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Khafid A ·Nurchayati Y · Hastuti ED · Setiari N

Vitamin C and total soluble solid content of crystal guava at different storage duration and ripeness

Abstract. Crystal guava (*Psidium guajava* var. 'Crystal') fruit is in great demand because of its delicious taste and high nutritional content. Storage aims to prevent postharvest damage to the fruit. However, storage that is too long causes morphological damage and decreased nutrients. This study aims to determine the effect of storage duration, fruit ripeness stage and the interaction between both factors on vitamin C and total soluble solids (TSS) content of crystal guava, and determine which treatment can produce the highest vitamin C and TSS. Fruits harvested simultaneously with three levels of ripeness based on the skin color: unripe fruit is dark green, ripe is light green, very ripe is yellowish green. Samples selected based on the same weight range. Storage was carried out for 0, 5, and 10 days at ± 10^{o}C. The study used a completely randomized design (CRD) with a 3x3 factorial pattern with two factors: storage duration and fruit ripeness level. Parameters observed were vitamin C, TSS, weight loss, diameter shrinkage, skin color and hardness. Data were analyzed using ANOVA and DMRT. Both treatments showed an interaction on vitamin C content. The best treatment was unripe fruit stored for ten days with 14.955 ppm of vitamin C. Both treatments did not show any interaction on TSS content. The best treatment was five days storage with TSS of 8.25 °Brix and very ripe fruit of 8.21 °Brix. Based on vitamin C, TSS content, and physical condition variables, the best guava fruit is unripe fruit stored for 10 days.

Keywords: Color · Physical · Postharvest · Quality · Softening

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Khafid A[1] · Nurchayati Y[1]* · Hastuti ED[1] · Setiari N[1]
[1] Departement of Biology, Faculty of Science and Mathematics, Diponegoro University, Jl. Prof Soedarto, SH. Tembalang, Semarang City 50275, Indonesia
*Correspondence: yulita.yoko@gmail.com

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Introduction

Crystal guava (*Psidium guajava* var. 'crystal') is a plant from the Myrtaceae family and is a cultivar of guava. Crystal guava is in great demand because it has a crunchy texture with few seeds and has many health benefits (Rosmalova, 2021), including helping to ward off free radicals, boosting the immune system, helping to maintain the health of heart, skin and digestive system (Sasmi et al., 2022).

People usually prefer to consume fruit with the best quality. Crystal guava fruit quality can be assessed from several aspects, such as physical condition, which includes fruit skin, hardness, color, and fruit size, as well as from the nutritional aspects such as total soluble solids (TSS), vitamin C and total acid (Kalsum et al., 2018; Kusumiyati et al., 2018; Romalasari et al., 2017).

Physical condition is one of the important criteria in determining fruit quality, because it can be seen directly. Fruits that have good physical condition are more attractive to consumers (Sembiring et al., 2020). Total soluble solids (TSS) can be used as fruit sweetness indicator because TSS component mosly are sugar compounds (Liu et al., 2010). This causes fruit that has a high TSS content to have a sweeter taste, so consumers prefer it. The content of vitamin C can also be used as fruit quality determiner because it is known to have health benefits when consumed, such as boost immune system (Carr & Maggini, 2017), as an antioxidant (Durán-Soria et al., 2021), and is important for skin health (Pullar et al., 2017). This is what causes fruit with a high vitamin C content to be considered good quality fruit.

One of the fruit quality determiner factors is its ripeness level. During the fruit ripening process, a series of physiological processes occur which cause changes in the biochemical content and structure of the fruit, such as pigments synthesis, starch breakdown, sugar, volatile compounds, and secondary metabolites synthesis (Pullar et al., 2017). Unripe fruits taste and texture are less delicious to consume, but the nutrients contained in them are still low compared to ripe fruits. So, the more ripe a fruit is, the better its quality (Maduwanthi & Marapana, 2017; Trong et al., 2019). However, fruit that is too ripe will also have decreased quality. Overripe fruit color will changes, have a strong scent and the fruit will become soft, making it easily damaged and get caught by disease (Pott et al., 2020).

Generally, fruits that been harvested by farmers are not sold immediately to consumers. This creates a time gap between the harvest and the consumption where fruits are being prone to be damaged. To prevent this, the fruit that has been harvested must be given a series of postharvest treatments. Postharvest treatments aims to minimize the level of damage to plant products after being harvested until they sold consumers (Mutiarawati, 2007). Storage is one of the postharvest treaments that aims to protect the fruit from damage (Sudjatha & Wisaniyasa, 2017). However, too long storage can also cause morphological damage to the fruit and reduce some of its nutritional value (Hong et al., 2012).

