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Effect of Different Pasteurization Temperatures on the Physicochemical and Microbiological Quality of Rock Melon Juice During Storage at 4°C

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ABSTRACT

Fruit juice, a popular beverage, is prone to spoilage due to its high moisture and nutritional content, which may pose health risks to consumers. This study aims to evaluate the changes in physicochemical and microbiological qualities of rock melon juice (RMJ) subjected to two pasteurization methods: mild-temperature-short-time (MTST) pasteurization at 70°C for 30 s and high-temperature-short-time (HTST) pasteurization at 85°C for 20 s. The juice was stored at 4°C for 21 days, with total soluble solids (TSS), titratable acidity (TA), pH, TSS/TA ratio, colour, total plate count (TPC), and yeast and mold counts assessed on days 1, 7, 14, and 21. Results indicated that pasteurization significantly affected ($p < 0.05$) the physicochemical and microbiological qualities of RMJ, except for TSS, TA, and pH. On day 1, both treatments increased the L* value (control: 68.16, MTST: 69.50, HTST: 69.70) while reducing a* (control: 20.15, MTST: 16.84, HTST: 15.87) and b* (control: 51.14, MTST: 46.67, HTST: 46.96) values, resulting in a lighter juice colour due to pigment degradation. MTST and HTST samples showed lower TPC (2.15 and 2.00 log CFU/mL) compared to the control sample (3.96 log CFU/mL) on day 1. During 21 days of storage, the microbial loads of pasteurized RMJ were observed at 2 log CFU/mL and lower. No significant differences ($p > 0.05$) were observed in TSS, TA, and microbiological quality between MTST- and HTST-treated RMJ after storage. HTST pasteurization is recommended to minimize colour changes, potentially enhancing juice acceptability and marketability.

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INTRODUCTION

Rock melon (*Cucumis melo* var. *reticulatus*) is a warm-season cucurbit species known for its distinctive spherical or oval shape,

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characterized by a rough, raised netting pattern covering its skin. Its orange flesh is filled with seeds. This delicious and nutritious fruit is enjoyed worldwide for its sweet and aromatic flavour and its phytonutrient content. It is highly beneficial for health since it offers high nutrition, antioxidants, phenolic compounds, ascorbic acid, carotenoids, essential vitamins, and minerals (Hassan et al., 2022). Rock melon is well-known for its juiciness and sweetness, and it is commonly enjoyed fresh as a healthy dessert or blended into refreshing juices. According to the Department of Agriculture (DOA) Malaysia, the cultivation of rock melons is primarily located in the states of Kedah, Kelantan, Pahang, and Sabah, with a total harvested area of 283.48 ha.

However, the short shelf life of rock melon juice (RMJ) is a significant drawback due to its high water content, which makes the juice vulnerable to microorganisms and degradation (Mandha et al., 2023). The untreated RMJ provides a conducive environment for microbial growth and supports metabolic activities due to its natural sugars, which serve as a source of nutrients for the microorganisms. This poses a critical risk to public health, as the consumption of juices contaminated with foodborne pathogens, such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, or *Salmonella* spp., can result in severe illness. Numerous foodborne outbreaks linked to pathogen-contaminated fruit juices have been reported globally. Furthermore, the degradation of untreated juice could lead to changes in its physicochemical properties, such as turbidity, colour and taste, which can adversely affect its overall quality (Salin et al., 2022). The physicochemical changes in fruit juices are primarily driven by enzymatic activity, which accelerates chemical reactions and results in the degradation of organic compounds. One key enzyme involved in this process is polyphenol oxidase (PPO), which is responsible for the browning of juices and unfavourably impacting their colour. These challenges underscore the necessity of implementing effective preservation methods to extend the shelf life of RMJ, ensuring microbial safety, and maintaining quality for consumer acceptance.

