

# **TROPICAL AGRICULTURAL SCIENCE**

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# Principal Component Analysis of Physicochemical Parameters and Microstructure Characteristics of Wampee Fruit Affected by Storage Temperatures

Hai Wang<sup>1</sup>, Feilong Yin<sup>2</sup>, Shurou Chen<sup>1</sup>, Ting Wei<sup>1</sup>, Ziyi Qin<sup>1</sup>, Jing Li<sup>1,3</sup>, Xia Li<sup>1,3</sup>, Xinhong Dong<sup>1,3\*</sup> and Hock Eng Khoo<sup>1,3</sup>

<sup>1</sup>Guangxi Key Laboratory of Electrochemical and Magneto-chemical Functional Materials, College of Chemistry and Bioengineering, Guilin University of Technology, Guilin 541006, China <sup>2</sup>College of Food and Biological Engineering, Guangxi Key Laboratory of Health Care Food Science and Technology, Hezhou University, Hezhou 542800, China <sup>3</sup>South Asia Branch of National Engineering Research Center of Dairy Health for Maternal and Child Health,

Guilin University of Technology, Guilin 541006, China

#### ABSTRACT

This study investigates the positive effect of two storage temperatures on physicochemical characteristics, texture and structure of wampee fruits. The fruits were treated by storing them at low (10°C) and room temperature (25°C) for eight days. The results showed that the low-temperature (10°C) treatment compared to the room temperature storage could reduce fruit decay rate and weight loss, inhibit  $O_2^-$  production rate, maintain higher total soluble solids, and slower increment in total flavonoids and phenolics. Principal component analysis (PCA) and partial least squares regression analysis further showed that weight loss was positively correlated with the content of total phenolics and flavonoids. The changes in the physiological indicators of the fruits were notably affected by storage temperature, especially in the early storage stages. Texture properties analysis indicated that the hardness and chewiness of the fruit at the low temperature (10°C) were significantly better

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*E-mail addresses:* wanghai990601@163.com (Hai Wang) Yfeilong1994@163.com (Feilong Yin) chenshurou0604@163.com (Shurou Chen) tingwei925@163.com (Ting Wei) h2oqzy@163.com (Ziyi Qin) 2017002@glut.edu.cn (Jing Li) biology754@163.com (Xia Li) dongxhok@126.com (Xinhong Dong) 2020153@glut.edu.cn (Hock Eng Khoo) \*Corresponding author than that at 25°C. Fruit colour values (L\*, C\* and h angle) of fruits at 10°C were also remarkably higher than that at 25°C. All the results suggested that low-temperature storage was a convenient and effective method to maintain the quality of the wampee and extend its shelf life compared to room temperature.

*Keywords*: Antioxidant, cold storage, oxygen radical, preservation, subtropical fruit

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#### INTRODUCTION

The fruit of *Clausena lansium* (Lour.) Skeels, also known as Chinese wampee (CW), is a tropical and subtropical evergreen fruit of the Rutaceae family in southern China. The wampee ripens from May to July, and the humid subtropical climate accelerates its decay. It will become brown overnight and lose edible value and commercial quality after 2–3 days (Zeng et al., 2020). The short shelf-life of wampee could be due to the fruit softening and peel browning. The increased fruit respiration rate during the warm ambient accelerates fruit ripening. The fruit's shelf life can be prolonged using several preservation techniques and storage conditions to solve this postharvest issue. These preservation techniques are modified atmosphere packaging, ethylene, oxalic acid,  $\gamma$ -aminobutyric acid, ultrasound and melatonin treatments. Low-temperature storage has been used as an effective way to keep fruits among many preservation technologies for fruits and vegetables.

The low fruit quality during room temperature storage is attributed to an increased atmospheric temperature (Mphaphuli et al., 2020). Sanchez et al. (2021) investigated the quality and shelf-life of Malaysian sweet potatoes, including moisture content (MC), soluble solids content (SSC), colour values and textural properties during different storage temperatures. Low-temperature storage of ripe pepper significantly inhibited the lipoxygenase activity, of which the low temperature inhibited ethylene production and delayed fruit senescence (Maalekuu et al., 2006). The enzymatic activities of several enzymes involved in the browning reaction are temperature-dependent. Therefore, low-temperature storage is used as an effective method for the postharvest preservation of fruits. Cai et al. (2010) found that too low temperature or structure, affecting the fruit taste. Fruit texture is another important indicator of a fruit's quality besides its appearance and physiological quality.