This study aims to determine the effect of storage duration, ripeness stage and their interaction on crystal guava fruit vitamin C and total soluble solids (TSS) content, and determine which treatment combination can produce the highest vitamin C and TSS.

Materials and Methods

This research was conducted in March-May 2022. Sampling was carried out at a crystal guava plantation in Kandri Village, Gunungpati District, Semarang City. Preparation and determination of vitamin C content was carried out at the Plant Structure and Function Biology Laboratory Department of Biology, Faculty of Science and Mathematics, Diponegoro University, and TSS content determination was carried out at the Food Engineering and Agricultural Products Engineering Laboratory, Faculty of Animal and Agricultural Science, Diponegoro University.

The materials used in this study included crystal guava fruit (*Psidium guajava* var. 'Crystal') which harvested from 6 years old tree, ascorbic acid and distilled water.

Research Design. The design used in this study was a 3x3 factorial complete randomized design (CRD). The first factor is the ripeness level of the crystal guava fruit, including unripe, ripe and very ripe fruit taking into account the color and the appearance of the fruit skin. The second factor was crystal guava's storage duration, including 0, 5, and 10 days in the refrigerator (±10^{o}C). Total guava fruit used in this research are 45 fruits. Vitamin C test was subjected to nine

treatments with three replications each, so the total was 27 fruits. While TSS test was subjected to nine treatments with two replications each, so the total was 18 fruits.

Table 1. Research Design

Maturity	Storage Duration		
	S1	S2	S3
M1	M1S1	M1S2	M1S3
M2	M2S1	M2S2	M2S3
M3	M3S1	M3S2	M3S3

Note :
M1 : Unripe S1 : 0 day storage
M2 : Ripe S2 : 5 day storage
M3 : Very Ripe S3 : 10 day storage

Crystal Guava Fruit Harvest and Selection. Crystal guava fruit is harvested simultaneously based on three levels of ripeness based the fruit skin color: unripe fruit is dark green, ripe fruit is light green and very ripe fruit is yellowish green. Crystal guava fruit then is weighed and selected based on the same weight range (200-250 gr). The fruits that were selected are also free of open or severe wounds.

Figure 1. Guava's Ripeness Level Based on Skin Color : (a) Unripe – Dark Green (b) Ripe – Light Green (c) Very Ripe – Yellowish Green

Storage. Guava Crystals that had been cleaned by wiping off dirt or other substances from fruit surface, wrapped in plastic wrap, and then stored in a plastic bag in the refrigerator at ±10 ^{o}C for 0 days, 5 days and 10 days.

Making CO_2 Free Aquadest. Making ascorbic acid standart solution or to do extraction from fruit requires CO_2-free distilled water. CO_2-free distilled water is made by heating distilled water for 5-10 minutes. After it boils, the distilled water is then left to cool into room temperature (Fauzana et al., 2022)

Test of Vitamin C. Testing for Vitamin C content was carried out destructively using the spectrophotometric method based on research conducted by Herlina & Muzdalifa (2020) with some modifications. The steps taken are as follows:

1) Determination of the Maximum Wavelength of Ascorbic Acid
   Ascorbic acid was used as a standard solution to determine vitamin C content using spectrophotometry method. An ascorbic acid solution with a concentration of 10 ppm was prepared, then the absorbance value was measured using a UV-Vis spectrophotometer with a series of wavelengths ranging from 260 to 270 nm (Dewi et al., 2018).
   The absorbance value of the measurement results will form a curve. The curve obtained will show the wavelength that produces the highest absorbance value. This wavelength is used to determine the vitamin C content of the sample.
2) Creating a Calibration Curve
   Serial of ascorbic acid dilutions were carried out with levels of 2, 4, 6, 8, 10 ppm. The ascorbic acid solution was measured for its absorbance value using the maximum wavelength that was previously obtained. A line equation graph is made where the x-axis is the concentration of ascorbic acid (ppm) and the y-axis is the absorbance value. The line obtained from the graph is a line equation with the formula y = bx + a.
3) Determination of Vitamin C Content
   Crystal guava fruit mashed with a mortar. Sample weighed 0.25 g, then put into a test tube, then added 5 ml of distilled water and centrifuged at 3500 rpm for 15 minutes. The sample was then filtered using filter paper and poured into an Erlenmeyer and then added 12 ml of distilled water. The sample then measured its absorbance value with a spectrophotometer with the optimum wavelength that had been obtained previously. The concentration of vitamin C is obtained by putting the absorbance value of the sample into the linear equation y = bx + a where y is the absorbance value, x is the concentration of vitamin C, a is a constant and b is the coefficient.