The quality and safety of fruit juices, including RMJ, are key concerns for consumers and the food industry. Thermal pasteurization is a common preservation technique used in the food industry. Pasteurization is a heat treatment to kill pathogens and reduce the number of spoilage microorganisms (Amine et al., 2023). The United States Food and Drug Administration (USFDA) implies a 5-log reduction of the most resistant microorganisms of public health concern that may occur in juices (United States Food and Drug Administration [USFDA], 2004). This will extend the juice shelf life due to reduced spoilage organisms. This method also helps inactivate enzyme activity that could lead to undesirable sensory and nutritive changes and affect the juice viscosity and off-flavour formation (Ongaratto & Viotto, 2016; Taranto et al., 2017).

However, thermal pasteurization comes with inherent drawbacks to the fruit juice. One primary concern is its effect on the colour, taste, and flavour of the final product, the consequence of pigment and flavour compound degradation during heat treatment. The heat treatment also leads to the degradation of bioactive compounds, including carotenoids and phenolic compounds (Ferreira et al., 2022). Therefore, understanding the changes in the physicochemical properties of RMJ is crucial for both the industry and consumers. Several pasteurization techniques are widely used in the food industry, such as high-temperature-short-time (HTST), mild-temperature-short-time (MTST), and low-temperature-long-time (LTLT).

It is also necessary to optimize the pasteurization temperature and duration to achieve optimal conditions that minimize quality changes in the juice (Mandha et al., 2023). For example, Vegara et al. (2013) suggested that the combination of low temperature and short pasteurization time (65°C for 30 s) of pomegranate juice can effectively inactivate microorganisms with minimal changes in their quality. Moreover, the storage duration of juice also influences the quality of juice, such as colour degradation due to residual enzyme activity during the thermal process and microbial stability due to microbial growth over time, which can compromise the product's safety. Marie-Michel et al. (2020) and Queirós et al. (2015) demonstrated that pasteurization conditions at 70°C for 30 s and 82°C for 20 s sufficiently eliminate microorganisms in cherry and watermelon juice during refrigeration storage for several weeks. Besides, the industrial thermal pasteurization conditions for premium juices are usually set at 71.1°C for 30 s, 90°C for 2 s, or 84°C for 20 s (Queirós et al., 2015). However, the effects of pasteurization on the physicochemical and microbiological properties of RMJ have not been extensively studied, leaving a gap in understanding how these treatments influence its storage stability as well as physicochemical and microbiological quality. Hence, the present study aims to evaluate the effect of MTST (70°C for 30 s) and HTST (85°C for 20 s) pasteurization on the physicochemical and microbiological quality of RMJ during storage at 4°C.

MATERIALS AND METHODS

Preparation of Rock Melon Juice (RMJ)

Ripe rock melon fruits were purchased from AEON Queensbay Mall Pulau Pinang, Malaysia, and immediately transported to the food processing laboratory at Universiti Sains Malaysia. The preparation of RMJ was carried out in several stages. Rock melon fruits were washed thoroughly under running water to remove any dirt or contaminants from the surface. Then, the rock melon fruits were cut in half crosswise, and the seeds were removed and discarded. The skin covering the fruits was slid off using a kitchen knife, leaving the orange-coloured flesh. The halved melons were cut into smaller pieces and transferred into a fruit juice extractor. The juice was collected, and the fruit pulp was discarded. No preservatives, sugar, or water were added to prepare a healthy juice.

Pasteurization of RMJ

In this experiment, two different pasteurization conditions were used: MTST (70°C for 30 s) and HTST (85°C for 20 s). In a warm water bath, RMJ was pasteurized in sterilized beakers covered with aluminium foil. The temperature of the juice at the centre of the tube was regularly monitored using a thermometer. The treatment time was measured after the juice samples reached the target temperature. Then, the RMJ was quickly cooled in an ice-water bath. The control was unpasteurized RMJ. The samples were then stored in 100 mL polyethylene terephthalate (PET) bottles, and the bottles were tightly closed with screw caps. The following terms were used to describe the different treatments in this study: control (freshly squeezed juice), MTST (pasteurization at 70°C for 30 s), and HTST (pasteurization at 85°C for 20 s). All treatments and analyses were conducted in duplicate. The microbiological and physicochemical parameters of the RMJs were analysed on the 1st, 7th, 14th, and 21st days of storage at 4°C.

Physicochemical Analysis

Determination of pH

The pH of RMJ was determined using a pH meter (Mettler Toledo Delta 320, China) at room temperature. The unpasteurized and pasteurized RMJ samples were placed in separate beakers, and the pH meter probe was inserted into the samples.