The literature reported significant correlations between texture parameters and storage quality in bananas and dates (Granados et al., 2014; Singh et al., 2013). Appropriate storage temperatures maintain the fruit quality. No previous study on texture analysis of wampee fruit has been published; a study compared the biochemical parameters of wampee stored at different storage temperatures. The results showed that the storage temperature of 8°C –10°C and 23°C–25°C did not significantly affect the biochemical parameters (total soluble solid, titratable acid and vitamin C content) of the matured post harvested wampee fruit during the 14 days of storage (Meng et al., 2021). The study also reported that the fruit samples stored at room temperature 23°C–25°C had lower peel lightness and hue (h) angle values. Their respiration rate was higher at room temperature. Another study also determined the browning index of wampee fruit stored at 4°C for 12 days (Zeng et al., 2020). Moreover, ethanol fumigation effectively reduced wampee peel browning and increased the antioxidant status of the peel during the storage of the fruit at 8°C for 12 days

(Shao et al., 2020). Therefore, we considered 10°C and 25°C as the storage temperatures of the wampee.

These studies did not determine the other physicochemical properties and perform a microstructure analysis of the wampee fruit peel, and no previous report on the comprehensive postharvest quality of wampee stored at different temperatures, especially all related physicochemical and biochemical characteristics, texture parameters and microstructure evaluation of the fruit peels. Thus, the primary objective of this study was to determine the influences of two storage temperatures and duration on the biochemical, physicochemical and texture parameters of wampee.

# MATERIALS AND METHODS

#### **Sample Preparation and Treatments**

Wampee was harvested at a commercial maturity stage from a plantation in Guilin, China. They had uniform shapes with good appearances, hard fruit texture and milky white pulp. The fruits were slightly ripened, with a mildly sour taste. A fully ripened wampee has a sweet taste with a citrus flavour. The harvested fruits had a maturity point of 8 out of 10 points and features like a light-yellow hue, round shape and similar sizes. All fruit samples were without disease or mechanical damage. Moreover, no peel browning was observed for all fruits.

All fruit samples (500 fruits) were divided into two groups, where 250 fruits from each group were packed into a foam box (lined with paper scraps to prevent the fruit from squeezing and scraping each other) and stored at 10°C and 25°C. A random sample of 50 fruits was respectively selected to determine the fruit quality values at 0, 2, 4, 6 and 8 days. The temperature selection was because 10°C is a more cost-effective storage temperature than the other lower temperatures. The literature also showed that a storage temperature higher than 10°C for wampee had significantly higher respiration rates (Meng et al., 2021). The fruit sample was also pre-treated with liquid nitrogen and stored at -80°C before further analyses.

# Decay Incidence, Weight Loss, pH and Total Soluble Solids

Wampee fruits were considered to decay if they had the characteristics of rot, visible fungal growth, or bacterial damage. The decay incidence was expressed as the percentage of diseased fruits to the total number of fruits (%). Weight loss rate of CW samples was calculated according to the method described by Singh et al. (2013). The pH values of the wampee samples were determined using a pH meter. In brief, the fruits stored at different temperatures were selected randomly, blended and filtered. The filtrates were measured for their pH value using a pH meter (INESA Scientific Instrument Co., Ltd., China).

Total soluble solid (TSS) of CW samples was measured by a handheld Boehmometer (LICHEN-BX Instrument Technology Co., Ltd., China). A random fruit sample was

pounded and filtered. A drop of clear liquid was obtained and measured on the prism of a handheld refractometer. The unit was determined as a Brix value (Xu et al., 2023). All analyses were performed based on three experimental replications.

#### Total Flavonoids, Total Phenolics and Vitamin C

Total phenolic content (TPC) was determined using a slightly modified method (Ghasemnezhad et al., 2011). Before TPC analysis, the methanolic extracts of wampee samples were centrifuged for 10 min at  $1,127 \times g$  (centrifugal force). The supernatant was subjected to determine TPC and total flavonoid content (TFC). In brief, 0.1 ml of the supernatant, 0.9 ml of distilled water and 0.4 ml of Folin reagent were mixed and reacted for 3 min at 25°C and then added with 1.0 ml of saturated sodium bicarbonate solution and left for 1 h at 25°C. The absorbance at 760 nm was determined, and the TPC was expressed as gallic acid equivalent. The aluminium chloride colourimetric assay was used to measure the TFC of the wampee sample (Karadeniz et al., 2005). TFC was expressed as rutin equivalent.

The vitamin C (VC) content was determined by referring to the method described previously with slight modification (Suntornsuk et al., 2002). A 3.0 g wampee sample was weighed, added with 2% hydrochloric acid solution, pulverised, filtered, and then transferred to a 100 ml volumetric flask. The filtrate (5.0 ml) was added with 0.5 ml of 10 g/L potassium iodide solution, 2.0 ml of 5 g/L starch solution and 2.5 ml of distilled water in a conical flask. The mixture was homogenised and titrated with potassium iodate solution. The amount of iodate solution (ml) used was recorded. For blank, 5.0 ml of 2% hydrochloric acid solution was used. All these analyses were performed based on three experimental replications.

