows the effectiveness of cold storage for pasteurised and unpasteurised red dragon fruit juices. After one week, the unpasteurised sample had a higher TPC and YMC than the initial week. In the following weeks, the unpasteurised sample's microbial counts increased significantly compared to pasteurised juice, reaching an uncountable range for TPC and a high value for yeasts and moulds by Week 4. Meanwhile, the pasteurised juice had a minimal increase for TPC, but YMC remained consistently below the detectable level of 2 log CFU/mL. A high TPC value indicates a shorter shelf life for the juice, as it may contain many bacteria that could lead to spoilage. Some spoilage organisms can survive pasteurisation, posing a risk of product spoilage during storage. Yeast and mould could grow in dragon fruit juice even at a low pH due to its acidophilic nature (Jerry & Bright, 2019). Spoilage bacteria and YMC found on the surface of fruits would be harmful if consumed without any treatment (Mengistu et al., 2022).

The pasteurised samples have shown a slight increase in TPC and maintained a consistently low YMC throughout the 4-week storage period. Pasteurisation kills most psychotropic bacteria, but some species and strains survive at refrigeration temperatures less than 7°C. However, the TPC is minimal and does not exceed the permitted level compared to before and during one month of storage. Thus, proper maintenance of refrigeration temperature after pasteurisation aids in ensuring the shelf life of juice is protected throughout storage until it reaches consumers.

The result is in line with a previous finding by Ma et al. (2020), who demonstrated that unpasteurised watermelon juice deteriorated rapidly due to microbial growth during storage. According to Mandha et al. (2023), pasteurisation effectively preserved a good microbial quality in watermelon juice throughout the storage period. However, the unpasteurised juice showed a gradual increase in TPC, reaching 8.33 log CFU/mL in the second week, which is higher than 6.05 log CFU/mL in this study. It might be due to the higher pH value of watermelon juice than dragon fruit juice, which limits microbial growth in more acidic environments. Additionally, Choo et al. (2022) reported that the microbial counts of pasteurised noni juice remained within acceptable levels throughout the 8-week storage period in refrigerated (4°C). This finding supports the suitability of refrigerated storage conditions in preserving the microbiological integrity of pasteurised noni juice.

Table 4
Microbiological analysis of unpasteurised and pasteurised (60°C, 60 s) red dragon fruit juice during storage at 4°C

	Microbiological analysis (log CFU/mL)	
	TPC	**YMC**
Unpasteurised		
Week 0	6.00 ± 0.18^{aA}	3.48 ± 0.23^{aA}
Week 1	6.03 ± 0.20^{aA}	4.28 ± 0.28^{bA}
Week 2	6.05 ± 0.15^{aA}	4.38 ± 0.23^{bA}
Week 3	$TNTC^{bA}$	5.32 ± 0.34^{cA}
Week 4	$TNTC^{bA}$	5.58 ± 0.73^{dA}
Pasteurised		
Week 0	3.75 ± 0.14^{aB}	$< LOD^{aB}$
Week 1	3.77 ± 0.25^{aB}	$< LOD^{aB}$
Week 2	3.79 ± 0.47^{bB}	$< LOD^{aB}$
Week 3	3.83 ± 0.32^{cB}	$< LOD^{aB}$
Week 4	3.86 ± 0.23^{dB}	$< LOD^{aB}$

Note. Values expressed as means ± standard deviations, Small letters within a column denote a significant difference ($p < 0.05$) within the same treatment group (either pasteurised or unpasteurised) across different storage weeks, $n = 3$, Capital letters within a column denote a significant difference ($p < 0.05$) between different treatment groups (pasteurised and unpasteurised) within the same storage week, $n = 3$, TNTC means too numerous to count, where the count of the colonies formed is > 300, LOD means the limit of detection, where the count of the colonies form is < 1