Test of TSS. TSS were measured destructively using a digital refractometer. Crystal guava fruit is crushed using food processor, then the extract is taken and dripped on the refractometer lens. The results will then appear automatically on the refractometer display. The units for the amount of TSS shown are units in ^{o}Brix (Ana et al., 2021).

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Fruit's Weight Loss Calculation. Crystal Guava weighed using scale before and after storage, then the weight loss percentage was calculated using the formula below :

$$\text{Weight Loss} = \frac{W1-Wn}{W1}\ x\ 100\%$$

Note :
W1 = Initial weight (gr)
Wn = Weight day-n storage (gr)

Fruit's Diameter Shrinkage Calculation. Crystal Guava diameter was measured using caliper before and after storage, then the diameter loss percentage was calculated using the formula below :

$$\text{Diameter Shrinkage} = \frac{d1-dn}{d1}\ x\ 100\%$$

Note :
d1 = Initial fruit diameter (mm)
dn = Fruit diameter day-n Storage (mm)

Fruit Physical Changes. Fruit Physical Changes were analyzed descriptively by observing fruit conditions before and after storage. Parameters observed included fruit color and hardness. Documentation is also carried out before and after the fruit is stored.

Data analysis. Quantitative data obtained from the study included vitamin C content, TSS, weight loss and diameter were analyzed using Two-Way ANOVA to see whether or not there was a significant effect between treatments. If there is, the analysis is carried on with DMRT (Duncan's Multiple Rage Test) at a significance level of 95% to determine the difference between treatments. Qualitative data including fruit visual, changes in fruit skin color and fruit hardness were observed and presented descriptively accompanied by documentation in the form of photos from the observations.

Result and Discussion

Ascorbic acid was used as a standard solution to determine the levels of vitamin C contained in crystal guava fruit using the spectrophotometric method. To determine the wavelength to be used, it is necessary to make a maximum wavelength curve beforehand. The ascorbic acid solution absorbance value measured at a wavelength of 260 to 270 nm (Dewi et al., 2018). Based on the measurements that have been made, the highest absorbance value is 0.483 at a wavelength of 265 nm (Figure 1.). Therefore, the wavelength used to measure the absorbance of vitamin C in crystal guava fruit is 265 nm.

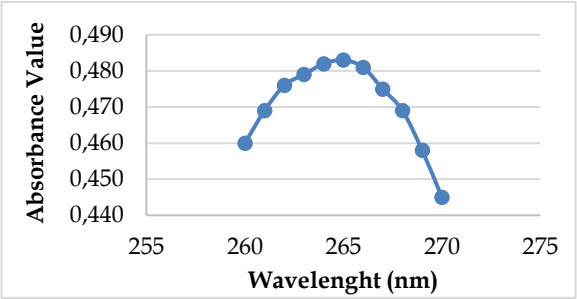

Figure 2. Maximum wavelength absorption curve for ascorbic acid.

Standard curve serves to determine the vitamin C content in a sample based on its absorbance value. The preparation of a standard curve was carried out by measuring the absorbance value of ascorbic acid solutions in stages with concentrations of 2, 4, 6, 8, and 10 ppm. Based on the test results, a standard curve is obtained with the line equation y = 0.0799x - 0.0209 (Figure 2.). The vitamin C content is determined by putting the absorbance value into the line equation that has been obtained, where the y variable is the absorbance value and the x variable is the vitamin C concentration.