Determination of Titratable Acidity (TA)

The TA of RMJ was expressed as citric acid content per unit volume (Marie-Michel et al., 2020). An amount of 5 mL RMJ sample was added to 50 mL of distilled water. The TA was determined by volumetric neutralization with 0.1 M sodium hydroxide solution, and phenolphthalein was used as the indicator. The acidity in the sample was obtained by considering the equivalent weight of citric acid, which is equal to 64.04 g/mol, according to the following formula [Equation 1] :

$$TA(\%) = \frac{V_{NaOH} \times N_{NaOH} \times 64.04}{V_{RMJ} \times 1000} \times 100 \qquad [1]$$

Determination of Total Soluble Solid (TSS). TSS was determined by dropping a few drops of RMJ on the prism of a Labart-90 copper refractometer with automatic temperature compensation (ATC, China) in terms of °Brix.

Colour Measurement. The colour measurement of the RMJ sample was determined using the Konica Minolta Spectrophotometer CM-3500d (Japan). The colour values were expressed as L* (whiteness or brightness/darkness), a* (redness/greenness), and b* (yellowness/blueness) at any time. The colour parameters were read thrice for each sample. Hue angle (h°), chroma (C*), and total colour difference (ΔE) were calculated from L*, a*, and b* values, using the formulas below [Equation 2-4]:

$$h° = tan^{-1}\frac{b^*}{a^*} \quad [2]$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad [3]$$

$$\Delta E^* = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2} \quad [4]$$

Microbiological Analysis

A volume of 1 mL RMJ sample was taken aseptically and transferred to 9 mL buffered peptone water (BPW) in a sterile dilution bottle. The solution was mixed thoroughly to create a 10^{-1} dilution. Then, 1 mL solution from the 10^{-1} dilution bottle was mixed with 9 mL BPW in a new sterile dilution bottle. This created a 10^{-2} dilution. The processes were repeated to create dilution factors of 10^{-3}, 10^{-4}, and 10^{-5}. An amount of 0.1 mL aliquots of each dilution was dispensed on plate count agar (PCA) and dichloran rose bengal chloramphenicol (DRBC) agar using the standard spread plate method. PCA was used to enumerate the total plate count, while DRBC agar was used for yeast and mold. The agar plates were incubated at a specific temperature: PCA was incubated at 37°C for 48 hours, and DRBC agar was incubated at 30°C for 7 days. After incubation, the visible colonies on each plate were counted. Counts of visible colonies were expressed as colony forming units per mL (CFU/mL) of sample and calculated using the formula below (Equation 5) :

$$CFU/mL = \frac{Number\ of\ colonies\ per\ plate\ \times\ dilution\ factor}{Volume\ of\ sample\ plated\ (mL)} \quad [5]$$

Statistical Analysis

Data obtained were subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS) 28.0 software (SPSS Inc., IBM). In this study, data were represented as mean values ± standard deviation (SD; $n = 2$). The significant differences between mean values of juice samples were determined by analysis of variance (one-way ANOVA) using Tukey's HSD (Honestly Significant Difference) test at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Effect of Different Pasteurization Temperatures on the Physicochemical Quality of RMJ

The changes in pH, TA, and TSS in pasteurized and unpasteurized RMJ samples were examined over a 21-day storage period, as shown in Table 1. No significant difference was observed between the pasteurized and unpasteurized RMJ in terms of pH, TA, and TSS. This suggests that the thermal process did not significantly affect these parameters. Nevertheless, a significant difference in pH and TA values between unpasteurized and pasteurized samples was shown on days 14 and 21 of storage.