# Hydrogen Peroxide Level and O<sub>2</sub><sup>-</sup> Production Rate

Hydrogen peroxide/peroxidase assay kit (Suzhou Keming Biotechnology Co. Ltd., China) was applied to determine the hydrogen peroxide ( $H_2O_2$ ) level (Cao et al., 2020). A 2.0 g wampee sample was weighed and added with 10 ml of the precooled acetone (Chengdu Kelong Chemical Co., Ltd., China) at -20°C. The mixture was agitated for 30 s, incubated in an ice water bath for 10 min and then centrifugated at 4°C 1,127 × g for 20 min. In triplicate measurements, the supernatant was collected and measured using an  $H_2O_2$  detection kit supplied by the Nanjing Jiancheng Bioengineering Research Institute Co., Ltd. (Jiangsu, China).

The  $O_2^-$  production rate was determined using the method described by Wang et al. (2015) with a slight modification. After weighing 2.0 g of the lyophilised sample, 10 ml of extraction buffer (50 mM, pH 7.8 in phosphate buffer) was added, mixed and agitated for 30 s. The mixture was kept in an ice bath for 10 min, followed by centrifugation at 1,127 × g for 30 min (4°C). The supernatant (1 ml) was added with 1 ml of phosphate

buffer (0.05 mol/L, pH 7.8) and 1 ml of 1 mM hydroxylamine hydrochloride of pH 3.2 (Thermo Scientific, Rockford, USA), shaken well, and kept at 25°C for 1 h. A 1 ml 17 mM p-aminobenzene sulfonic acid solution of pH 2.5 (Runfeng Synthetic Technology Co., Ltd., China) pre-dissolved in boiling water and 1 ml of 7 mM α-naphthylamine solution of pH 7.1 (Macklin Biochemical Technology Co., Ltd., China) (prepared using 75% aqueous acetic acid solution as solvent) were pipetted into the reacting sample solution and then mixed well. The absorbance was measured at 530 nm in triplicate measurements.

# **Colour Attributes and Texture Profile Analysis**

Colour attributes such as lightness (L\*), red-green (a\*), yellow blue (b\*), chroma (C\*) and h angle of the fruit peel were determined using a fully automatic colourimeter (Ma et al., 2021). An analytical sample of 30 fruits was measured for L\*, a\*, b\*, C\* and h values. A three-point measurement (front, side and back) of the fruit was performed for each fruit. A single experimental replication was considered for analysing all colour attributes of each fruit.

The texture profile of wampee samples was determined using the method described by Požrl et al. (2010) with slight modification. The texture parameters were hardness, springiness, chewiness, cohesiveness, resilience and stringiness. The mode set was texture profile analysis (TPA). An analytical sample of 30 fruits was analysed for each texture parameter with a single measurement.

# **Microstructure Measurement**

The microstructure of the wampee peels was measured using a scanning electron microscope (SEM). The samples (five pieces of the peel) were soaked into a solution of 2% (Wuhan Kangdeli Chemical Co., Ltd., China). The peels were then rinsed three times with the corresponding phosphate buffer for 15 min and dehydrated in ethanolic solutions of 50%, 70%, 80% and 90% at 15 min each (Qin et al., 2022). The ethanol was purchased from Fuyu Chemical Company (China). The samples were freeze-dried before the electron microscope scanning. All SEM analyses were done based on three sample replications.

# **Statistical Analysis**

All data were presented as means  $\pm$  standard errors of different sample replicates. The data for biochemical characteristics, colour attributes and textural parameters were statistically analysed using Statistical Package for Social Sciences (SPSS) version 25.0 software. The significant differences between the two variables were analysed using a one-way analysis of variance (ANOVA) coupled with Duncan's multiple range test. The PCA was also determined using SPSS software for selected biochemical and physicochemical parameters of wampee peels, with a significance value set at p < 0.05.

#### RESULTS

#### Decay Incidence, Weight Loss, pH and Total Soluble Solids

Table 1 shows the quality parameters of wampee samples stored at two temperatures (10°C and 25°C). The results showed that the TSS values of the fruit samples decreased with the increasing storage duration. The decreasing rate of the fruit samples stored at 10°C was significantly lower than those kept at 25°C (p<0.05). In contrast, the fruit samples stored at 10°C throughout the storage period. In this study, the weight loss rate of wampee samples kept at 10°C was significantly lower than the fruit samples stored at 25°C (p<0.05).