Effect of Storage on Physicochemical Properties

Table 5 presents the physicochemical properties of unpasteurised and pasteurised (60°C for 60 s) red dragon fruit juice samples for 4 weeks of cold storage. After titration, TA was determined by observing the colour changes in the dragon fruit juice sample. TA in the unpasteurised sample steadily increases, reaching 0.38%, while the pasteurised sample shows a more controlled increase, reaching 0.20%. Both samples' pH fluctuated, decreasing to 4.64 at week 1 and gradually increasing until week 4. TSS remained relatively stable in both samples. Colour parameters (L*, a*, b*, h°, C) demonstrate a darkening trend, which is more noticeable in the pasteurised sample. Hence, pasteurisation had no notable impact on the pH and TSS of dragon fruit juice compared to unpasteurised samples, but a significant difference can be observed for colour parameters. These findings align with the previous research on watermelon juice enriched with L-citrulline by Tarazona-Díaz et al. (2017).

Additionally, the sudden increase of pH and TA after week 1 in this study is similar to the previous research by Adedokun et al. (2022) on the storage stability of black plum, baobab, and pineapple juice blends. In that study, the authors found that the pH remained stable during the initial two weeks at 4°C. However, a slight pH reduction occurred from week 2 to week 4 due to biological processes within the juice samples.

TA of the unpasteurised dragon juice increased drastically from 0.17% to 0.27% after one week. It may be due to the growth of microorganisms in unpasteurised juice, which have contributed to forming organic acids such as lactic acid bacteria via metabolic processes. Additionally, yeasts and other microorganisms capable of fermenting sugars could increase the production of organic acids (Aneja et al., 2014). However, the TA has resulted in non-significant changes in the later weeks due to the sugars in the dragon fruit juice, which microorganisms may have utilised during the initial phase, reducing the acid production rate. It is supported by a previous finding by Sandle (2016), who stated that the nutrient requirements vary widely among microorganisms, in which most derive energy by metabolising simple sugars like fructose.

For the pasteurised sample, there was a slight increase in the TA, from 0.10 to 0.15%, possibly due to a lower microorganism count than in the unpasteurised sample. The finding is similar to Unluturk and Atilgan (2015), who identified a drastic increment in TA of untreated white grape juice compared to pasteurised juice. Besides, the pasteurised samples maintained a constant TA (0.15% to 0.20 %) from week 1 to week 4 due to slowed enzymatic reactions in the juice, which indicates that oxidative enzymes have a significant enzymatic activity in the pulp of dragon fruit. The activity of enzymes such as polyphenol oxidase (PPO) and peroxidase (POD) in fruit juices is influenced by the concentration of oxygen present in the environment. Changes in the fruit juice's flavour components, which might affect the juice's acidity, can also result from browning reactions facilitated by PPO

Table 5
Physicochemical analysis of unpasteurised and pasteurised (60°C, 60 s) red dragon fruit juice during storage at 4°C