Table 1
Physicochemical quality of the pasteurized and unpasteurized RMJ samples

Parameter	Treatment	Storage period (day)			
		1	7	14	21
pH	Control	6.80 ± 0.04^{Aa}	6.90 ± 0.05^{Aa}	5.61 ± 0.28^{Ba}	5.20 ± 0.04^{Ba}
	MTST	6.73 ± 0.01^{Aa}	6.88 ± 0.11^{Aa}	6.92 ± 0.16^{Ab}	6.95 ± 0.06^{Ab}
	HTST	6.71 ± 0.02^{Aa}	6.97 ± 0.23^{ABa}	7.25 ± 0.04^{ABb}	7.37 ± 0.16^{Bb}
TA (%)	Control	0.12 ± 0.01^{Aa}	0.09 ± 0.00^{Aa}	0.21 ± 0.01^{Aa}	0.39 ± 0.08^{Ba}
	MTST	0.10 ± 0.00^{Aa}	0.10 ± 0.0^{Aa}	0.11 ± 0.01^{Ab}	0.10 ± 0.03^{Ab}
	HTST	0.10 ± 0.01^{Aa}	0.09 ± 0.02^{Aa}	0.10 ± 0.01^{Ab}	0.11 ± 0.01^{Ab}
TSS (°Brix)	Control	8.00 ± 0.00^{ABa}	7.75 ± 0.35^{Aa}	7.75 ± 0.35^{Aa}	8.25 ± 0.35^{ABa}
	MTST	7.75 ± 0.35^{Aa}	8.25 ± 0.35^{Aa}	7.50 ± 0.71^{Aa}	8.00 ± 0.00^{Aa}
	HTST	7.75 ± 0.35^{Aa}	8.25 ± 1.06^{Aa}	8.50 ± 0.71^{Aa}	7.75 ± 0.35^{Aa}
TSS/TA ratio	Control	67.14 ± 7.91^{Aa}	86.11 ± 3.93^{Aa}	37.05 ± 4.18^{Ba}	21.57 ± 3.78^{Ba}
	MTST	77.50 ± 3.53^{Aa}	82.50 ± 3.53^{Aa}	68.34 ± 2.35^{Ab}	83.33 ± 23.57^{Aa}
	HTST	81.95 ± 9.82^{Aa}	98.57 ± 12.12^{Aa}	89.45 ± 0.78^{Ac}	71.25 ± 12.37^{Aa}

Note: Results are expressed as means ± standard deviations from a duplicate samples. Means values with the same superscript uppercase letter within the same row indicate no significant difference by storage time ($p > 0.05$). Mean values with the same superscript lowercase letter within the same column indicate no significant difference by processing method ($p > 0.05$). Control (unpasteurized RMJ); MTST (pasteurization at 70°C for 30 s); HTST (pasteurization at 85°C for 20 s). Abbreviations: TSS, total soluble solid; TA, titratable acidity

pH

The pH of pasteurized samples increased towards neutral, while the unpasteurized sample decreased and became more acidic by day 14. The pH of unpasteurized RMJ decreased significantly from 6.80 to 5.20 after cold storage, which was similar to some studies (Ferreira et al., 2022; Lagnika et al., 2017; Techakanon & Sirimuangmoon, 2020). The decreased pH could be due to the lactic acid production by the lactic acid bacteria, which also reflected the increase of bacterial counts in the unpasteurized sample during storage.

Another reason for the decrease in pH is the dissociation of citric acid from the fruits (Kong et al., 2020). A slight increase in pH was found in MTST and HTST samples after 21 days. The increased pH trend was also observed in sweet cherry juice, as reported by Queirós et al. (2015). The increase in pH was attributed to the acid hydrolysis of some polysaccharides into disaccharides like starch into sucrose, fructose, and glucose, which is believed to have altered the taste of fruit juice by making it sweeter and less sour (Rehman et al., 2014). There were no significant changes in pH between the MTST and HTST samples, which aligns with the findings by Kong et al. (2020) and Vegara et al. (2013). This stability might be due to the pasteurization temperatures in MTST and HTST that were not high enough to cause significant degradation of citric acid, which has a decomposition temperature of 165°C (Tsioptsias et al., 2024). Additionally, the buffering capacity of RMJ may have contributed to maintaining its pH despite thermal treatment.

