Journal of Food, Nutrition and Diet Science
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Original Research

Effects of ascorbic acid and kelulut honey as antibrowning agent on the quality of minimally processed jackfruit (Artocarpus heterophyllus)

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Abstract:
Minimally processed jackfruit is gaining popularity for its economic importance to the fruit industry, but its high susceptibility to enzymatic browning has been its limitation. This study determines the effects of applying ascorbic acid (AA) and kelulut honey (KH) on the shelf life of minimally processed jackfruit (MPJ) stored at chill and ambient temperature for 12 days. The quality of treated and untreated minimally processed jackfruit was evaluated by weight loss, firmness, colour (L* and b*), ascorbic acid content and browning inhibition. A microbiological analysis was performed to estimate the shelf-life. The MPJs dipped in 3% ascorbic acid and 3% kelulut honey and stored at 4°C showed the least weight loss of 0.75% and 0.42%, respectively, and the significantly least reduction in firmness of 67.93% and 65.24%, respectively. Ascorbic acid was found to be more efficient in reducing the discolouration of MPJ compared to kelulut honey. Depending on the temperature, the 3% KH dipping solution is more efficient than ascorbic acid in inhibiting the browning activity of MPJ with an inhibition of 9.29%, retaining ascorbic acid content (16.45 mg/100 g) and inhibiting microbial growth (6.20 log CFU/g) of MPJ up to 9 days of storage.

Keywords: Jackfruit, Anti-browning agent, Stingless bee kelulut honey, Ascorbic acid, Storage

Introduction

Jackfruit, also known as Artocarpus heterophyllus, is a nonseasonal fruit that commonly thrives in warm and humid regions with the highest yield production in June and December [1-2]. Originated from India, jackfruit has been widely distributed to other countries. India, Bangladesh, Myanmar, Thailand, Vietnam, China, the Philippines, Indonesia, Malaysia and Sri Lanka are the countries with the highest jackfruit production [3]. The growing cultivation of jackfruit is mainly driven by the fruit's value-added properties that can be processed into products such as pickles, wine, chips, jam and dehydrated snacks [4]. In addition to the fruit, other parts of the tree also play a useful role. Multi-purpose jackfruit tree can provide food, timber, medicines, animal feed, and industrial products [5-7]. The unripe jackfruit is also considered as a potential vegetarian meat, as its texture is very similar to that of chicken meat [8]. In South and Southeast Asia, the locals consume jackfruit as their staple food to replace wheat, corn, and rice, which are easily affected by climatic changes [9]. Despite the versatility

Received: Oct.23, 2023; Revised: Nov.14, 2023; Accepted: Nov.15, 2023; Published: Nov.28, 2023

DOI: https://doi.org/10.55976//fnds.12023122144-56

of jackfruit and its high nutritional value, its market value has been stagnated for years due to insufficient government funding and lack of training in handling the fruit. Some proper methods should be proposed to solve these problems, since fruit production is expected to be in high demand worldwide in the coming years [10].

Jackfruit tree is easy to grow and can withstand climate change to produce high yields. However, this does not guarantee the fruit's marketability. Inappropriate handling of the highly perishable jackfruit subsequently leads to fruit dumping problem. The Food and Agricultural Organization of the United Nations reported that 75% of jackfruit production in India is wasted [11]. Previous report mentioned that consumers' preference for jackfruit depends significantly on the fruit's texture, sweetness and aroma. Approximately 82% of consumer acceptance is based on the jackfruit taste. Food manufacturers are working to produce a fresh, safe, ready-to- eat and highly nutritious food to meet the current consumer trend. It is common practise to sell fresh jackfruit in the markets as a whole fruit or separate them in fruitlets packed in polyethylene bags. Transporting a whole jackfruit is relatively a difficult process, mainly because of its irregular shape and size, which can weigh up to 45 or 50kg [12-14]. Selling of whole jackfruit seems to be uneconomical since only 30% to 35% of the whole fruit is edible [15]. Jackfruit cutting is labour intensive, which is inconvenient for urban consumers. Minimal processing serves as an alternative method for food preservation because of sensory, quality and nutritional aspects. Separating the jackfruit fruitlets from the whole fruit for transportation can lead to tissue wounding, which alters the physical properties of the fruitlets. Limitations of minimally processed fruit include microbial decay, shrivelling, colour changes or browning, texture changes, and the development of off-flavour [16]. Browning can be prevented by dipping the freshly cut fruits in an aqueous solution containing anti-browning agents [17]. Ascorbic acid is commonly used in freshly cut fruit to reduce the growth of microorganisms and to decrease the activity of polyphenol oxidase [18]. However, current studies are more interested in using natural anti-browning agents in freshly cut fruits to compare and balance the temporary browning inhibition of synthetic anti- browning agents [19]. Kelulut honey is a natural anti-browning agent with distinct and unique phenolic and flavonoid compounds that are responsible in antioxidant, anti-inflammatory and antibacterial antioxidant activities [20]. A study stated that kelulut honey was effective in inhibiting the browning reaction of Kajari melon [21].

Minimally processed jackfruit (MPJ) is gaining popularity for its economic importance to the fruit industry, but its high susceptibility to enzymatic browning has been its limitation. This study determines the effects of applying ascorbic acid (AA) and kelulut honey (KH) from stingless bees on the shelf life of minimally processed jackfruit stored at chill and ambient temperature for 12 days.

Materials and methods

Materials

The Jackfruit used in this study was cultivar J33, purchased from Syarikat Perniagaan NG in Kuala Lumpur, Malaysia. It was chosen based on its appearance as described by Saxena [22], which has a brownish yellow skin colour without physical blemishes or deformities. The Kelulut honey was obtained from Malaysian Agriculture Research and Development Institute, Serdang, Malaysia. The honey was produced by Malaysian stingless bees (Heterotrigona itama), which has a dark amber colour with a pH of 3.6. The ascorbic acid was supplied by Fisher Scientific, USA.

Preparation of KH dipping solution

The 1% KH dipping solution was prepared by mixing 10 ml of KH with 990 ml distilled water. Three different concentrations of KH dipping solution were prepared (1, 2 and 3) % and labelled as 1%KH, 2%KH and 3%KH, respectively [21].

