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INFLUENCE OF STORAGE TEMPERATURE ON VISCOSITY, COLOUR, EMULSION AND VITAMIN E STABILITY OF PINK GUAVA JUICE FORTIFIED WITH VITAMIN E

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Abstract:

This study was undertaken to assess the changes in viscosity, colour, emulsion stability, and vitamin E stability of pink guava juice fortified with vitamin E (PGJD) during 180 days of storage at 5°C, 15°C, and 25°C. PGJD with a combination of 70% (w/v) Xanthan Gum (XG), 30% (w/v) Carboxyl methyl cellulose (CMC), 0.8% (w/v) Polysorbate 80 (P80), and 225mg vitamin E was prepared and stored for 180 days at 5°C, 15°C, and 25°C. For every 30 days of storage, viscosity, emulsion, colour, and vitamin E stability were determined using a viscometer, chromameter, emulsion stability index (ESI), and high-performance liquid chromatography (HPLC), respectively. The result indicates that the viscosity of PGJD increased significantly slower when stored at a lower storage temperature. In addition, emulsion stability will be maintained for approximately 30 days longer when stored at a lower temperature as opposed to a higher temperature. The L* value of the colour increased for all samples, however, the C* values indicated red colour intensity from PGJD was gradually fading through time. In addition, the H° value of all PGJD samples exhibited a steady increase during storage, whereas vitamin E exhibited a consistent deterioration pattern. The findings of the present study can be used as a guideline for beverage manufacturers and retailers in selecting the best storage temperature for their products.

Keywords:

Pink Guava Juice, Storage Temperature

Introduction

Pink guava is a delectable fruit with a rich aroma and a flavour that is somewhere between sweet and tart. It is a very good source of antioxidants, carotenoids, flavonoids, and triterpenoids, as well as dietary fibre and pectin. In comparison to conventional guava juice, pink guava juice is preferred due to its appealing colour and higher nutritious content, such as lycopene. Pink guava juice has the potential to be sold due to its numerous health benefits. Several researchers interested in the topic of vitamin fortification of fruit juice, particularly guava juice, reported that the addition of vitamin E to fruit juice will significantly improve daily vitamin consumption. However, when pink guava juice is stored, it loses its ascorbic acid, lycopene, non-reducing sugars, and sensory properties (Aishah et al., 2016; Bujang et al., 2016; Mousa., 2020; Rashid et al., 2018; Youssef et al., 2017; Kanwal et al., 2016; Sinchaipanit et al., 2015). The objective of this study is to evaluate the changes in viscosity, colour, emulsion stability, and vitamin E stability during 180 days of storage at 5°C, 15°C, and 25°C. In order to prolong the overall quality of pink guava juice, it is necessary to store those juices at the correct temperature. The finding of this study can serve as a guideline for beverage manufacturers and retailers to establish the optimal storage temperature for fruit juice products.

Literature Review

Pink Guava

Pink guava (*Psidium guajava* L.) is a member of the vast Myrtaceae or Myrtle family. It is indigenous to tropical and warm subtropical regions, originated in Central America, and is cultivated extensively in Perak, Malaysia. The guava has a green exterior and pink flesh with a sweet flavour. The pink guava is one of the easiest fruits to process in the food industry due to the fact that the entire fruit is edible, and its soft texture makes it simple to transform into a puree or fruit juice. Pink guava has a rich tropical aroma, colour, aroma, flavour, and functional properties. It is an excellent source of carotenoids, flavonoids, triterpenoids, pectin, and dietary fibre, as well as phytochemicals such as ascorbic acids, anthocyanins, and ellagic acids (Hashim & Ismail, 2022; Suwanwong & Boonpangrak, 2021; Nagarajan et al., 2019; Campoli et al., 2019; Lamo al., 2019; Ninga et al., 2018; Moon et al., 2018; Garbanzo et al., 2017; Aishah et al., 2016).

Pink Guava Juice

According to Aishah et al. (2016), the pink guava fruit pulp is not susceptible to browning and may be processed into a pink guava juice drink. It has marketable potential due to an increased awareness of the consumer about the importance of having a healthy choice of drink instead of common caffeine-containing beverages such as coffee, tea, or carbonated soft drinks (Hashim & Ismail, 2022). In Malaysia, pink guava juices are marketed with total soluble solids ranging from 9.9°Brix to 10.63°Brix and pH ranged between 3.46 and 3.98 (Hashim & Ismail, 2022). Pink guava juice is preferred compared to regular guava juice because of the presence of lycopene, which accounts for more than 80% of its total carotenoid content (Nagarajan et al., 2019; Campoli et al., 2019; Aishah et al., 2016).

Vitamin E Fortification in Fruit Juice

In recent years, vitamin fortification of fruit juice, particularly guava juice, has been examined by a number of researchers. Hence, the addition of vitamin E to fruit juices will significantly increase daily vitamin consumption (Hashim & Ismail, 2022; Fuente et al., 2020; Ephrem et al., 2018). Vitamin E is a powerful chain-breaking antioxidant that eliminates oxygen radicals

and halts free radical chain reactions in the human body. According to Whitney et al. (2018), the recommended daily consumption of vitamin E (tocopherol and tocotrienol equivalents) for healthy humans is 10 mg (or 15 IU) per day. Vitamin E has good benefits on cardiovascular disease, cancer prevention, the immune system, and delaying the ageing process, and is associated with a considerable reduction in the risk of Alzheimer's disease among older persons (Lui et al., 2018; Alzoubi et al., 2019).