TA

The TA of unpasteurized RMJ significantly increased after 21 days as the pH decreased. The TA of unpasteurized RMJ significantly increased after 21 days, corresponding with a decrease in pH. A significant difference in TA was observed between pasteurized and unpasteurized RMJ during days 14 to 21 of storage, paralleling the changes in pH. The increase in TA for the unpasteurized RMJ can be attributed to microbial activity, particularly the fermentation of sugars by naturally occurring microorganisms, leading to the production of organic acids such as lactic and acetic acids. The accumulation of these acids contributes to higher TA values and a lower pH. In contrast, pasteurization inactivates most spoilage microbes, slowing acid production and preserving juice stability over time (Ağçam et al., 2018; Mandha et al., 2023).

TSS

No significant changes in TSS were observed in both pasteurized samples, while a slight decrease in TSS was recorded in the unpasteurized sample. This is because intense pasteurization condition effectively inhibits yeast growth, preventing further changes (Techakanon & Sirimuangmoon, 2020). In pasteurized RMJ, TSS is expected to remain relatively constant since microbial activity is inhibited, preventing sugar breakdown. However, in unpasteurized RMJ, TSS may decrease due to microbial fermentation, where sugars are metabolized into organic acids and other byproducts.

TSS/TA Ratio

The increase of TA in control samples over time resulted in a decrease in the TSS/TA ratio, from 67.14 to 21.57. The decrease in TSS/TA ratio influenced the flavour profile of RMJ as the acidity became stronger (Roongruangsri et al., 2013). The MTST sample was predicted to be slightly sweeter than the HTST on day 21 because the TSS/TA ratio in MTST was higher.

The TSS, TA, and pH of unpasteurized RMJ were 8.00 °Brix, 0.122%, and 6.80, respectively. The TSS of unpasteurized RMJ was similar to many juice products, which has the range between 5 to 16 °Brix (Babarinde et al., 2019; Basak et al., 2022; Kasim & Kasim, 2014; Mandha et al., 2023). The variation depends on the fruit variety, growth condition, maturity, and climate (Lazano, 2006). TSS in the RMJ mainly contains sugars, acids, and other nutritional compounds, such as vitamins and minerals. The pH value of unpasteurized RMJ was typically higher than other fruits, such as orange juice (pH 2.90; Azzouzi et al., 2018), pineapple juice (pH 4.10; Lagnika et al., 2017), sweet cherry juice (pH 3.68; Queirós et al., 2015), mango juice (pH 4.83; Babarinde et al., 2019), and passion fruit (pH 3.39; Kaddumukasa et al., 2017). The finding indicates that RMJ is generally sweeter and less tart than other fruit juices.

According to the Codex Alimentarius, the minimum °Brix value of melon juice is 8.0 °Brix. In this study, the TA of RMJ ranged from 0.09% to 0.39%, while TSS was between 7.50 to 8.50 °Brix. The slightly lower value of TSS could be due to the absence of additives such as sugars to increase the solid content in the juice. Therefore, the incorporation of additives to enhance solid content may be explored to meet the specified criteria and to ensure compliance with established quality benchmarks for fruit juice products.

Colour

Table 2 shows the colour changes in pasteurized and unpasteurized RMJ during storage. The value L* represents the lightness, a* depicts the redness, and b* indicates the yellowness of the juice. The RMJ juice is a light-coloured juice, i.e., dominant with a yellow-orange colour.

All colour components of pasteurized RMJ significantly change compared to the unpasteurized RMJ, indicating that the thermal process can significantly affect the colour of juice. Pasteurized RMJ had higher L* values but lower a* and b* values than unpasteurized RMJ, which resulted in a lighter colour and the loss of the red and yellow colours. Lee and Coates (1999) also reported a similar observation of slight increases in L* value after pasteurization in grapefruit juices, which was attributed to partial precipitation of unstable and suspended particles in the juices. Carotenoids are the pigments in rock melon responsible for the orange colour of the flesh. However, carotenoids are unstable when exposed to heat and light; hence, degradation occurs during thermal pasteurization, leading to the reduction of the red and yellow colours (Sharma et al., 2008). The isomerization of carotenoids at high temperatures also contributed to the colour changes (Santhirasegaram et al., 2013).