Preparation of AA dipping solution

The 1% AA dipping solution was prepared by mixing 10 g of ascorbic acid with 990 ml distilled water. Three different concentrations of AA dipping solutions were prepared (1, 2 and 3) % and labelled as 1%AA, 2%AA and 3%AA, respectively [23].

Preparation of MPJ

The MPJ were cut manually along the axis using sharp-edged stainless-steel knives and the bulbs were removed from rind by hand. The fleshy pericarps of bulbs were cut in half and the seeds were removed. The half-cut fleshy pericarps of bulbs were washed with distilled water and divided evenly before further analysis.

MPJ bulbs were completely immersed in each type of dipping solution (1%K1, 1%K2, 1%K3, 1%A1, 1%A2 and 1%A3) for 15 min at ambient temperature, with a fruit to dipping solution ratio of 1:10 (weight: volume). MPJ immersed in 1000mL distilled water served as a control. The immersion time was fixed at 15 minutes, as it is the minimum time that provides the highest strength value (12.76 g) [24]. The dipped MPJ bulbs were drained with a sieve and stored in a cooler (Protech, Malaysia) at a temperature of 4±1˚C and in a humidity chamber (JIM, United Kingdom) for storage study at ambient temperature (25˚C; 70-80% RH value). The control and treated MPJ were stored in polystyrene containers and periodically analysed for physiological, microbiological and sensory evaluation. The packed MPJ stored at 25˚C was evaluated every day, while the MPJ stored at 4˚C was evaluated every 3 days. The result of each analysis was reported as

the mean of three sample replicates to obtain statistical validity.

Determination of weight loss

Weight loss was determined according to the method described by Chien, Sheu and Yang [25]. The weight loss (%) of MPJ was calculated using Equation 1.

$$\text{Weight loss} = [(W1 - W2)/ W1] \times 100\% \quad (1)$$

where;
W1 = Sample weight before storage
W2 = Sample weight after storage

Determination of firmness reduction

The reduction in MPJ firmness was determined according to the method described by Xu [26]. The MPJ firmness reduction was analysed using a texture analyser (Stable Micro System, United Kingdom). The firmness reduction (%) was calculated using Equation 2.

$$\text{Firmness reduction} = [(F1 - F2)/F1] \times 100\% \quad (2)$$

where;
F1 = Firmness value before storage
F2 = Firmness value after storage

Colour evaluation

The colour was evaluated by using Minolta Colourimeter Spectrophotometer (Minolta Co. Ltd., Japan) [27]. The L* value represents sample lightness (0-black to 100-very light), a positive b* value represents yellow and a negative b* value represents blue. Only L* and b* values were analysed, since the fruitlets used have a relatively yellow colour.

Ascorbic acid content

Ten grams of MPJ were weighed, ground and mixed with a 3% metaphosphoric acid (HPO3) solution. The MPJ-HPO3 mixture was titrated against the dye (2, 6-dichlorophenol- indophenol) until a pink colour formed (end point). The dye solution was standardized by titrating with a standard ascorbic acid solution. Standardization of the dye solution was carried out by pipetting 10 ml of ascorbic acid standard solution into a 100 ml flask. The titration was done until a permanent faint pink colour formed. The volume of dye solution required to change the colour of 10 ml of ascorbic acid was recorded [28]. The value of titrate was recorded and calculated using Equation 3 to get the ascorbic acid content. The results were expressed in mg per 100 g of pulp.

$$\text{Ascorbic acid (mg/100g of pulp)} = (ABVT \times 100)/ (VAWS) \quad (3)$$

where;
A = Titre
B = Dye factor
VT = Total volume made up from titration
VA = Aliquot volume
WS = Sample weight taken

Polyphenol oxidase (PPO) inhibition assay

Five grams of MPJ were homogenized with 20 ml of 0.05 M phosphate buffer at pH 6. The supernatant was centrifuged at 4000 rpm for 20 min at 4˚C (Sigma, USA) and transferred into a 50 ml volumetric flask. The supernatant acts as a polyphenol oxidase enzyme and was preserved in an ice bath. The reaction mixture for the PPO assay contained 3.8 ml of 0.05 M phosphate buffer (pH 6), 1.0 ml of 0.2 M catechol solution and 0.2 ml of the supernatant as enzyme solution [29]. The absorbance was measured using a UV-VIS spectrophotometer (Shimadzu, Japan) recorded at 420nm every 5 min until the value remained constant [30]. The enzyme activity of each sample after treatment was expressed as percentage inhibition of enzyme activity [30]. The percentage inhibition of polyphenol oxidase was calculated using Equation 4.

$$\text{Enzyme activity inhibition (\%)} = (\Delta A420 \text{ control} - A420 \text{ treatment}) \times 100 / A420 \text{ control}) \quad (4)$$

where;
Δ = change in A420 between any time during the experiment and the initial time.

Microbiological analysis

The microbiological growth of the MPJ was counted using the total plate count method. Approximately 10 g of the sample treated with the selected treatment concentration was homogenized in 90 ml of sterile peptone water solution. Plating was carried out using the pour plate technique. After serial dilution, 1 ml of the homogenized MPJ was poured into each sterilized petri plates, added with nutrient agar, and left for incubation at 37 ˚C for 48 h. The number of microorganisms was expressed as log CFU g-1 of the sample [63].

Statistical analysis

The results obtained were analysed with one-way analysis of variance (ANOVA) using Minitab16 software.

Results

Weight loss

From Table 1, the weight loss of control MPJ, MPJ dipped in AA at three different concentrations and MPJ dipped in KH at three different concentrations show an increasing pattern throughout the storage days under both storage temperatures. At 25˚C storage, the MPJs dipped with 3% AA and 3% KH show 1.12% and 1.72% of weight loss, respectively, significantly less than the control, for which the weight loss was 3.88% after 3 days of storage. However, at 4 ˚C storage, the MPJs dipped with 3% AA and 3% KH, the weight loss was only 0.75% and 0.42%, respectively, significantly less than the control

(2.79%) even after 12 days of storage. These results indicate a strong influence of storage temperatures and dipping solutions on the weight loss of MPJs. A higher concentration of dipping solution gives a better reduction in weight loss of MPJs for both AA and KH dipping solutions.