As shown in Table 1, the fruit samples kept at  $10^{\circ}$ C had a lower peel decaying rate than those stored at 25°C. On day eight, the peel decaying rate of the fruit samples kept at 25°C was four times higher than that of 10°C. The wampee samples stored at the low temperature had better fruit quality than those at 25°C. TSS of the wampee samples kept at 10°C was significantly higher than the room temperature storage. The TSS of the fruit samples that were stored at 25°C had a decreasing trend. The reduction was significantly higher than the low-temperature storage starting from day two (p<0.05). The acidity trend (based on pH value) of the fruit samples stored at 10°C remained relatively constant during storage. However, the acidity of the fruits in the 25°C group was significantly (p<0.05) reduced on day eight.

#### Total Flavonoids, Total Phenolics and Vitamin C

The TPC and TFC of wampee samples stored at 10°C and 25°C had an increasing trend. The TPC of the low-temperature storage samples had a slower increment than those kept at 25°C (Table 1). The results also showed that the TFC content of the fruit samples was significantly increased after four days of storage at 25°C (p<0.05). Similar to the reported TPC values, the TFC of the fruit samples stored at 10°C had a slower increment. On the other hand, the VC of the fruit samples kept at both temperatures decreased from day 0 until day 6. The VC values of these fruit samples significantly increased to over four times higher on day 8 than on day 6. The fruit samples stored at 10°C had a higher increase in VC than those at 25°C.

#### Hydrogen Peroxide Content and O<sub>2</sub><sup>-</sup> Production Rate

The  $H_2O_2$  accumulation in the wampee samples kept at both storage temperatures showed an increasing trend with the extended storage times (Table 1). Starting from day 4, the fruit samples refrigerated at 10°C had a significantly slower increment in the level of  $H_2O_2$ than the samples stored at 25°C (p<0.05). The increasing  $O_2^-$  production trend in both fruit samples was unlike the  $H_2O_2$  level. The wampee samples stored at room temperature showed increasing levels of  $O_2^-$ , but the  $O_2^-$  values of the fruit samples kept at 10°C fluctuated. The baseline value was higher than those determined in the samples stored at room temperature for two days.

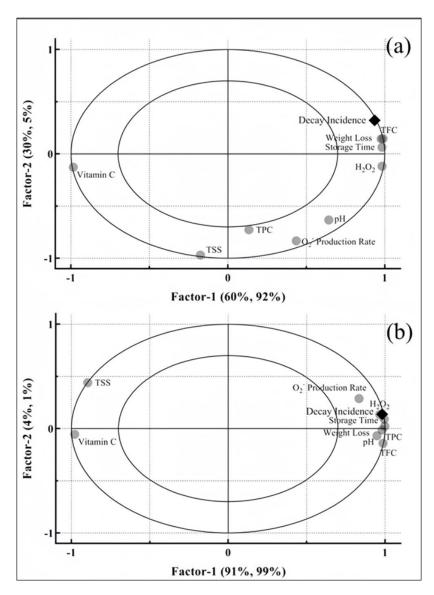
|   | Day 0 | 0 |       | Day 2 | y 2   |    |       | Day 4 | y 4    |    |       | Day 6 | 7 <b>6</b> |   |       | Day 8  | y 8   |    |
|---|-------|---|-------|-------|-------|----|-------|-------|--------|----|-------|-------|------------|---|-------|--------|-------|----|
| rameters  |       |   | 10°C  |       | 25°C  |    | 10°C  |       | 25°C   |    | 10°C  |       | 25°C       |   | 10°C  | U<br>U | 25°C  | 0  |
| Weight loss (%)   | 0.00  |   | 0.39  | L L   | 1.68  | q  | 0.72  | ef    | 2.55   | ပ  | 1.05  | e     | 3.92       | 9 | 1.75  | q      | 5.46  | а  |
| Decay rate (%)  | 0.00  | а | 0.00  | а     | 16.67 | ပ  | 3.33  | ab    | 46.67  | q  | 10.00 | bc    | 60.00      | e | 13.33 | ပ      | 83.33 |    |
| Hd  | 3.18  | а | 3.36  | bc    | 3.36  | bc | 3.36  | bc    | 3.39   | cd | 3.33  | q     | 3.41       | р | 3.33  | q      | 3.51  | 9  |
| TSS (Brix)  | 16.30 | р | 16.70 | q     | 15.77 | Ļ  | 17.47 | ab    | 15.80  | Ļ  | 16.53 | ပ     | 15.77      | Ĥ | 16.00 | e      | 14.53 | 50 |
| TPC (g /kg)   | 59.57 | e | 58.41 | e     | 63.07 | q  | 63.88 | p     | 67.16  | ပ  | 60.14 | e     | 77.65      | q | 59.44 | e      | 88.08 |    |
| TFC (g/kg)  | 0.06  | o | 0.09  | q     | 0.11  | cd | 0.11  | cd    | 0.13   | ပ  | 0.12  | cd    | 0.16       | q | 0.18  | q      | 0.25  |    |
| Vitamin C (mg/kg)   | 4.42  | Ч | 3.85  | 50    | 2.68  | р  | 3.25  | Ļ     | 2.60   | p  | 3.09  | e     | 1.19       | q | 16.62 | ပ      | 5.08  |    |
| $H_2O_2 \pmod{kg FW}$   | 0.89  | e | 1.20  | р     | 1.22  | р  | 1.33  | р     | 1.43   | ပ  | 1.41  | ပ     | 1.57       | q | 1.58  | q      | 1.70  |    |
| O <sub>2</sub> <sup>-</sup> production rate<br>(mmoL/min/kg FW) | 0.28  | р | 0.37  | cd    | 0.15  | e  | 0.57  | ab    | 0.26 d | р  | 0.45  | bc    | 0.67       | а | 0.38  | cd     | 0.69  | 9  |