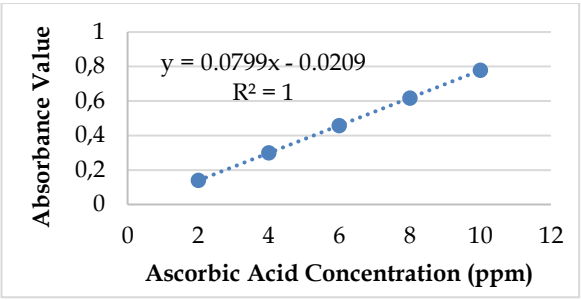

Figure 3. Ascorbic Acid Standard Curve

The results of ANOVA showed that the combination of duration of storage and ripeness level treatment had a significant effect on the vitamin C content of crystal guava fruit. The highest vitamin C content was found in the M1S3 treatment with 14.955 ppm. The lowest vitamin C content was found in the M1S1 treatment of 5.760 ppm (Table 2).

Unripe crystal guava fruit showed an increase in vitamin C content until the 10th day of storage (Table 2.). This is presumably because the fruit continues to produce vitamin C during storage. Kubo (2014) explained that storage in

cold temperatures (0-15 °C) is known to reduce the rate of metabolism in fruit, but not all metabolic processes are stopped or suppressed to the same extent. Tsaniklidis et al. (2014) in their research showed that enzymes that play a role in the synthesis of ascorbic acid such as L-galactono 1,4 lactone dehydrogenase (GalLDH) and GDP-Mannose-3'5'-epimerase (GME) were still active in tomatoes stored at room temperature. 10° C

Table 2. Content of vitamin C (ppm) of crystal guava fruit at different treatments of storage duration and ripeness level.

Matu-rity	Storage Duration			
	S1	S2	S3	Mean
M1	5.760^{i}	8.933^{gh}	14.955^{abc}	9.883
M2	7.183^{hi}	12.541^{cde}	9.995^{efg}	9.906
M3	9.882^{fg}	11.346^{def}	14.874^{bc}	12.034
Mean	7.608	10.940	13.274	

Note: Numbers followed by the same letters are not significantly different in the DMRT test at a significance level of 95% (α=5%)

Cells experienced stress in the form of chilling injury due to low temperature storage in the range of 15 °C to 0 °C (Kubo, 2014). Plants produce high amounts of H_2O_2 which are Reactive Oxidative Species (ROS) as a response to cold stress (Slesak et al., 2007). ROS compounds can cause cell damage that leads to cell death (Smirnoff & Wheeler, 2000). Ascorbic acid has the main function as a reducing agent for H_2O_2 which is produced both in the process of photosynthesis and in stress (Tsaniklidis et al., 2014). Wang et al. (2017) stated that ascorbic acid acts as a cofactor for the enzyme ascorbate peroxidase (APx) which plays a role in reducing H_2O_2, producing monodehydroascorbate (MDHA) and H_2O. This process occurs in the cell wall.

L-ascorbate + H_2O_2 ⇌ Monodehydroascorbate + H_2O

The amount of ascorbic acid in fruit remains high in cold storage due to the recycle. Smirnoff (2018) explained that monodehydroascorbate originating from the oxidation process by the APx enzyme will be reduced by the monodehydroascorbate reductase (MDHAR) enzyme using NADH, producing ascorbic acid again.

2 Monodehydroascorbate + NADH + H^+ ⇌ 2 Ascorbic acid + NAD^+

There are conditions where MDHA can be broken down into ascorbic acid and dehydroascorbic (DHA). Ascorbic acid can also be produced from the reduction of DHA by the enzyme dehydroascorbate reductase (DHAR) with the help of glutathione which is an antioxidant compound.

2 Glutathione + Dehydroascorbate ⇌ Glutathione Disulfide + Ascorbic acid

The vitamin C content in unripe crystal guava fruit was lower at 0 and 5 days of storage because the fruit was not fully ripe yet. The content of vitamin C crystal guava fruit is higher when the fruit becomes more ripe. Kartika (2016) states that the highest vitamin C content occurs when the fruit becomes more ripe. This increase occurred due to the biosynthesis of vitamin C from glucose contained in the fruit. Fenech *et al*. (2019) explained that the most dominant biosynthesis of ascorbic acid in plants occurs through the Smirnoff-Wheeler (SW) synthesis pathway. Ascorbic acid in this pathway is synthesized from the sugar molecule D-glucose-6-phosphate. The sugar content in ripe fruit is higher than in unripe fruit (Dewi et al., 2017; Dolkar et al., 2017), so that ripe fruit can produce more vitamin C because there is more substrate available.