Firmness reduction

Table 2 shows the firmness reduction for MPJs stored at different temperatures. The firmness of control MPJ, MPJs dipped in AA at three different concentrations and MPJs dipped in KH at three different concentrations continues to decrease throughout the storage days at both temperatures. Initially, on day 1 of storage at 25˚C, the MPJ dipped with 2% AA and 1% KH both has the least firmness reduction of 2.24% and 3.80%, respectively, which are significantly lower than control MPJ with firmness reduction of 7.25%. However, the firmness of MPJs dipped with KH and AA shows a prominent reduction, subsequently up to day 3 that is significantly higher than the firmness reduction of control MPJ. At chill temperature (4˚C), initially on day 3, MPJ dipped with 3% AA and 2% KH both has the significantly least firmness reduction as compared to that of control MPJ. However, throughout 12 days of storage at 4˚C, MPJ dipped with 3% AA (67.93%) and 3% KH (65.24%) shows the least firmness reduction as compared to other concentrations and significantly less than that of control MPJ (80.00%). In comparison between storage temperatures on day 3, the firmness reduction occurred to all the MPJs stored at 25˚C is more significant as compared to all the MPJs stored at 4˚C. The different storage temperatures and dipping solutions exert significant influence on the increasing or decreasing of firmness reduction in the MPJs.

Colour

Table 3 shows that on day 0 of storage at 25˚C, all of the MPJs dipped with AA and KH of three different concentrations have a darker yellow colour than that of control MPJ. The L* values of the dipped MPJs are significantly lower than that of control MPJ. However, after 3 days of storage, the results show that only the MPJs dipped with 3% AA have the highest L* value and a significantly higher b* value than the control MPJs, which show a lighter and more intense yellow colour than the other MPJs. Only the MPJ dipped with 3% AA shows increasing L* and b* values throughout storage, while the other MPJs show either increased L* value with decreased b* value (MPJs dipped with 1% AA, 1% KH, 3% KH), or decreased in both L* and b* values (control MPJ, MPJs dipped with 2% AA and 2% KH).

On day 0 of storage at 4˚C, all MPJs dipped with AA and KH of three different concentrations have a brighter yellow colour than that of control MPJ, except for the MPJs dipped with 2% and 3% AA, which have a darker, less yellow colour than the control MPJ. However, there is a gradual decrease in both L* and b* values of all MPJs during the 3 days of storage, which is lesser than the decrease in MPJs stored at 25˚C. A significant decrease in the L* and b* values of all MPJs can be observed after 6 days of storage. After 12 days, all MPJs dipped with AA of three different concentrations have significantly higher L* and b* values than that of control MPJ. The dipped MPJs with AA show a brighter yellow colour than that of control MPJ and KH dipped MPJs. The MPJs dipped with KH of all concentrations have significantly lower b* values, which signifies a less yellow colour than that of control MPJ.

From these results, it can be seen that the AA dipping solution is significantly better in enhancing MPJ's yellow colour when stored at 25˚C and is significantly better in reducing discolouring of MPJ's yellow colour when stored at 4˚C. The storage temperature poses a significant influence on the dipping solution's function to enhance or maintain the MPJ's colour.

Table 1. Weight loss of MPJ during storage at ambient and chill temperature

Storage temperature	Storage (days)	Weight loss of MPJ (%)						
		Control	1% AA	2% AA	3% AA	1% KH	2% KH	3% KH
25˚C	0	-	-	-	-	-	-	-
	1	2.63 ± 0.17^{a}	1.76 ± 0.04^{b}	1.24 ± 0.10^{c}	0.76 ± 0.06^{d}	0.23 ± 0.05^{e}	0.11 ± 0.03^{f}	0.06 ± 0.03^{g}
	2	3.49 ± 0.26^{a}	2.32 ± 0.18^{b}	1.68 ± 0.04^{c}	0.52 ± 0.08^{ef}	0.90 ± 0.10^{d}	0.62 ± 0.03^{e}	0.42 ± 0.07^{f}
	3	3.88 ± 0.32^{a}	2.52 ± 0.44^{b}	1.81 ± 0.21^{d}	1.12 ± 0.76^{f}	1.96 ± 0.46^{c}	1.89 ± 0.92^{d}	1.72 ± 0.72^{e}
4 ˚C	0	-	-	-	-	-	-	-
	3	0.61 ± 0.10^{h}	0.60 ± 0.17^{h}	0.38 ± 0.03^{j}	0.14 ± 0.06^{k}	0.43 ± 0.08^{i}	0.38 ± 0.07^{j}	0.17 ± 0.06^{k}
	6	1.20 ± 0.16^{h}	0.74 ± 0.13^{i}	0.61 ± 0.05^{j}	0.37 ± 0.02^{k}	0.74 ± 0.03^{i}	0.69 ± 0.09^{ij}	0.27 ± 0.08^{l}
	9	1.93 ± 0.92^{h}	1.16 ± 0.42^{j}	1.05 ± 0.47^{j}	0.61 ± 0.09^{m}	1.36 ± 0.76^{i}	0.82 ± 0.06^{l}	0.42 ± 0.08^{n}
	12	2.79 ± 0.85^{h}	1.37 ± 0.45^{j}	1.25 ± 0.62^{j}	0.75 ± 0.05^{m}	1.66 ± 0.79^{i}	1.12 ± 0.59^{l}	0.42 ± 0.08^{n}

Values are expressed as mean ± standard deviation (n=3). Means with different letters within the same row are significantly different at the level of $p<0.05$. Notes: AA1- 1% Ascorbic acid; AA2- 2% Ascorbic acid; AA3- 3% Ascorbic acid; KH1- 1% kelulut honey; KH2- 2% kelulut honey; KH3- 3% kelulut honey

Table 2. Firmness reduction of MPJ during storage at ambient and chill temperature