	TA (%)	pH	TSS (°Brix)	L*	a*	b*	h°	C	ΔE
Unpasteurized									
Week 0	0.17 ± 0.02^{aA}	5.05 ± 0.01^{aA}	10.6 ± 0.1^{aA}	13.5 ± 0.15^{aA}	43.28 ± 0.01^{aA}	21.44 ± 0.01^{aA}	25.83 ± 0.10^{aA}	49.20 ± 0.10^{aA}	-
Week 1	0.27 ± 0.10^{bA}	4.64 ± 0.01^{bA}	11.1 ± 0.03^{bA}	4.92 ± 0.01^{bA}	25.02 ± 0.01^{bA}	7.82 ± 0.01^{bA}	17.36 ± 0.01^{bA}	26.21 ± 0.01^{bA}	-
Week 2	0.32 ± 0.26^{cA}	4.91 ± 0.10^{cA}	11.1 ± 0.03^{bA}	5.15 ± 0.04^{cA}	29.29 ± 0.04^{cA}	8.83 ± 0.01^{cA}	16.78 ± 0.26^{cA}	30.59 ± 0.17^{cA}	-
Week 3	0.33 ± 0.36^{cA}	4.93 ± 0.04^{cA}	11.1 ± 0.03^{bA}	5.15 ± 0.03^{cA}	29.65 ± 0.29^{cA}	8.86 ± 0.26^{cA}	16.64 ± 0.01^{cA}	30.95 ± 0.04^{cA}	-
Week 4	0.38 ± 0.10^{dA}	4.98 ± 0.02^{dA}	11.0 ± 0.02^{bA}	5.92 ± 0.02^{dA}	32.64 ± 0.02^{dA}	10.23 ± 0.01^{dA}	17.40 ± 0.01^{dA}	34.21 ± 0.03^{dA}	-
Pasteurized									
Week 0	0.10 ± 0.01^{aB}	5.01 ± 0.01^{aB}	10.6 ± 0.03^{a}	13.43 ± 0.03^{aA}	43.96 ± 0.01^{aA}	22.44 ± 0.01^{aB}	27.04 ± 0.01^{aB}	49.36 ± 0.02^{aB}	1.05 ± 0.02
Week 1	0.15 ± 0.20^{bB}	4.64 ± 0.01^{bB}	11.0 ± 0.02^{bA}	6.48 ± 0.02^{bB}	32.09 ± 0.01^{bB}	10.28 ± 0.01^{bB}	17.76 ± 0.01^{bB}	33.70 ± 0.01^{bB}	7.65 ± 0.04
Week 2	0.17 ± 0.10^{bB}	4.86 ± 0.01^{cB}	11.0 ± 0.03^{bA}	6.73 ± 0.02^{cB}	34.16 ± 0.01^{cB}	11.59 ± 0.26^{cB}	18.74 ± 0.36^{cB}	36.07 ± 0.01^{cB}	5.50 ± 0.03
Week 3	0.19 ± 0.10^{cB}	4.92 ± 0.01^{dB}	10.9 ± 0.02^{cA}	6.97 ± 0.02^{dB}	34.68 ± 0.01^{cB}	12.02 ± 0.10^{dB}	19.12 ± 0.10^{dB}	36.70 ± 0.26^{dB}	6.52 ± 0.10
Week 4	0.20 ± 0.10^{cB}	4.99 ± 0.02^{eB}	10.9 ± 0.03^{cA}	6.44 ± 0.01^{eB}	33.47 ± 0.02^{dB}	11.1 ± 0.36^{eB}	18.35 ± 0.10^{eB}	35.26 ± 0.36^{eB}	1.31 ± 0.10

Note. Values expressed as means ± standard deviations; Small letters within a column denote a significant difference ($p < 0.05$) within the same treatment group (either pasteurised or unpasteurised) across different storage weeks, n = 3, Capital letters within a column denote a significant difference ($p < 0.05$) between different treatment groups (pasteurised and unpasteurised) within the same storage week, $n = 3$, TA = Titratable acidity, TSS = total soluble solids, L* = Lightness, a* = Redness, b* = Yellowness, H° = Hue angle, C = Chroma, ΔE = Colour difference

and POD enzymes. A study by Shaik and Chakraborty (2023) mentioned that the enzyme activity remains below 10% when the treated lime juice is stored at temperatures of 4°C. Thus, cold storage is efficient in maintaining the acidity of pasteurised fruit juice.

The pH of unpasteurised and pasteurised samples decreased from 5.05 and 5.01, respectively, to 4.64 in week 1. Acids found naturally in fruit juice, such as citric and malic acids, can be degraded. Oxidation of citric and malic acids can form intermediate compounds such as oxalic acid and oxaloacetic acid. These reactions might include enzymatic or chemical activities that degrade bigger acid molecules into simpler ones, reducing pH (Jerry & Bright, 2019). However, for both samples, a slight increase in pH can be detected from week 1 to week 4. It might be due to the effectiveness of cold storage, which avoids further chemical reactions that could turn the juice highly acidic and affect its sensory characteristics of sweetness and sourness. TSS increased slightly from 10.6 to 11.1 and 11.0 for unpasteurised and pasteurised samples at week 1 because of the conversion of carbohydrates to simple sugars (Mandha et al., 2023). During cold storage, the TSS for both samples showed only a slight change; the overall values ranged from 10.9 to 11.01. Cold storage will inhibit enzymatic activity, which could increase the solid content in the juice. Storage temperature can impact the rate of enzymatic and chemical reactions. Higher temperatures may accelerate these reactions, leading to a more rapid increase in TSS (Babarinde et al., 2019). This finding is similar to Wang et al. (2022), who reported that the TSS increased initially due to starch hydrolysis and then decreased after the starch had been completely digested.