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# **Principal Component Analysis**

As shown in Figure 1a, the variations explained by the first (PC1) and second (PC2) principal components were 76.8% and 10.5%, respectively. The results showed that overall responses to the 10°C treatment were more closely associated with each other than those in the 25°C fruit, regardless of storage duration. PC1 can discern the differences in quality parameters of the wampee treated with the two storage temperatures.

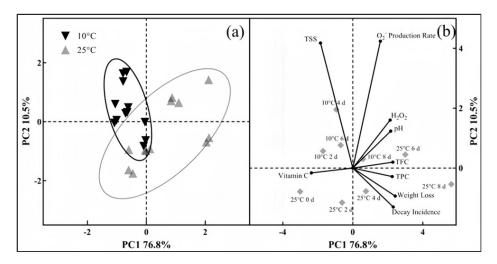


*Figure 1*. Loadings and scores of principal component analysis for Wampee samples. (a) Biplot of the biochemical indicators and (b) loading and score plots. These plots showing components 1 and 2 are explained by 90% of the total variation

Six indicators (decay rate, weight loss, TPC, TFC, pH values and  $H_2O_2$  level) of the wampee samples contributed the most to the PC1 score, while TSS and  $O_2^-$  production rate were explained by PC2 (Figure 1b). PC1 distinguished the differences in the variables between the wampee samples kept at day 6 and day 8 for both temperatures. This indicates that changes in weight loss, TPC, TFC, pH values and  $H_2O_2$  levels in the sample have arisen from storage at 25°C. The differences in these quality parameters for the samples between day 2 and day 4 of storage were better distinguished in PC2. It shows that the changes in TSS and  $O_2^-$  production rate of the samples on day 2 versus day 4 were due to the low-temperature storage.

#### Partial Least Squares Regression Analysis

The decay incidence of the wampee samples was selected as the dependent variable (Y). The other indicators were independent variables (X). The partial least squares regression (PLSR) model was also developed (Figure 2). Most X and Y variables lie between  $R^2 = 50\%$  and 100% confidence ellipses, indicating that the model has a higher confidence. TFC, weight loss, storage time, H<sub>2</sub>O<sub>2</sub> and VC content were the variables with significant effects on the decay incidence under low-temperature storage. As a comparison, four new factors (TSS, TPC, pH and O<sub>2</sub><sup>-</sup> production rate) affected the wampee decay incidence under room temperature storage.



*Figure 2.* Partial least squares regression biplots for the correlation between decay incidence and postharvest physiology of (a) low-temperature storage and (b) room-temperature storage

#### **Colour Attributes**

The colour attributes of the wampee peel stored for 2, 4, 6 and 8 days are presented in Table 2. The values of all colour attributes of the fruit sample kept at 25°C for eight days

# were significantly lower than $10^{\circ}$ C (p<0.05), except for a\* value. No significant change in the a\* value was observed between the samples kept at two different temperatures for eight days (p>0.05). The trend of C\* values of the samples stored at 10°C was similar to that of L\* values. However, a significant variation was reported for the C\* value of the peel sample kept at 25°C, with a significantly elevated C\* value on day 2 and reduced after day 4 (p<0.05). The changes in the h value of the samples kept at 10°C were similar to that of 25°C. However, Table 2 shows a significant decrease (p<0.05) in b\* values under the low-temperature storage.