Storage temperature	Storage (days)	Firmness reduction of MPJ (%)						
		Control	1% AA	2% AA	3% AA	1% KH	2% KH	3% KH
25˚C	0	-	-	-	-	-	-	-
	1	7.25±0.21^{d}	7.39±0.23^{c}	2.24±0.38^{g}	8.21±0.14^{b}	3.80±0.18^{f}	12.78±0.09^{a}	6.68±0.15^{e}
	2	13.36±0.21^{d}	7.65±0.20^{e}	5.91±0.22^{f}	17.64±0.24^{c}	59.80±0.17^{a}	13.44±0.21^{d}	24.48±0.10^{b}
	3	13.36±0.21^{d}	47.42±0.13^{d}	44.00±0.47^{f}	45.24±0.20^{e}	60.55±0.32^{a}	55.01±0.18^{b}	31.60±0.36^{g}
4˚C	0	-	-	-	-	-	-	-
	3	12.20±0.06^{j}	12.76±0.19^{i}	8.63±0.11^{k}	6.83±0.34^{l}	18.12±0.12^{h}	1.29±0.20^{n}	5.03±0.16^{m}
	6	21.25±0.13^{j}	24.63±0.17^{i}	13.35±0.35^{m}	13.89±0.23^{l}	32.52±0.10^{h}	7.28±0.10^{n}	18.67±0.29^{k}
	9	33.73±0.32^{j}	33.63±0.32^{j}	23.05±0.23^{l}	29.76±0.33^{k}	43.12±0.08^{i}	49.22±0.23^{h}	33.92±0.25^{j}
	12	80.00±0.26^{h}	71.41±0.24^{j}	71.07±0.25^{j}	67.93±0.35^{l}	69.28±0.19^{k}	72.97±0.31^{i}	65.24±0.29^{m}

Values are expressed as mean ± standard deviation (n=3). Means with different letters within the same row are significantly different at the level of p<0.05. Notes: AA1- 1% Ascorbic acid; AA2- 2% Ascorbic acid; AA3- 3% Ascorbic acid; KH1- 1% kelulut honey; KH2- 2% kelulut honey; KH3- 3% kelulut honey

Table 3. Colour (L* and b* values) of MPJ during storage at ambient and chill temperature

Storage temperature	Colour value	Storage (days)	Colour value						
			Control	1% AA	2% AA	3% AA	1% KH	2% KH	3% KH
25˚C	L*	0	59.63±0.75^{a}	54.39±0.45^{b}	52.23±0.70^{d}	38.68±0.47^{g}	46.96±0.34^{e}	53.61±0.31^{c}	40.09±0.72^{f}
		1	57.57±0.10^{a}	48.77±0.21^{e}	52.52±0.30^{c}	43.59±0.60^{f}	51.41±0.65^{d}	53.71±0.34^{b}	40.81±0.12^{g}
		2	49.72±0.91^{d}	51.60±0.82^{c}	56.80±0.54^{b}	39.35±0.26^{g}	47.81±0.85^{f}	48.99±0.79^{e}	58.66±0.95^{a}
		3	52.24±0.21^{c}	56.16±0.71^{b}	49.57±0.65^{e}	56.88±0.22^{a}	47.3±0.32^{f}	51.17±0.33^{d}	47.23±0.52^{f}
	b*	0	32.14±0.79^{k}	37.02±0.47^{h}	33.06±0.16^{j}	20.46±0.6^{n}	30.32±0.64^{l}	33.49±0.29^{i}	21.63±0.27^{m}
		1	38.00±0.74^{h}	31.62±0.23^{l}	33.73±0.12^{k}	24.63±0.24^{m}	34.59±0.90^{j}	34.82±0.29^{i}	22.33±0.95^{n}
		2	19.61±0.31^{l}	35.29±0.32^{i}	36.57±0.41^{h}	21.00±0.52^{k}	16.82±0.82^{m}	20.98±0.76^{k}	31.15±0.31^{j}
		3	19.84±0.65^{l}	27.32±0.23^{j}	30.76±0.43^{h}	29.58±0.41^{i}	18.21±0.80^{m}	20.48±0.34^{k}	18.15±0.76^{m}
4˚C	L*	0	63.02±0.02^{d}	68.27±0.70^{a}	62.80±0.62^{e}	57.96±0.39^{f}	67.41±0.51^{b}	67.12±0.30^{c}	67.28±0.52^{c}
		3	63.51±0.50^{d}	66.98±0.85^{a}	60.94±0.05^{f}	56.98±0.67^{g}	66.76±0.62^{b}	65.88±0.51^{c}	62.89±0.15^{e}
		6	56.07±0.67^{e}	55.50±0.22^{f}	53.65±0.23^{g}	58.36±0.33^{d}	64.04±0.72^{a}	63.82±0.34^{b}	61.91±0.42^{c}
		9	48.51±0.22^{e}	53.52±0.12^{b}	56.40±0.21^{a}	51.39±0.76^{c}	46.82±0.21^{g}	48.35±0.92^{f}	49.45±0.92^{d}
		12	45.09±0.50^{g}	50.38±0.67^{c}	52.57±0.13^{a}	47.90±0.65^{b}	44.88±0.43^{f}	46.39±0.50^{e}	46.59±0.05^{d}
	b*	0	41.12±0.77^{l}	38.28±0.62^{m}	41.93±0.23^{k}	37.14±0.85^{n}	46.70±0.62^{i}	45.14±0.85^{j}	47.93±0.15^{h}
		3	39.89±0.92^{j}	36.83±0.29^{m}	39.55±0.20^{i}	35.86±0.50^{n}	47.61±0.85^{i}	47.91±0.92^{k}	38.94±0.92^{l}
		6	31.85±0.02^{n}	36.14±0.07^{l}	37.09±0.70^{k}	32.47±0.43^{m}	45.98±0.60^{i}	46.39±0.71^{h}	39.68±0.52^{j}
		9	27.14±0.20^{j}	34.37±0.60^{h}	34.25±0.61^{h}	29.60±0.90^{i}	22.73±0.71^{l}	25.22±0.65^{k}	21.09±0.32^{m}
		12	25.60±0.77^{k}	31.41±0.8^{i}	32.41±0.93^{h}	27.10±0.43^{j}	22.40±0.64^{m}	24.48±0.75^{l}	20.50±0.12^{n}

Values are expressed as mean ± standard deviation (n=3). Means with different letters within the same row are significantly different at the level of p<0.05. Notes: AA1- 1% Ascorbic acid; AA2- 2% Ascorbic acid; AA3- 3% Ascorbic acid; KH1- 1% kelulut honey; KH2- 2% kelulut honey; KH3- 3% kelulut honey

Browning inhibition

Table 4 shows that browning inhibition increased for the MPJ dipped with solution of higher concentration. The 3% AA solution presents a higher browning inhibition than the 1% and 2% AA solution with browning inhibition of 9.54% and 5.40% after 3 days of storage at 25˚C and 4˚C, respectively. In addition, the 3% KH solution causes a higher browning inhibition than 1% and 2% KH solution with browning inhibition of 12.11% and 3.62% after 3 days of storage at 25˚C and 4˚C, respectively. The browning activity of MPJ is significantly reduced by dipping MPJs in AA and KH solutions, as all of the dipped MPJs have significantly higher browning inhibition than the control MPJ at both storage temperatures.