Table 2
Colour changes of pasteurized and unpasteurized RMJ samples

Colour components	Treatment	Storage period (day)			
		1	7	14	21
L*	Control	68.16 ± 0.23^{Aa}	65.19 ± 0.05^{Ba}	59.98 ± 0.83^{Ca}	57.23 ± 0.09^{Da}
	MTST	69.50 ± 0.13^{ABb}	68.54 ± 1.09^{Ab}	68.97 ± 2.31^{Ab}	74.73 ± 0.38^{Bb}
	HTST	69.70 ± 0.06^{Ab}	70.17 ± 0.83^{Ab}	70.75 ± 1.66^{Ab}	73.27 ± 0.08^{Ac}
a*	Control	20.15 ± 0.37^{Aa}	19.34 ± 0.14^{Aa}	19.03 ± 0.30^{Aa}	19.40 ± 0.30^{Aa}
	MTST	16.84 ± 0.10^{Ab}	17.08 ± 0.30^{Ab}	15.52 ± 1.33^{Aab}	10.33 ± 0.32^{Bb}
	HTST	15.87 ± 0.82^{Ab}	14.79 ± 0.16^{ABc}	13.62 ± 1.41^{ABa}	11.68 ± 0.02^{Bc}
b*	Control	51.14 ± 0.69^{Aa}	54.72 ± 0.03^{Aa}	54.96 ± 0.53^{Aa}	53.24 ± 2.65^{Aa}
	MTST	46.67 ± 0.01^{Ab}	46.96 ± 0.29^{Ab}	41.06 ± 1.61^{Bb}	32.31 ± 0.8^{Cb}
	HTST	46.96 ± 0.02^{Ab}	48.96 ± 0.81^{Ab}	49.06 ± 1.09^{Ac}	48.12 ± 0.13^{Aa}
Hue angle (°)	Control	68.50 ± 0.10^{Aa}	70.53 ± 0.12^{Aa}	70.91 ± 0.11^{Aab}	69.96 ± 1.20^{Aa}
	MTST	70.16 ± 0.11^{ABab}	70.01 ± 0.21^{ABa}	69.32 ± 0.88^{Aa}	72.27 ± 0.93^{Ba}
	HTST	71.33 ± 0.91^{Ab}	73.20 ± 0.09^{Ab}	74.51 ± 1.20^{Bb}	76.36 ± 0.01^{Bb}
Chroma	Control	54.97 ± 0.78^{Aa}	58.04 ± 0.07^{Aa}	58.16 ± 0.60^{Aa}	56.67 ± 2.38^{Aa}
	MTST	49.61 ± 0.03^{Ab}	49.96 ± 0.35^{Ab}	43.90 ± 1.98^{Bb}	33.93 ± 0.67^{Cb}
	HTST	49.57 ± 0.24^{Ab}	51.14 ± 0.82^{Ab}	50.92 ± 1.43^{Ac}	49.52 ± 0.13^{Ac}
ΔE	Control	-	4.75 ± 0.63^{Aa}	9.10 ± 0.48^{Ba}	11.24 ± 0.28^{Ca}
	MTST	5.73 ± 0.73^{Aa}	5.28 ± 1.24^{Aa}	11.22 ± 1.36^{Ba}	22.24 ± 039^{Cb}
	HTST	6.18 ± 1.36^{Aa}	6.14 ± 0.06^{Aa}	7.41 ± 2.73^{Aa}	10.35 ± 0.53^{Aa}

Note: Results are expressed as means ± standard deviations from a duplicate sample. Mean values with the same superscript uppercase letter within the same row indicate no significant difference by storage time ($p > 0.05$). Mean values with the same superscript lowercase letter within the same column indicate no significant difference by processing method ($p > 0.05$). Control (unpasteurized RMJ); MTST (pasteurization at 70°C for 30 s); HTST (pasteurization at 85°C for 20 s)

On day 21 of storage, the L* values of MTST continued to increase significantly, from 69.50 to 74.72. MTST sample showed a significant difference from HTST, resulting in a lighter colour on day 21, as shown in Figure 1. The a* and b* values of MTST and HTST also showed a significant difference, in which MTST had a higher reduction of colour pigments than HTST. This situation could be due to the heating duration of MTST (30 s), i.e., longer than HTST (20 s), causing a more severe degradation. The b* value of HTST at day 21 of storage showed no significant changes throughout the storage period and no significant difference with the unpasteurized RMJ. Therefore, HTST resulted in better colour retention compared to MTST.