| Appearance       | Storage temp              | oeratures (°C)            |
|------------------|---------------------------|---------------------------|
|                  | 25                        | 10                        |
| Lightness (L*)   |                           |                           |
| 2                | $36.27 \pm 1.01^{bc}$     | 40.52±0.65ª               |
| 4                | $35.73 \pm 0.75^{cd}$     | $35.72 \pm 0.48^{cd}$     |
| 6                | $33.43 \pm 0.49^{de}$     | $36.75 \pm 0.44^{bc}$     |
| 8                | 31.33±0.59°               | $38.23 \pm 0.44^{ab}$     |
| Red-green (a*)   |                           |                           |
| 2                | 15.34±0.46°               | 15.71±0.60°               |
| 4                | $16.67 \pm 0.36^{bc}$     | 18.65±0.41ª               |
| 6                | $17.56{\pm}0.29^{\rm ab}$ | $17.31 \pm 0.31^{ab}$     |
| 8                | $16.40{\pm}0.29^{\rm bc}$ | $16.71 \pm 0.35^{bc}$     |
| Yellow blue (b*) |                           |                           |
| 2                | $25.29{\pm}0.88^{cd}$     | $30.36{\pm}0.47^{a}$      |
| 4                | 26.33±0.59°               | $26.51 \pm 0.38^{bc}$     |
| 6                | $23.74{\pm}0.44^{de}$     | $27.01 \pm 0.36^{bc}$     |
| 8                | 22.09±0.41°               | $28.32 \pm 0.35^{b}$      |
| Chroma (C*)      |                           |                           |
| 2                | 29.76±0.79°               | $34.39{\pm}0.30^{a}$      |
| 4                | 31.54±0.49 <sup>b</sup>   | $32.83{\pm}0.20^{\rm ab}$ |
| 6                | 29.60±0.48°               | 32.30±0.26 <sup>b</sup>   |
| 8                | $27.62{\pm}0.44^{d}$      | $33.11 {\pm} 0.26^{ab}$   |
| Hue (h)          |                           |                           |
| 2                | 58.26±1.17 <sup>b</sup>   | $62.56{\pm}1.17^{a}$      |
| 4                | $56.90 \pm 0.88^{bc}$     | $54.69 {\pm} 0.92^{cd}$   |
| 6                | $53.35 \pm 0.41^{d}$      | $57.13 \pm 0.70^{bc}$     |
| 8                | $53.23{\pm}0.52^{d}$      | 59.31±0.72 <sup>ab</sup>  |

Table 2Lightness, chroma and hue of wampee samples stored at different temperatures

*Note.* Data are presented as mean  $\pm$  standard error (n=30, sample replicate). Different superscript lowercase letters (<sup>a-e</sup>) denote a significant difference (p<0.05)

#### **Texture Characteristic**

Table 3 presents the TPA data of wampee samples stored at 10°C and 25°C. No significant changes in TPA values were observed between the sample variables (p>0.05). The hardness value of the samples stored at 10°C increased gradually with increasing storage duration, and it dropped after six days of storage. However, the springiness and resilience under room temperature storage decreased significantly (p<0.05). The stringiness values of the fruit samples kept at 10°C were slightly reduced with increasing storage times of up to six days, whereas the fruit samples stored at 25°C for six to eight days had a significant increase in stringiness values (p<0.05).

Table 3

| Texture of | characteristics | of wampe | e samples store | d at different | temperatures |
|------------|-----------------|----------|-----------------|----------------|--------------|
|            |                 |          |                 |                |              |

| Days  | Hardness          | Springiness              | Chewiness         | Cohesiveness                 | Resilience                  | Stringiness                  |
|-------|-------------------|--------------------------|-------------------|------------------------------|-----------------------------|------------------------------|
| 10 °C |                   |                          |                   |                              |                             |                              |
| 2     | 257±21°           | $0.86{\pm}0.01^{a}$      | $184 \pm 15^{bc}$ | $0.83{\pm}0.01^{a}$          | $0.84{\pm}0.02^{a}$         | $0.28{\pm}0.03^{\rm b}$      |
| 4     | $330\pm32^{bc}$   | $0.87{\pm}0.01^{a}$      | $234\pm22^{a}$    | $0.82{\pm}0.01^{a}$          | $0.84{\pm}0.02^{a}$         | $0.23{\pm}0.03^{\rm b}$      |
| 6     | 431±28ª           | $0.88{\pm}0.01^{a}$      | $305{\pm}23^{ab}$ | $0.81{\pm}0.01^{ab}$         | $0.83{\pm}0.02^{a}$         | $0.18{\pm}0.04^{\text{b}}$   |
| 8     | $391{\pm}26^{ab}$ | $0.85{\pm}0.01^{ab}$     | $266 \pm 17^{bc}$ | $0.80{\pm}0.01^{\text{abc}}$ | $0.79{\pm}0.01^{\text{ab}}$ | $0.43{\pm}0.11^{\text{ab}}$  |
| 25 °C |                   |                          |                   |                              |                             |                              |
| 2     | $325 \pm 32^{bc}$ | $0.86{\pm}0.01^{ab}$     | $221\pm20^{bc}$   | $0.80{\pm}0.02^{\text{abc}}$ | $0.85{\pm}0.03^{a}$         | $0.30{\pm}0.05^{\mathrm{b}}$ |
| 4     | $310{\pm}45^{bc}$ | $0.79{\pm}0.02^{\circ}$  | 178±24°           | $0.75{\pm}0.02^{bc}$         | $0.71{\pm}0.03^{\circ}$     | $0.29{\pm}0.07^{\rm b}$      |
| 6     | 267±27°           | $0.82{\pm}0.02^{\rm bc}$ | 165±21°           | $0.74{\pm}0.04^{\circ}$      | $0.75{\pm}0.04^{\rm bc}$    | $0.60{\pm}0.10^{a}$          |
| 8     | 275±37°           | $0.82{\pm}0.02^{\rm bc}$ | 173±26°           | $0.76{\pm}0.02^{bc}$         | $0.71{\pm}0.03^{\circ}$     | 0.69±0.12ª                   |