At 25˚C, the browning inhibition fluctuates for certain dipped MPJs, including MPJs dipped with 2% AA, 3% AA, and 2% KH solutions, which have an increased browning inhibition from day 2 to day 3 of storage. The results may be due to the uneven ripening processes occurring on the MPJ's skin. At 4˚C, it can be seen that a significant increase in browning inhibition of all dipped MPJs happened on day 9 of storage with the highest for MPJs dipped with 2% AA and 3% AA solutions, being 19.27% and 35.45%, respectively.

The results also show that the KH solution is more efficient in inhibiting browning activity of MPJ, with MPJs dipped with 3% KH solution has the highest browning inhibition of 12.11% and 9.29% at 25˚C and 4˚C, respectively, by the end of the storage period.

Ascorbic acid content

Table 5 shows that the ascorbic acid content of all

MPJ decreases throughout the storage period at both temperatures. All MPJs dipped with AA and KH solutions at all concentrations have a significantly higher ascorbic acid content than the control MPJ at both temperatures. The presence of AA and KH significantly retained the ascorbic acid content of MPJs from decreasing. A higher concentration of dipping solution retains more ascorbic acid of MPJs, resulting in a less reduction of ascorbic acid content throughout the storage period. The ascorbic acid content of MPJ dipped in a 3% AA solution and MPJ dipped in a 3% KH solution is higher than that of MPJs dipped in 1% AA, 2% AA, 1% KH and 2% KH solutions.

Both at 25°C and 4°C, MPJs dipped in 3% KH solution have the highest ascorbic acid content of 18.03mg/100g and 16.45mg/100g after 3 days and 12 days of storage, respectively. This shows a better retention of ascorbic acid content by KH solution than that of AA solution.

The reduction in the ascorbic acid content of MPJs is significantly affected by the different storage temperatures. The chill temperature significantly decreases the reduction rate of ascorbic acid content in MPJs. The ascorbic acid content of control MPJ stored at 25°C is reduced by 65% from day 1 to day 3 of storage, while the ascorbic acid content of control MPJ stored at 4°C is reduced by only 30% from day 1 to day 3.

Microbiological analysis

Table 6 shows that the microbial load of MPJs general increases towards the end of the storage period at both storage temperatures. All MPJs dipped with AA and KH solutions at all concentrations have a lower microbial load than control MPJ at both storage temperatures, indicating a significant effect of AA and KH solution in inhibiting or reducing microbial growth on MPJ. The microbial growth on MPJ is greatly inhibited when the concentration of dipping solution is higher. At 25°C, the MPJs dipped with 3% AA and 3% KH solutions show the least microbial load after 2 days with 6.87 log CFU/g and 6.35 log CFU/g, respectively. The only MPJs that still have acceptable microbial load after 2 days of storage at ambient temperature include MPJs dipped with 3% AA, 2% KH and 3% KH solutions. The control MPJ shows the fastest microbial growth, with the microbial load exceeding an acceptable limit after only 1 day of storage.

The results show that the chill temperature can significantly reduce the microbial growth in all MPJs. The control MPJ shows an acceptable microbial load up to day 5 of storage at chill temperature, with all the dipped MPJs also showing a significantly lower microbial load than the control. Although both AA and KH solution have a reducing effect on the MPJs microbial growth, the 3% KH solution shows a better effect compared to the AA solution with the lowest microbial load of 6.20 log CFU/g after 9 days of storage.

Table 4. Browning inhibition of MPJ during storage at ambient and chill temperature

Storage temperature	Storage (days)	Browning inhibition (%)						
		Control	1% AA	2% AA	3% AA	1% KH	2% KH	3% KH
25°C	0	-	-	-	-	-	-	-
	1	4.02±0.04g	5.73±0.18e	8.74±0.06d	11.47±0.04b	4.48±0.06f	10.44±0.09c	12.12±0.16a
	2	2.27±0.23g	2.69±0.02f	4.60±0.01e	8.67±0.09b	5.54±0.17d	6.60±0.18c	14.71±0.15a
	3	1.02±0.08g	1.81±0.03f	6.87±0.10d	9.45±0.07c	4.61±0.11e	10.48±0.03b	12.11±0.04a
4°C	0	-	-	-	-	-	-	-
	3	2.06±0.07l	2.16±0.04k	3.54±0.19j	5.40±0.14h	0.81±0.06n	1.72±0.16m	3.62±0.08i
	6	1.03±0.12n	1.09±0.11m	5.12±0.07k	6.64±0.05j	1.11±0.07l	8.24±0.24i	11.65±0.08h
	9	1.69±0.18n	11.82±0.12l	19.27±0.52j	16.91±0.21k	4.63±0.09m	22.55±0.07i	35.45±0.49h
	12	1.24±0.06n	4.63±0.10l	2.51±0.11m	6.76±0.18i	4.87±0.09k	6.53±0.13j	9.29±0.08h

Values are expressed as mean ± standard deviation (n=3). Means with different letters within the same row are significantly different at the level of $p<0.05$. Notes: AA1- 1% Ascorbic acid; AA2- 2% Ascorbic acid; AA3- 3% Ascorbic acid; KH1- 1% kelulut honey; KH2- 2% kelulut honey; KH3- 3% kelulut honey

Table 5. Ascorbic acid content of MPJ during storage at ambient and chill temperature