The drastic drop in colour values for unpasteurised and pasteurised samples at week 1 compared to initial values is due to the cold storage that slowed down or temporarily inhibited enzymatic and chemical reactions responsible for colour development or stability. Enzymatic browning reactions due to PPO and POD enzymes, oxidation or other chemical processes contributing to colour changes are typically temperature dependent. Lower temperatures can slow these reactions, reducing colour intensity (Wibowo et al., 2015). Furthermore, unpasteurised and pasteurised samples exhibited noticeable changes in their colour characteristics over the four weeks. The L* values showed a consistent increase in both samples, indicating that the colour of the juice became lighter. Similarly, the a* and b* values were consistently increased in unpasteurised and pasteurised samples.

Despite variations in the hue angle (h°) values, no consistent trend was observed over the four weeks, suggesting fluctuations in the dominant wavelength of colour without a distinct shift for unpasteurised. It might be due to a growing trend in the microbial count, especially psychrotrophic bacteria, which causes chemical changes that lead to improper colour degradation (Hwang et al., 2022). In addition, chroma values increased over the four weeks for unpasteurised and pasteurised samples, indicating an increment in colour saturation. For L*, a*, b*, h° and C values, there was a sudden decrease at week 4 for pasteurised samples from three weeks of gradual increase. Dragon fruit consists of enzymes,

including polyphenol oxidase, which can catalyse enzymatic browning reactions. Even though pasteurisation is intended to deactivate enzymes, residual enzymatic activity may still exist. This remaining activity may eventually result in browning (Zhu et al., 2019).

For the first week, pasteurised samples showed the highest colour difference (7.65), and there was only a slight change at week 4 (1.34). Lee and Coates (2003) observed an increase in the b* value after thermal processing of orange juice at 90°C for 30 s. Similarly, Xu et al. (2015) found that thermal processing at 110°C for 8.6 s increased the yellowness of an orange juice blend, while Igual et al. (2014) discovered an increase in the b* value of grapefruit juice after heat treatment at 80°C. These findings support that the higher the temperature, the greater the changes in the colour parameters of the fruit juice. Moreover, chemical changes, including enzymatic reactions, influence the colour change of the juice during storage. Thus, pasteurisation leads to a noticeable alteration in colour, yet maintaining a lower temperature lessens this effect and preserves the vibrant colour of the juice.

Effect of Storage on Vitamin C Concentration

Table 6 shows the vitamin C content of unpasteurised and pasteurised samples at cold storage. Initially, the unpasteurised sample had a significantly higher vitamin C concentration of 37.4 mg/100 mL. However, the vitamin C concentration decreased significantly ($p < 0.05$) over the four-week storage period. This decline can be due to the natural sensitivity of vitamin C to numerous environmental variables, such as air, light, and temperature (Ajibola et al., 2009). Factors such as oxygen exposure during handling and storage contribute to the breakdown of vitamin C in fruit juices, resulting in a progressive drop in concentration (Mieszczakowska-Frąc et al., 2021). There is a significant difference ($p < 0.05$) among unpasteurised and pasteurised samples, especially during week 4. Both samples resulted in a declining trend for vitamin C content. This result is similar to findings by Mgaya-Kilima et al. (2015) and Alim et al. (2023), who concluded that vitamin C degradation during storage is one of the most essential factors affecting the quality of the juice.