Crystal guava fruit that is very ripe also experiences a similar condition to unripe fruit. The vitamin C content of very ripe fruit also increases up to 10 days of storage. In addition, the vitamin C content of very ripe fruit was higher than other fruit at 0 and 5 days of storage. The vitamin C content of very ripe fruit on the 10th day of storage tends to be the same as that of unripe fruit, although it is lower.

The high content of vitamin C in very ripe fruit in each storage treatment is thought to be related to its ripeness level. Fruits can synthesize ascorbic acid in greater quantities due to the supply of additional substrates for synthesizing ascorbic acid, one of which is pectin. This pectin comes from the process of softening the cell walls of the fruit, where when the fruit becomes more ripe, hydrolysis of pectin compounds will occur in the cell walls (Sharma et al., 2015). This pectin will then be used as a substrate for the synthesis of ascorbic acid via the D-galacturonic pathway (Fenech et al., 2019). This is also reinforced by the condition of very ripe fruit, which has a softer

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fruit hardness level than other ripeness levels (Table 8.).

Ripe crystal guava fruit vitamin C content increase at 5 days of storage, then decreased at 10 days of storage (Table 2.). This is presumably because vitamin C is used as an oxidative defense, while glucose, which is used as a substrate for synthesizing vitamin C, is diverted to the respiration process which increases in the climacteric phase of ripe fruit. The climacteric phase is a phase that occurs in several types of fruit where there will be a drastic increase in respiration rate (Saltveit, 2019). Low temperatures can reduce respiration rate, but not completely stopped (Bal, 2013; Luengwilai & Beckles, 2013).

Saltveit (2019) explained that carbohydrates will be broken down into glucose, then through the process of glycolysis, glucose is processed to produce other compounds, such as pyruvic acid, NADPH and ATP and glucose-6-phosphate. Glucose and Glucose-6-phosphate are substrates that act as materials for synthesizing vitamin C. In the process of respiration, these two compounds are used to produce energy. Glucose is used through the Krebs cycle process and oxidative carboxylation (glucose) to produce ATP and NADPH, while glucose-6-phosphate is used through the Pentose-Phosphate Shunt process or also called the phosphoglutanean pathway.

Based on the results of ANOVA, the combination of treatments between storage duration and ripeness level did not show any interaction with total soluble solids (TSS) content. However, each variable independently shows a significant influence. The best storage duration treatment was treatment P2, with an average TSS content of 8.25 °Brix and the lowest TSS content in treatments P1 and P3 were 7.23 and 7.01 °Brix, respectively. The level of fruit ripeness with the highest TSS content was at T3 of 8.21 °Brix and the lowest was at T1 of 6.95 °Brix and T2 of 7.33 °Brix (Table 3).

The total soluble solids (TSS) content of crystal guava increased in more ripe fruit. In the storage duration treatment, the TSS content of crystal guava also increased at 5 days of storage (Table 3.). The same thing was also reported by Dolkar et al. (2017) and Hong et al. (2012) where the TSS content of guava fruit increases in more ripe fruit and in longer storage. This is because during storage, hydrolysis of starch compounds occurs, changing them from insoluble to became soluble (Bishnoi et al., 2017; Dolkar et al. , 2017).

Table 3. Tss content (°brix) of crystal guava fruit at different treatments of storage duration and ripeness level.

Ripeness	Storage Duration			
	S1	S2	S3	Mean
M1	7.05	7.10	6.70	6.95[b]
M2	6.63	8.53	6.85	7.33[b]
M3	8.03	9.13	7.48	8.21[a]
Mean	7.23[q]	8.25[p]	7.01[q]	

Note: Numbers followed by the same letters are not significantly different in the DMRT test at a significance level of 95% (α=5%)

Sharma et al. (2015) stated that guava produces various enzymes that break down polysaccharide compounds, including the enzymes pectin galacturonase (PG), pectin methyl esterase (PME), cellulase and β-D-galactosidase. Payasi et al. (2009) explained that PG enzymes play a role in hydrolyzing glycosidic bonds in galacturonic acid which is a component of pectin. The PME enzyme works by removing the methyl group from galacturonic acid from pectin. Cellulase enzymes play a role in hydrolyzing β-1,4 glucan bonds in cellulose and xyloglucan. The β-D-galactosidase enzyme plays a role in removing galactosyl groups in pectin and xyloglucan. This is confirmed by Dolkar et al. (2017) research, which is the pectin content in guava decreased when it became more ripe along with increasing PME enzyme activity. This was supported by the finding of decrease in hardness during cold storage in guavas (Hong et al., 2012), tomatoes (Luengwilai & Beckles, 2013) and plums (Bal, 2013).