Storage temperature	Storage (days)	Ascorbic acid (mg/100g)						
		Control	1% AA	2% AA	3% AA	1% KH	2% KH	3% KH
25°C	0	24.82 ± 0.34^{a}	24.22 ± 0.40^{b}	22.117 ± 0.17^{f}	23.14 ± 0.24^{e}	22.02 ± 0.07^{g}	24.14 ± 0.17^{c}	23.81 ± 0.18^{d}
	1	15.77 ± 0.21^{g}	16.65 ± 0.23^{f}	18.42 ± 0.38^{d}	19.81 ± 0.14^{c}	18.25 ± 0.18^{e}	21.04 ± 0.09^{b}	21.86 ± 0.15^{a}
	2	9.23 ± 0.21^{f}	14.99 ± 0.20^{d}	13.92 ± 0.22^{e}	17.03 ± 0.24^{c}	18.04 ± 0.17^{b}	19.77 ± 0.21^{a}	18.44 ± 0.36^{b}
	3	8.68 ± 0.13^{g}	13.82 ± 0.13^{e}	12.05 ± 0.47^{f}	16.54 ± 0.22^{c}	16.22 ± 0.32^{d}	17.21 ± 0.18^{b}	18.03 ± 0.10^{a}
4°C	0	24.82 ± 0.36^{h}	24.22 ± 0.28^{i}	22.17 ± 0.11^{m}	23.14 ± 0.24^{l}	22.02 ± 0.12^{n}	24.14 ± 0.19^{j}	23.81 ± 0.08^{k}
	3	17.45 ± 0.17^{m}	18.68 ± 0.19^{l}	19.80 ± 0.11^{k}	20.03 ± 0.34^{j}	20.87 ± 0.12^{i}	21.02 ± 0.20^{h}	21.17 ± 0.16^{h}
	6	15.85 ± 0.14^{m}	16.76 ± 0.17^{l}	18.59 ± 0.35^{k}	19.27±0.23j	19.76 ± 0.10^{i}	20.45 ± 0.10^{h}	20.41 ± 0.29^{h}
	9	11.95 ± 0.22^{n}	14.05 ± 0.32^{m}	14.5 ± 0.23^{l}	15.04 ± 0.33^{k}	17.61 ± 0.08^{j}	18.22 ± 0.23^{i}	18.57 ± 0.25^{h}
	12	7.88 ± 0.25^{n}	11.22 ± 0.24^{m}	11.79 ± 0.25^{l}	14.98 ± 0.35^{k}	15.82 ± 0.19^{j}	16.34 ± 0.31^{i}	16.45 ± 0.29^{h}

Values are expressed as mean ± standard deviation (n=3). Means with different letters within the same row are significantly different at the level of $p<0.05$. Notes: AA1- 1% Ascorbic acid; AA2- 2% Ascorbic acid; AA3- 3% Ascorbic acid; KH1- 1% kelulut honey; KH2- 2% kelulut honey; KH3- 3% kelulut honey

Table 6. Total microbial count of MPJ during storage at ambient and chill temperature

Storage temperature	Storage (days)	Total microbial count (log CFU/g)						
		Control	1% AA	2% AA	3% AA	1% KH	2% KH	3% KH
25°C	0	1.69	1.55	1.52	1.46	1.53	1.38	1.47
	1	7.08	6.70	6.43	5.14	7.49	6.30	5.13
	2	8.50	7.58	7.10	6.87	7.75	6.43	6.35
4°C	0	1.71	1.53	1.55	1.45	1.51	1.55	1.46
	5	6.46	5.69	5.49	5.60	6.59	5.31	5.19
	9	7.52	6.87	6.61	6.77	7.17	6.32	6.20

Discussions

Weight loss and firmness reduction

The weight and firmness of fruit indicates the fruit's quality and its physiological changes that occurred throughout its storage. The data presented in Table 1 shows that the weight loss increased with storage time in both the control and dipped MPJs, but it was significantly reduced at chill temperature and with dipping solution. At the end of the storage period at 25°C and 4°C, the weight loss was lowest for MPJ dipped with 3% AA and 3% KH solutions, of 1.12% and 0.61%, respectively. The fruit's weight loss during storage is a natural phenomenon caused by the water loss from the fruit, leading to shrivelling and loss of brightness [31]. The respiration and transpiration intensity of the fruit influences the rate of water loss in fruits [32, 31]. A study on strawberries showed a significant correlation between the fruit's weight loss with its respiration and transpiration rate, with a higher respiration or transpiration rate leading to a greater water loss in fruits [33]. The heat generated during the respiration process leads to temperature elevation within the fruit which in turn increases internal water vapor pressures leading to increased transpiration [34]. The application of honey dipping acts as a natural film covering the MPJs skin, thus reducing the water loss by transpiration [35]. A previous study showed that the application of 10% honey to freshly cut papaya had prevented the fruit's weight loss to a greater extent compared to aloe vera gel [36]. In another study, 20% honey was found to be more competent in reducing weight loss of grapes to only 0.39%, significantly less than the control (0.54%) and modified atmosphere packaging (0.41%) [16]. The decrease in weight loss of MPJs dipped with ascorbic acid could be due to the fact that ascorbic acid acts as an antioxidant, slows down the ripening process, and thus subsequently reduce the rate of water loss [37].

Both the control and dipped MPJs show a significantly lower weight loss at the lower temperature. After 3 days of storage, the control MPJ (3.88%), MPJs dipped with 3% AA (1.12%) and 3% KH (1.72%) stored at 25°C showed a significant higher weight loss as compared to the control MPJ (0.61%), MPJs dipped with 3% AA (0.14%) and 3% KH (0.17%) stored at 4°C. A lower temperature causes a comparatively lower physiological loss of fruit weight [38]. The chill condition slows down biological reactions and microbial growth, thus reduces the respiration rate and weight loss [39].

Firmness reduction of MPJ was determined to evaluate the freshness of the minimally processed jackfruits [30]. Table 2 shows that the firmness of MPJ decreased throughout the storage period, indicating a shrivelled condition and moisture loss of MPJs. A similar decreasing trend was seen in the firmness of pear stored at 0°C for 150 days [40]. The MPJs stored at chilled temperature showed a lower ($p<0.05$) firmness reduction compared to the MPJs stored at ambient temperature. The higher firmness reduction of the MPJs stored at 25°C could be caused by the high solubilization and depolymerization of cell wall components including pectin at a higher temperature [25]. It is generally caused by the rapid hydrolysis of starch to sugar and pectin cell wall degradation, which is associated with fruit ripening [41]. A previous study on tomato has shown that the firmness of tomatoes reduced slightly at 2˚C-8˚C and a higher reduction occurred when stored at 16˚C [42]. A drastic decrease in firmness was observed in MPJ on day 3 of storage at ambient temperature and on day 12 of chill storage, that may be caused by the ripening process of climacteric fruits as the endogenous ethylene level increases over time [43].