Vitamin C concentration in the pasteurised sample also decreased across the four weeks, starting in Week 0 at 33.9 mg/100 mL. Despite being heat treated at 60°C, 60 s the pasteurised sample showed a similar trend of vitamin C breakdown as unpasteurised sample. This decline can be due to persistent oxidative reactions even after pasteurisation (Hernandez et al., 2006). Although pasteurisation helps to reduce the activity of enzymes that degrade vitamin C, it does not eliminate the impact of oxygen exposure and other variables that contribute to the deterioration of this sensitive nutrient during long periods of storage. It is supported by Tiencheu et al. (2021), who found a decrease in vitamin C after 1 month of storage at the refrigerating temperature for pawpaw, pineapple, and watermelon

Table 6
Vitamin C analysis of unpasteurised and pasteurised (60 °C, 60 s) red dragon fruit juice during storage at 4°C

	Vitamin C concentration (mg/100 mL)
Unpasteurised	
Week 0	37.4 ± 0.10^{aA}
Week 1	28.3 ± 0.01^{bA}
Week 2	25.0 ± 0.10^{cA}
Week 3	22.9 ± 0.10^{dA}
Week 4	19.8 ± 0.26^{eA}
Pasteurised	
Week 0	33.9 ± 0.01^{aB}
Week 1	28.1 ± 0.26^{bB}
Week 2	22.0 ± 0.06^{cB}
Week 3	20.7 ± 0.10^{dB}
Week 4	17.8 ± 0.26^{eB}

Note. Values expressed as means ± standard deviations; Small letters within a column denote a significant difference ($p < 0.05$) within the same treatment group (either pasteurised or unpasteurised) across different storage weeks, $n = 3$, Capital letters within a column denote a significant difference ($p < 0.05$) between different treatment groups (pasteurised and unpasteurised) within the same storage week, $n = 3$

juices. Due to the unstable nature of vitamin C, the decrease in vitamin C is identified in juices with increased storage duration because of oxidation during storage.

In Week 4, the unpasteurised sample's vitamin C content dropped to 19.8 mg/100 mL, whereas the pasteurised sample's concentration decreased to 17.8 mg/100 mL. This finding is similar to Wurlitzer et al. (2019), who found that vitamin C in tropical fruit juice was degraded during storage with a considerable loss of 30% to 40% from its initial concentration. In addition, Mandha et al. (2023) found that cold storage had an adverse impact on vitamin C in mango juice by reducing it from 61.1 to 33.9 mg/100 mL due to the presence of oxygen, which led to oxidation. Despite the drop in both samples, the pasteurised sample's vitamin C concentration is close to the unpasteurised sample. This implies that although pasteurisation reduced the vitamin C content of red dragon fruit juice, it still contributes to an acceptable range for consumers' daily intake. According to the US FDA, the daily value for vitamin C is set at 90 mg to support immune function and overall health. In comparison, the Ministry of Health Malaysia (MOH) recommends a reference nutrient intake of 70 mg for maintaining adequate vitamin C levels in adults. Pasteurised juice retains a significant quantity of vitamin C (17.8 mg) even after week 4, making it efficient for consumption as it provides 19.7% of the daily value of vitamin C and 25.4% of the reference nutrient intake of vitamin C as recommended by the FDA and MOH respectively.

CONCLUSION

The study revealed that pasteurisation significantly impacted red dragon fruit juice's microbiological, physicochemical, and vitamin C properties. Both high (80°C) and low (60°C) temperature pasteurisation effectively reduced microbial counts, especially at 60-second durations. Physicochemical analysis showed no significant effects on total acidity (TA) and total soluble solids (TSS) but noticeable changes in colour and pH, particularly at 80°C, where pigment degradation occurred. Vitamin C content decreased with higher temperatures and longer pasteurisation times due to heat sensitivity. Unpasteurised juice saw rapid microbial growth over four weeks of storage, while pasteurised juice maintained acceptable bacterial counts. Pasteurisation did not significantly affect pH and TSS but affected colour throughout storage. Both unpasteurised and pasteurised juices experienced a decline in vitamin C content over time, with unpasteurised juice showing more significant losses due to enzymatic and oxidative degradation. Thus, the study determined that pasteurisation at 60°C for 60 s balances microbiological quality and has minimal impact on physicochemical characteristics and vitamin C concentration.

ACKNOWLEDGEMENTS

The authors extend their gratitude to Assoc. Prof. Dr. Cheng Lai Hoong for her valuable guidance and expertise in vitamin C analysis, particularly for suggesting key modifications. They also thank the laboratory assistants of the Food Technology Division, School of Industrial Technology, USM, for their assistance in preparing the necessary chemical reagents and equipment and for their support throughout the research.

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