The TSS content of crystal guava decreased at 10 days of storage in all ripeness level (Table 3). The decrease in TSS occurred because of the sugar compounds in the fruit were used for various metabolic processes, such as for the biosynthesis of vitamin C (Fenech et al., 2019), fruit development and as a source of energy (Durán-Soria et al., 2021; Saltveit, 2019). This is also supported by the increase of crystal guava fruit vitamin C content during storage (Table 2), where the content of sugar compounds is partly intended to synthesize vitamin C as a defense mechanism from oxidative stress due to low temperatures.

The results of ANOVA showed that there was no interaction between the combination of ripeness level and storage duration treatments on crystal guava fruit weight loss. When viewed from each treatment individually, the ripeness

level did not have a significant effect on weight loss (Table 4), but there was a tendency for the highest fruit weight loss to occur in the T2 ripeness treatment, which was 0.004%. Storage duration treatment has a significant effect on fruit weight loss. Storage duration that causes the highest weight loss is the P3 of 0.005%.

Table 4. Weight loss (%) of crystal guava fruit at different treatments of storage duration and ripeness levels.

Ripeness	Storage Duration		
	S2	S3	Mean
M1	0.001	0.004	0.002[a]
M2	0.001	0.007	0.004[a]
M3	0.001	0.004	0.002[a]
Mean	0.001[q]	0.005[p]	

Note: Numbers followed by the same letters are not significantly different in the DMRT test at a significance level of 95% (α=5%)

Crystal guava fruit weight were loss during 5 and 10 days of storage (Table 4). Fruit weight loss occurs due to reduced water content in the fruit, due to the fact that the fruit stored after being harvested still undergoes a transpiration process. Transpiration is a process where water molecules from inside the fruit cells will come out into the environment. The longer the storage duration, the longer the transpiration process takes, so that the weight loss is also higher (Lawati et al., 2021; Kalsum et al., 2018). The rate of weight loss is influenced by several aspects, including environmental factors such as temperature and humidity (Kusumiyati et al., 2018).

There was a tendency for weight loss to be higher in ripe crystal guava fruit compared to unripe and very ripe fruit. This is because the ripe crystal guava fruit is in the climacteric phase, where the rate of cellular respiration is at its peak. Respiration is the process of breaking down sugar compounds stored in cells. Sugar compounds such as glucose are broken down into CO2 and H2O and produce energy that will be used in the development process (Saltveit, 2019). The breakdown of glucose compounds causes the biomass of crystalline guava to decrease. Kusumiyati et al. (2018) stated that the fruit that has been harvested continues to undergo metabolism, where the food reserves stored in the vacuoles will be used, while the fruit no longer gets a supply of nutrients from the tree because it has been picked.

The results of ANOVA showed that there was no interaction between storage duration and fruit maturity level on the diameter shrinkage of crystal guava fruit. Likewise, each treatment independently did not have a significant effect. The highest weight loss was found in immature crystal guava fruit and fruit stored for 10 days (Table 5.)

Table 5. Diameter shrinkage (%) of crystal guava fruit at different treatment duration of storage and ripeness levels.

Ripeness	Storage Duration		
	S2	S3	Mean
M1	0.002	0.005	0.003[a]
M2	0.001	0.004	0.002[a]
M3	0.000	0.002	0.001[a]
Mean	0.001[p]	0.003[p]	

Note: Numbers followed by the same letters are not significantly different in the DMRT test at a significance level of 95% (α=5%)

Table 6. Visual of Crystal Guava Fruit at Different Treatments of Storage Duration and Ripeness Levels

Ripeness	Storage Duration		
	S1	S2	S3
M1			
M2			
M3			

In Table 5., it can be seen that unripe fruit after being stored for 5 the diameter are shrunk by 0.002% from the initial diameter, after being stored for up to 10 days, the diameter shrinkage increased to 0.005%. Ripe fruit diameter shrunk by 0.001% from the initial diameter after being stored for 5 days, and increased to 0.004% after being stored for up to 10 days. Very ripe fruit when stored for 5 days did not experience a diameter shrinkage, but after being stored for 10 days the diameter shrunk by 0.002% from the initial diameter. The average shrinkage in diameter for 5 days of storage was 0.001% and for 10 days of storage was 0.003%. The reduction in fruit diameter is thought to be due to the transpiration process in the fruit. Transpiration

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causes a reduction in the number of water molecules contained in cells because they move out into the environment (Lawati et al., 2021). This causes the cell volume to decrease, resulting in a reduced size of the crystal guava fruit.