After 3 days of storage at 25°C, the firmness reduction of control MPJ is lesser than all the MPJs dipped in AA and KH solutions of three different concentrations. However, at 4°C, the firmness decreased at a slower rate for the first 3 days of storage and started to accelerate throughout the subsequent days. At the end of the storage period, all dipped MPJs have significantly less firmness reduction compared to the control MPJ, with MPJ dipped with 3% KH showing the least firmness reduction of 65.24%. This could be related to the existence of honey, which acts as a film to prevent weight loss and maintain the firmness of MPJ. As mentioned by Jeon and Zhao [44], 10% honey coating the freshly cut apples was able to

prevent weight loss due to transpiration and maintain the firmness of the apples.

Colour

Colour is a very important parameter for determining the quality of a fruit and its acceptance by consumers [45]. Table 3 shows that the L* and b* values for MPJs varied significantly ($p<0.05$) during the 3 days of storage at ambient storage. The variation could be caused by the fact that the whole jackfruit has different ripening stages as it ripens from top to bottom [46]. However, only the MPJ dipped in 3% AA (L*=56.88, b*=29.58) shows both higher L* and b* values than the control MPJ (L*=52.24, b*=19.84) after 3 days of storage at 25°C. This may be due to the ascorbic acid acting as a radical scavenger and reducing agent to prevent MPJ from forming a darker or bluish skin colour. Although both AA and KH of all concentration show the capability in minimizing L* and b* values from decreasing, 3% AA shows the best result in maintaining L* and b* values to be significantly higher than the control MPJ.

For MPJ stored at chill temperature, all treated MPJ showed ($p<0.05$) a decrease in L* and b* values throughout the storage period. A significant decrease in L* and b* values of all MPJs is prominent after 6 days of storage. Some moulds grow faster at the end of the storage period, which can cause the significant decrease in L* and b* values, simultaneously with the ripening process [33]. The decrease in the L* value of the control and dipped MPJs indicates the darkening of bulb tissue, which could be attributed to the skin browning of the fruit surface due to the oxidation of polyphenols and the formation of dark pigment [47]. Figure 1-3 shows the colour change of control and dipped MPJs, which can visually indicate the decrease in L* and b* values at day 12 of chilled storage. Based on the visual observation, the control showed a paler yellow colour with bluish hints (L*=45.09, b*=25.60) compared to MPJ dipped in 3% AA (L*=47.90, b*=27.10) and MPJ dipped in 3% KH (L*=46.59, b*=20.50). The low b* value in MPJ dipped in 3% KH could be due to spoilage by moulds, which alter the metabolic activities and pigments of MPJ, subsequently causing a less yellowish colour [33].

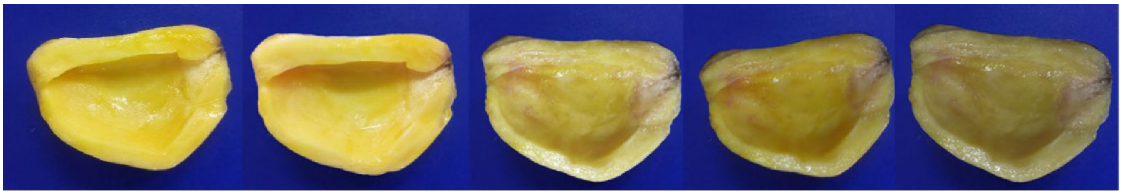

Figure 1. Colour change of control MPJ during chill storage for 0, 3, 6, 9, 12 days, respectively

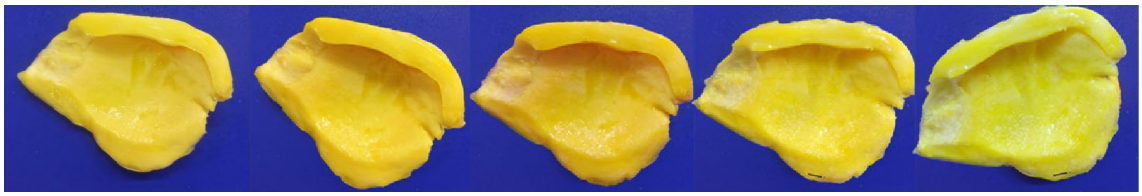

Figure 2. Colour change of 3% AA dipped MPJ during chill storage for 0, 3, 6, 9, 12 days, respectively

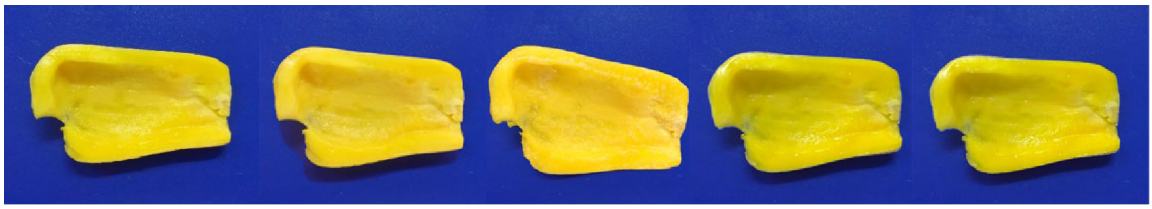

Figure 3. Colour change of 3% KH dipped MPJ during chill storage for 0, 3, 6, 9, 12 days, respectively

Browning inhibition

Table 4 shows the browning inhibition of control and dipped MPJ. It can be observed that the browning inhibition decreases with increasing storage time. Browning inhibition is related to polyphenol oxidase activity, with higher browning inhibition indicating lower polyphenol oxidase activity. Based on the results, browning inhibition increases with increasing concentration of AA and KH solution. A previous study on the effect of different AA concentration on mung bean sprouts showed that 0.2% AA inhibited browning by 2.61%, while 2% AA inhibited browning by 16.60% [48]. Another study on the effect of different honey concentration coating on freshly cut nectarine showed that 50% honey inhibited browning more than 10% honey [22, 49]. Browning inhibition is the highest in MPJ dipped in 3% KH both at ambient and chilled storage. Kelulut honey is known as a potent antioxidant carrier, containing ascorbic acid content, small peptides, flavonoids and other phenols. These compounds have the ability to decrease the activities of polyphenol oxidase [50]. Jeon and Zhao [44] suggested that honey has an inhibitory effect against superoxide anion radicals in the pericarp tissue of litchi fruit, which resulted in the inhibition of the enzymatic browning reaction. Gacche [51] also considered that honey is an inhibitor of polyphenol oxidase activity in apple juice. Ascorbic acid prevents browning by its free radical scavenging mechanism and reducing power [52]. The enzymatic browning of apple slices dipped in ascorbic acid solution was inhibited due to the reduction of quinones by ascorbic acid back into their original polyphenol compounds [53].