Meanwhile, significant changes in the colour components during storage were detected in the unpasteurized RMJ. The L* value of unpasteurized RMJ decreased significantly from 68.16 to 57.23 during the storage period, which led to a darker colour. Rabie et al. (2015) also reported a decrease in the L* value of untreated physalis juice. Likewise, Mandha et al. (2023) stated that the colour compounds in unpasteurized RMJ were unstable due to

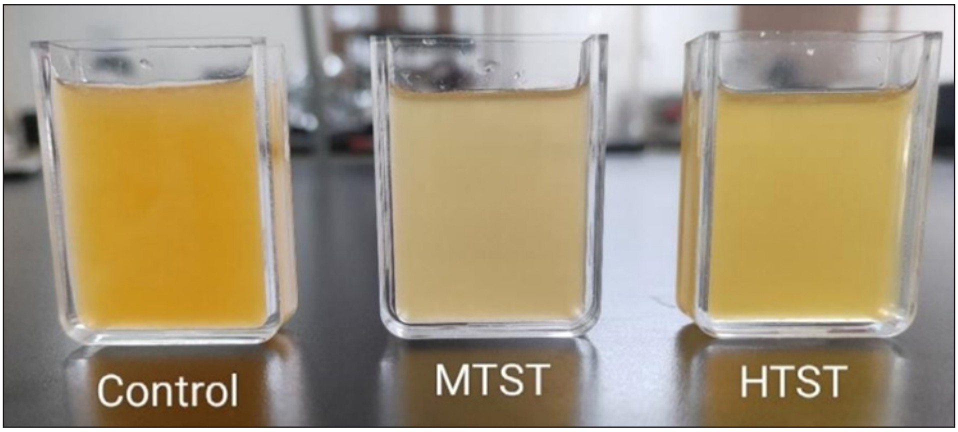

Figure 1. Colour of pasteurized and unpasteurized RMJ samples at day 21 of storage

residual enzymatic activities. However, HTST samples exhibited an increase in L* and b* values during the storage period. This could be due to enzymes like PPO and peroxidase (POD), which are responsible for the inactivation of the browning reactions during the thermal process.

A greater increase in hue angle was found in HTST samples compared to the MTST and control, indicating HTST samples had a slight shift toward a more yellowish appearance. Chroma, which represents colour intensity, maintained relatively higher values in the control samples (54.97 to 56.67), indicating stable saturation. MTST samples exhibited a significant chroma reduction (49.61 to 33.93), suggesting pigment breakdown. HTST samples showed minimal changes in chroma (49.57 to 49.52), demonstrating that shorter treatment time better preserves colour intensity. MTST samples showed the highest colour degradation, with ΔE reaching 22.24 on day 21, indicating significant visible colour loss. HTST samples had the lowest ΔE values, which confirmed superior colour retention compared to MTST.

Effect of Different Pasteurization Temperatures on the Microbiological Quality of RMJ

Table 3 shows the result of microbiological analysis for total plate count and yeasts and mold count in pasteurized and unpasteurized RMJ samples during storage. After pasteurization of RMJ, the MTST and HTST samples resulted in a significant decrease of microbial load for both counts, indicating that the pasteurization process had successfully inactivated the microorganisms to a lower level. This result is congruent with previous studies, which reported the reduction of microbial count in pasteurized juices, with yeast and mold counts below the detection limit (Hu et al., 2020; Kong et al., 2020; Mandha et al., 2023; Queirós et al., 2015). The decrease in microbial load was due to the heat that killed the microorganisms by denaturing their enzymes and disrupting their cell membrane, rendering them unable to survive or reproduce effectively.

There was a gradual increase in the total plate count of unpasteurized RMJ during the 21-day storage period from 3.96 to 8.59 log CFU/mL, which was considered spoiled as the total plate count exceeded 5 log CFU/mL. According to Ma et al. (2020), unpasteurized fruit juice should be consumed within 4 hours of preparation to ensure its safety and quality. The pasteurized samples were found to have a total plate count of below 2.00 log CFU/mL on day 21 of storage, indicating a significant difference from the unpasteurized samples.