*Note.* Data are presented as mean  $\pm$  standard error (n=30, sample replicate). Different superscript lowercase letters (<sup>a-e</sup>) denote a significant difference (p<0.05)

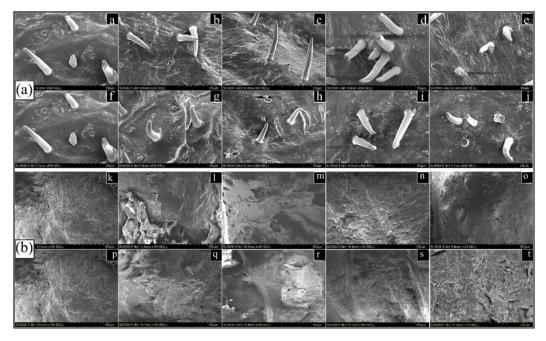
#### **Microstructures of Wampee Peel**

Figure 3a depicts the exocarp microstructures of wampee samples stored at 10°C and 25°C. Notable changes were observed in the microstructures during the storage of wampee samples. The result showed that the exocarp surface of the fruit was lined with a uniform, dense and waxy layer; the stomata were evenly distributed on the surface; and villi of different lengths were observed. At the top layer of the fruit, more villi were clustered, usually 2–5 villi in each cluster.

On the contrary, the villi were solitary on the surface. The increasing storage time caused a gradual fall off of the hypha and waxy layer, and the stomata gradually changed from smooth round edges to irregular ellipses Figure 3a [a–e]. As shown in Figure 3a [h], some wedge-shaped damage to the waxy layer is observed. Figure 3a [i] shows that rod-shaped and granular pathogenic infections appeared around the base of the epidermal villi of the samples stored at 25°C for up to six days. In contrast, the fruit samples kept at 10°C

had intact and waxy surfaces, flake cracks were not seen, and the texture remained intact with fewer fractures and no pathogenic infection.

Figure 3b shows the endocuticle structures of wampee samples stored at  $10^{\circ}$ C [k–o] and  $25^{\circ}$ C [p–t]. It can be seen from Figure 3b that many cavities formed between the parenchyma and vascular bundle on the lining of the outer epidermis and the outer epidermis. The parenchyma gradually shrunk and curled with the increase in storage time, resulting in the deepening of the longitudinal depth of the cavities, which is presented in Figure 3b [o and r]. On the contrary, the low-temperature storage could maintain better tissue denseness and orderliness than those kept at 25°C. It indicates that the low temperature maintains the structure of wampee peels.



*Figure 3*. Microstructure of wampee peel samples during storage at 10°C and 25°C for eight days. (a) peel surface, scale bar of 100  $\mu$ m; (b) inner peel, scale bar of 500  $\mu$ m

# DISCUSSION

Temperature controls various physiological changes during fruit storage. Our work showed that the surface of the matured wampee stored at 10°C was brighter and had fewer black spots. The results are consistent with the findings reported in the literature that the fruit samples stored at 25°C had a significantly higher decay rate than that of the other two lower temperatures (6°C and 10°C) (Hong et al., 2013). A storage temperature below 9°C could cause chilling injuries to mandarin oranges and develop off-flavours (Morales et al., 2020). The weight loss rate is also consistent with the previous report (Požrl et al., 2010).

The positive finding could be that fruit respiration is the most active at 25°C. The results also showed that the fruit samples stored at room temperature had a significantly lower fruit quality starting from day 4 of storage than those refrigerated at a low temperature.