On the day 9 of chill storage, the percentage of browning inhibition increased dramatically from 11.65% for MPJ dipped in 3% KH to 35.45%. However, the browning inhibition decreased to 9.29% on day 12 as polyphenol oxidase activity declined with the decrease in browning inhibition. High polyphenol oxidase activity, which related to high browning activity, occurred when the MPJ began to rot, with polyphenol oxidase acting against pathogen [54].

A study conducted on the "Huangguan" pear found that at low temperature, PPO activity decreases alongside with the expression of PPO1 and PPO5 genes, which are positive regulators for browning [55]. A low storage temperature induces less vibration of the enzyme, which reduces the reaction of the enzyme with other molecules [55]. Thus, less enzymatic activity occurs. Simultaneously, a low temperature prevents the degradation of phenolic compounds, which are responsible for antioxidative and anti-browning activities. The presence of KH also increases the amount of ascorbic acid available, which suppresses reactive oxygen species causing the browning [56]. A combination of these factors, which may occur simultaneously, contributes to the slow browning of MPJ stored at low temperature.

At a higher temperature, the enzyme vibrates more vigorously, which leads to an active reaction of the enzyme [55]. Due to the rapid reaction and degradation, fewer antioxidative and anti-browning compounds are able to maintain available throughout the storage [57]. Thus, faster browning was observed in MPJ stored at higher temperature as compared to lower temperature.

Ascorbic acid content

Table 5 shows the results of ascorbic acid content for the control and the dipped MPJs under both storage conditions. A general decreasing trend was observed in the ascorbic acid content of the MPJ throughout the storage period. Ascorbic acid is a sensitive compound in fruits and vegetables and is readily degraded by oxygen, light, heat, enzymes and metals. An overall observation showed that the ascorbic acid content is higher in MPJs dipped in AA and KH of higher concentrations. Ascorbic acid has the ability to produce oxidative stress, which eventually maintains the ascorbic acid content [48]. A study by Yousuf and Srivastava [21] found that 15% of honey can retain 56 mg of AA/100g, while 10% honey can retain 30 mg of AA/100g.

The lowest ascorbic acid content (8.68 mg/100g and 7.88 mg/100g) throughout the storage was found in the control MPJ both at ambient and chill storage. The higher ascorbic acid loss in storage at ambient temperature could be greatly favoured by the existence of higher level of oxygen diffusion compared to chilled MPJ. The presence of oxygen promotes the AA oxidation to dehydroascorbic acid, which is later converted into diketogulonic acid [58].

At the end of storage, the highest values (18.44 mg/100g and 16.45mg/100g) of ascorbic acid were found in the MPJ dipped in 3% KH. Similar results were reported by Yousuf and Srivastava [21], where honey treatment retains the highest value of ascorbic acid on freshly cut Kajari melon after storage at 4˚C. The application of honey as coating serves as a protective layer that controls the permeability of oxygen and carbon dioxide and decreases the ascorbic acid autoxidation. Therefore, ascorbic acid content is maintained longer, and the respiration rate of coated fruit was reduced [59].

Fruits undergo browning during postharvest storage due to the process of cellular respiration and photosynthesis [60]. These processes produce by-products called reactive oxygen species, which contributes to the browning of plant tissue [56]. KH acts as an outsource antioxidant supply, which helps in the antioxidant system of the MPJ cells, to enhance the redox buffer regulation and eliminate the reactive oxygen species [61].

A study reported that the honey of stingless bees consists of various phenolic compounds including flavonoids (naringenin, aromadendrin, taxifolin, isoquercetin, scopoletin, quercetin, eriodyctiol), umbelliferone, carnosol and syringaldehyde [62].

Microbiological analysis

Table 6 presents the results of the microbiological analysis of the control and dipped MPJs at ambient and chilled temperature. The total number of microbial counts

on the control MPJ increased from 1.69 to 7.08 and 8.50 log CFU/g at the end of the ambient storage, meanwhile it increased from 1.71 to 7.52 log CFU/g at the end of chilled storage. The dipped MPJs also showed a increase in total microbial counts with the increasing of storage time. A previous study also showed the same pattern of result with a increase in microbial count upon storage of chemically treated and freshly cut papaya [63].

A higher concentration of AA and KH on the MPJ effectively inhibited the growth of microorganisms. The lowest total microbial count was found in MPJ dipped in 3% KH both in storage at ambient and chilled temperature. Kelulut honey has several characteristics related to antimicrobial activity, which include lower food pH, an osmotic effect, hydrogen peroxide production and phytochemical factor [64]. Since the maximum limit for total plate count is log 7 CFU/g, in storage at ambient temperature, MPJs dipped in 3% AA, 2% KH and 3% KH lasted for two days for safe consumption, while in storage at chilled temperature, the other MPJs (except control) lasted for nine days for safe consumption [65].

Conclusion

Applied as anti-browning agents in this study, the ascorbic acid and kelulut honey have significantly affected the quality of MPJ throughout the fruit's storage period. The MPJ dipped in 3% AA and 3% KH show the least weight loss and firmness reduction, which are significantly lower than that of control MPJ. The rate of weight loss and firmness reduction is significantly dependent on temperature. Both ascorbic acid and kelulut honey can reduce discolouration in the MPJ, however, the ascorbic acid is better in retaining the bright yellow colour of MPJ as compared to kelulut honey. The MPJ dipped in 3% KH shows the highest inhibition of browning activity, a better retention of ascorbic acid content and the least microbial load with 6.20 log CFU/g even after 9 days of storage. Kelulut honey at 3% concentration can be one of the effective natural anti-browning agents to maintain the quality of MPJ and can be practically used widely in fruit industry.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

Authors wish thank the Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

Data availability statement

All data generated or analysed during this study are included in this published article.

Authors' contribution

N. F. Mohd Fuad and N. Hussain; methodology, validation, N. F. Mohd Fuad; investigation, K. Rajentran; resources, N. Azhar; data curation, N. F. Mohd Fuad; writing-original draft preparation. N. F. Mohd Fuad; writing-review and editing, W. N. A. S. Wan Mohd Zul; supervision, N. Hussain. All authors have read and agreed to publish the manuscript.

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