Table 3
Microbiological quality of pasteurized and unpasteurized RMJ samples

Colony count (log CFU/mL)	**Treatment**	**Storage period (day)**			
		1	**7**	**14**	**21**
Total plate count	Control	3.96 ± 0.46^{Aa}	6.43 ± 0.04^{Ba}	8.47 ± 0.01^{Ca}	8.59 ± 0.23^{Ca}
	MTST	2.15 ± 0.21^{Ab}	2.69 ± 0.13^{Ab}	3.17 ± 0.45^{Ab}	$< 2.00^{Bb}$
	HTST	2.00 ± 0.00^{Ab}	2.60 ± 0.42^{Ab}	2.80 ± 0.71^{Ab}	2.00 ± 0.00^{Ac}
Yeasts & mold count	Control	3.18 ± 0.25^{Aa}	2.65 ± 0.07^{Aa}	2.15 ± 0.21^{ABa}	$< 2.00^{Ba}$
	MTST	$< 2.00^{Ab}$	$< 2.00^{Ab}$	$< 2.00^{Ab}$	$< 2.00^{Aa}$
	HTST	$< 2.00^{Ab}$	$< 2.00^{Ab}$	$< 2.00^{Ab}$	$< 2.00^{Aa}$

Note: Results are expressed as means ± standard deviations from a duplicate sample. Mean values with the same superscript uppercase letter within the same row indicate no significant difference by storage time ($p > 0.05$). Mean values with the same superscript lowercase letter within the same column indicate no significant difference by processing method ($p > 0.05$). Control (Unpasteurized RMJ); MTST (pasteurization at 70°C for 30 s); HTST (pasteurization at 85°C for 20

On the contrary, the result of yeast and mold counts in the unpasteurized RMJ showed a gradual decrease of microbial load as the storage period increased, from day 1 (3.18 log CFU/mL) to day 21 (below 2.00 log CFU/mL). This result contradicted some studies in which the yeast and mold counts in unpasteurized juice continuously increased during the storage period at 4°C (Hu et al., 2020; Huang et al., 2018; Juliet et al., 2020; Leneveu-Jenvrin et al., 2020; Mandha et al., 2023; Queirós et al., 2015; Techakanon & Sirimuangmoon, 2020; Yildiz et al., 2021). Since the growth condition of yeast and mold is between 10°C to 35°C, the decrease in yeast and mold counts might be due to the long refrigeration storage condition (4°C), which slows down and prevents their growth. The yeast and mold counts of pasteurized samples was less than 2.00 log CFU/mL and remained unchanged for the 21 days of storage.

The shelf life of the pasteurized RMJ was expected to be longer than 21 days. Santhirasegaram et al. (2015) also found that fruit juice undergoing thermal pasteurization has a shelf life exceeding 5 weeks with almost no microbial growth. Commercial fruit juice products typically have a shelf life of several weeks, depending on their packaging, concentration, and storage conditions (Ashurst, 2016). If the quality of RMJ samples in this study were observed for a longer period, HTST might have a longer shelf life than MTST.

CONCLUSIONS

In conclusion, thermal pasteurization and storage significantly affected the physicochemical and microbiological qualities of RMJ, except for the TSS, TA, and pH. All the physicochemical and microbiological parameters of unpasteurized RMJ showed a significant difference during the 21 days of cold storage. The TSS and TA significantly increased, while the pH decreased to become more acidic. The colour of the juice became darker. The total plate count increased while yeast and mold counts decreased with the storage period. There were significant differences between the colour and pH of the HTST (85°C for 20 s) and MTST (70°C for 30 s) juices but no significant difference in the microbiological quality. The HTST-treated juice had a significant increase in pH at day 21 of storage, while MTST-treated juice had no significant difference. Furthermore, MTST-treated juice resulted in a lighter colour than HTST. The HTST-treated RMJ was better at retaining the juice quality as it had a minor colour change compared to MTST-treated RMJ. The HTST pasteurization method is recommended for its minimal colour changes and comparable pH stability to MTST.

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