Storing matured and ripe wampee samples at 25°C could maintain their freshness for up to three days. The reason was that decreased storage temperature reduced the respiration rate of fruit. Storing the fruits at 25°C increases the ripening process. The quality rating of the fruit samples is closely related to the peel decay rate. The decay process of frozen fruits is faster than that of those kept in the chiller, probably because the inner membrane of the frozen peel is disrupted, which releases polyphenol oxidase and leads to peel decay. TSS of the wampee samples stored at 10°C was significantly higher than the room temperature storage. It indicates that 10°C inhibited sugar metabolism inside the cell wall of the wampee. This finding is consistent with a previous report that low-temperature storage reduced the loss of TSS in Chinese bayberries stored for up to 14 days (Yang et al., 2007). The main reason for the decrease in TSS was the increased carbohydrate metabolism.

In this study, the VC content of the wampee samples stored at room temperature for up to six days showed a decreasing trend, but the low-temperature storage inhibited the VC loss in fruit samples during the 8-day storage. Citrus fruit typically has a higher acidity than many other fruits. Due to wampee being a citrus fruit, the low pH value is attributed to VC and phenolic acid content. The ripe wampee has a higher pH because the ripened fruit has low acidity. Also, low-temperature storage reduced the ripening process. Carmona et al. (2017) found that higher temperatures increased gene expression of the flavonoid pathway in blood orange. However, the low-temperature storage did not affect total phenolics and VC in the red raspberries (Mullen et al., 2002). High-temperature storage induced oxidative stress on the fruit. The high level of  $H_2O_2$  in the fruit tissue denotes increased oxidative products during the prolonged storage period was possibly due to the antioxidative activity in the fruit pulp. The positive correlations between total phenolic content,  $O_2^-$  production rate and decay incidence at higher temperatures were due to the accelerated degradation of phenolic compounds.

The results of this study showed that the brightness of wampee peels reduced during storage at ambient temperature, the colour saturation decreased, and the peel brightness faded. This could be observed from the wampee peel, where the peel colour changed from bright golden yellow to matte brown. However, the low-temperature storage was favourable in maintaining the brightness and colour tone of the fresh wampee. The low temperature also maintained the fruit's pleasant appearance, quality and shelf life.

This finding is consistent with a previous report that the low-temperature storage preserved the freshness, colour and appearance of the Chinese bayberry fruit at the prolonged storage duration (Yang et al., 2007). The report from Carmona et al. (2017)

also confirmed that a higher temperature promoted the accumulation of flavonoids in the fruit peel, which resulted in a high C\* value. These colour attributes are closely related to peel browning. The samples stored at 25°C had a higher decay rate and reduced lightness values than those at 10°C. The higher L\* value denotes a brighter peel. The value of C\* indicates the saturation of the colour; the higher C\* value shows a more intense colour. A higher h value represents a better hue. Usually, the colour of the fruit changed from green to brownish red, while the b\* value remained stable during the postharvest period (Wang et al., 2020).

The springiness and resilience under room temperature storage decreased, which can be attributed to the fact that the stiffness coefficient of the fruit was affected by temperature (Singh & Reddy, 2006). Higher temperatures stimulated pectinamethylesterase and polygalacturonase responses, leading to fruit hardness decay (da Silva et al., 2021). The TPA data showed that the wampee samples stored at 10°C had better texture characteristics than those at 25°C. It could also maintain a better quality of the fruit.

Dehydration of fruit causes changes in the microstructures of the fruit peel. The weight loss determined for the wampee samples denotes fruit dehydration. The dehydrated capsule membrane of the fruit peel cracked, and the sac structure completely collapsed and deformed. Pathogen infections may also be associated with fruit dehydration, and the respiration, transpiration and metabolism rates were the highest. The energy and water consumed were also the largest (Teixeira et al., 2011). These are related to the loose histiocyte and huge intercellular space between the pedicel. Hence, the organisation structure deformed due to prolonged storage time and water loss.

# CONCLUSION

This study showed that low-temperature storage (10°C) significantly improved the quality of wampee samples, including quality parameters, texture properties and microstructure of wampee peel. It had positive effects against weight reduction and wampee peel browning, inhibited loss of TSS, free fatty acids, phenolic compounds and VC, and reduced accumulation of reactive oxygen species compared with the fruit samples stored at 25°C. The texture and microstructure analysis of the peel samples further confirmed that the lowtemperature storage was more effective in maintaining better texture and tissue integrity. Therefore, storage of wampee at 10°C is a more economical and effective method for maintaining the quality of the fruit. It also reduced fruit senescence and peel browning. Extending storage periods and comparing a few different storage temperatures are suggested for future studies to obtain a more effective way of maintaining fruit quality. The physical factors contributing to fruit decay are the alternative recommendations for future research.

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