Analysis of the physical condition of crystal guava fruit was carried out qualitatively. The physical conditions of the fruit are presented in tabular form including visual fruit (Table 6.), skin color (Table 7.) and fruit hardness (Table 8.). There are differences in the physical conditions of crystal guava fruit with different ripeness levels and after being stored at different duration.

Based on observations, unripe has a dark green skin color. After being stored for 5 and 10 days, the color of the unripe fruit skin changed to yellowish green. Ripe fruit has a bright green skin color, and turns yellowish green on storage for 5 and 10 days. Very ripe fruit has a yellowish green skin color, and turns greenish yellow after 5 days of storage, then becomes bright yellow after 10 days of storage. Color scale were created to help to understand more of the color difference between treatments (Table 7).

Table 7. Skin color number scale of crystal guava fruit at different treatments of storage duration and level of ripeness.

Ripeness	Storage Duration		
	S1	S2	S3
M1	1	3	3
M2	2	3	3
M3	3	4	5

Description: 1 = dark green; 2 = Bright green; 3 = Yellowish green; 4 = Greenish yellow; 5 = Bright yellow

The color change that occurs in the skin of the crystal guava is due to the process of changing the pigment in the fruit skin. This color change also indicates that the fruit is becoming more ripe. Choo (2018) stated that chlorophyll degradation causes the color of the fruit to change. Kapoor et al. (2022) added that ethylene produced during fruit ripening functions as a signaling compound, where ethylene signals specific transcription factors, activating chlorophyll catabolic genes (CCG) which play a role in degrading chlorophyll in fruit peels.

There are differences in the level of fruit hardness in fruit with different ripeness. The fruit also experienced changes in the level of hardness after being stored for different durations (Table 8). Unripe fruit has a hardness rating on the very hard scale. After being stored for 5 and 10 days, the fruit becomes softer, the level of hardness becomes "hard". Ripe fruit has a hardness level on the "hard" scale, then after being stored for 10 days it becomes softer, the hardness level becomes "soft". Very ripe fruit has a hardness level on the "hard" scale, changing to "soft" after being stored for 5 days and becoming "very soft" after being stored for 10 days (Table 8).

Table 8. Hardness of crystal guava fruit at different treatments of storage duration and ripeness levels.

Ripeness	Storage Duration		
	S1	S2	S3
M1	++++	+++	+++
M2	+++	+++	++
M3	+++	++	+

Note : ++++ = Very Hard; +++ = Hard; ++ = Soft; + = Very soft

This change in hardness of the crystal guava fruit is due to the softening of the fruit cell walls during fruit ripening. Pectin is one of the dominant compounds in composing the middle lamella. During maturation, changes occur in the parenchyma cell wall, especially in the middle lamella. Pectin undergoes a change from insoluble to dissolved (Paniagua et al., 2014; Payasi et al., 2009). Several enzymes play a role in this process, including the pectin galacturonase (PG) enzyme which works to hydrolyze the glycosidic bonds of galacturonic acid (a component of pectin), the pectin methyl esterase (PME) enzyme which functions to hydrolyze the methyl-ester bonds of galacturonic acid which make up pectin and enzymes. β-D-galactosidase plays a role in removing the galactosyl group in pectin (Paniagua et al., 2014; Payasi et al., 2009; Sharma et al., 2015).

Conclusion

Based on the research that has been done, it can be concluded several things as follows:

1. Storage duration affects the crystal guava fruit's vitamin C and total soluble solids (TSS) content.
2. The level of fruit ripeness affects the crystal guava fruit's vitamin C and TSS content.
3. There is an interaction between the duration of storage and the level of ripeness on the vitamin C content of crystal guava fruit
4. Treatment with the best results:

a. The combination of treatments to produce crystal guava fruit with a high vitamin C content was unripe fruit which was stored for 10 days.
b. The best treatment to produce crystal guava fruit with the highest TSS was very ripe fruit and fruit stored for 5 days.
c. Based on vitamin C, TSS, and the fruit's physical condition, the best crystal guava fruit was the unripe ones that is stored for 10 days

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