Guava Juice Degradation During Storage

Fruit juice will undergo natural degradation during storage due to several factors such as storage temperature, duration, and environmental exposure. Degradation in fruit juice quality is normally judged by monitoring the loss of certain quality attributes during storage at a selected temperature and duration. Several studies were conducted and reported that pink guava juice stored for several periods in various temperatures showed a significant degradation in terms of ascorbic acid, lycopene, antioxidant, total phenolic content, anthocyanin, and colour (Aishah et al., 2016; Bujang et al., 2016). In addition, increment of the bacterial count, degradation of ascorbic acid, visible sedimentation, and sensory attributes deterioration was also reported on white guava juice (Mousa., 2020; Rashid et al., 2018; Youssef et al., 2017; Kanwal et al., 2016; Sinchaipanit et al., 2015). Other pink guava products such as nectar also degraded during storage in terms of ascorbic acid, lycopene, non-reducing sugars, and sensory attributes (Ordóñez-Santos et al., 2010; Kanwal et al., 2016; Bal et al., 2014).

Methodology

The study was conducted using a quantitative approach and requires approximately nine months to complete. During laboratory work, data were collected manually and analysed statistically using statistical computer software. Below are elaborated explanations of the particular research methods.

Raw Materials

Golden Hope Food and Beverages Sdn Bhd supplied the pink guava puree and flavour enhancer, while Sime Darby Bioganic Sdn Bhd supplied the vitamin E. Additional ingredients, including citric acid, food colouring, food preservatives, food stabilisers, and emulsifiers, were acquired from Meilun Food Chemical Sdn Bhd, while sugar was acquired from a local grocery.

Preparation of Pink Guava Juice Drink Fortified with Vitamin E (PGJD)

PGJD was prepared by using a formulation adopted from Hashim & Ismail (2021) with modifications such as pink guava puree (12%), sugar (9%), citric acid (0.15%), food colouring (0.0006%), flavour enhancer (0.046%), preservative (0.015%), water (77.6%), stabiliser (0.2%), vitamin E (0.05%) and emulsifier (0.8%). The ingredients were homogenised with a laboratory homogenizer at 20,000 rpm for five minutes and then heated at 100°C for five minutes before being hot-filled into glass bottles. The finished product was cooled to room temperature immediately.

Determination of Viscosity

Viscosity was measured using the method of Phan et al., (2021) with slight modification, the analysis was performed using a rotatory viscometer (V1-L, Myr, Tarragona, Spain). At room temperature, a PGJD sample with a volume of 900 ml was poured into a beaker with a capacity of 1000 ml, and the viscosity of the sample was measured with an L1 spindle at a rotation speed of 200 revolutions per minute. After taking three separate readings, an average was calculated.

Determination of Colour

The colour changes were determined using a chromameter (CR400, Minolta, Japan). The method used was adapted from Hashim & Ismail (2021). The colour indices were measured using CIE L* C* H° colour space (The International Commission on Illumination, Vienna, Austria). Colour coordinates for L*, a*, and b* were measured, and other colour parameters were calculated, with chroma (C*) being the quantitative attribute of colour intensity or saturation. The higher the chroma value, the more saturated the colour. Chroma values were calculated as the following equation;

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2}$$

Hue angle (H°) is the qualitative attribute of the colour expressed as (0°/360°) red, (90°) yellow, (180°) green, and (270°) blue and calculated using the following equation below;

$$\text{Hue angle} = (\tan^{-1} a^*/b^*)$$

Determination of Emulsion Stability Index (ESI)

The emulsion stability of PGJD was calculated using the emulsion stability index (ESI) equation according to Hashim & Ismail (2021). The monitoring was done in triplicate samples. A higher ESI value indicated greater emulsion stability. The physical stability test was measured every 7 days until the storage period ended at 180 days. Results with ESI below 95% were considered unacceptable. The ESI index was measured as a percentage by using the following equation below;

$$ESI = \frac{(HE - (HC + HS))}{HE} \times 100$$



Emulsion height (HE), height of cream layer (HC) and height of sedimentation phase (HS)

Determination of Vitamin E (HPLC)

Vitamin E was determined by using a method from Hashim & Ismail (2021). 10 µL sample was injected into high-performance liquid chromatography (HPLC) (1200 Series, Agilent, USA). The mobile phase used was an 88:4:8 mixture of acetonitrile, chloroform, and ethyl acetate. The HPLC flow rate was set at 1.3 ml/min and the wavelength at 292 nm, and the vitamin E was detected using a UV detector and analytical column C18.

Statistical Analysis

All data obtained were calculated as mean values of triplicate analysis and then subjected to one-way ANOVA using SPSS software.

Result and Discussion

Viscosity Changes in PGJD During Storage

Figure 1 shows the viscosity values of PGJD stored for 180 days at 5°C, 15°C, and 25°C. The results also show that all of the different samples stored for 180 days at different temperatures produced the same viscosity pattern, with the viscosity value of all samples gradually increasing. The results also show that on day 1, PGJD stored at 5°C had the highest significant viscosity value (34.33 ± 1.34 mPa.s), while PGJD stored at 25°C had the lowest significant viscosity value (17.33 ± 1.33 mPa.s). After 180 days of storage, PGJD stored at 25°C had the highest significant viscosity value (64.67 ± 2.73 mPa.s), while PGJD stored at 5°C had the lowest viscosity (53.67 ± 1.51 mPa.s). The results showed that the viscosity of PGJD stored for 180 days at 25°C increased faster than PGJD stored at 15°C and 5°C. Figure 1 shows how the viscosity line for PGJD stored at 25°C crossed the lines for the other two samples. Hence, indicated that by using higher temperatures during the storage process, the viscosity of PGJD increased significantly faster than samples that were stored at a lower temperature range.

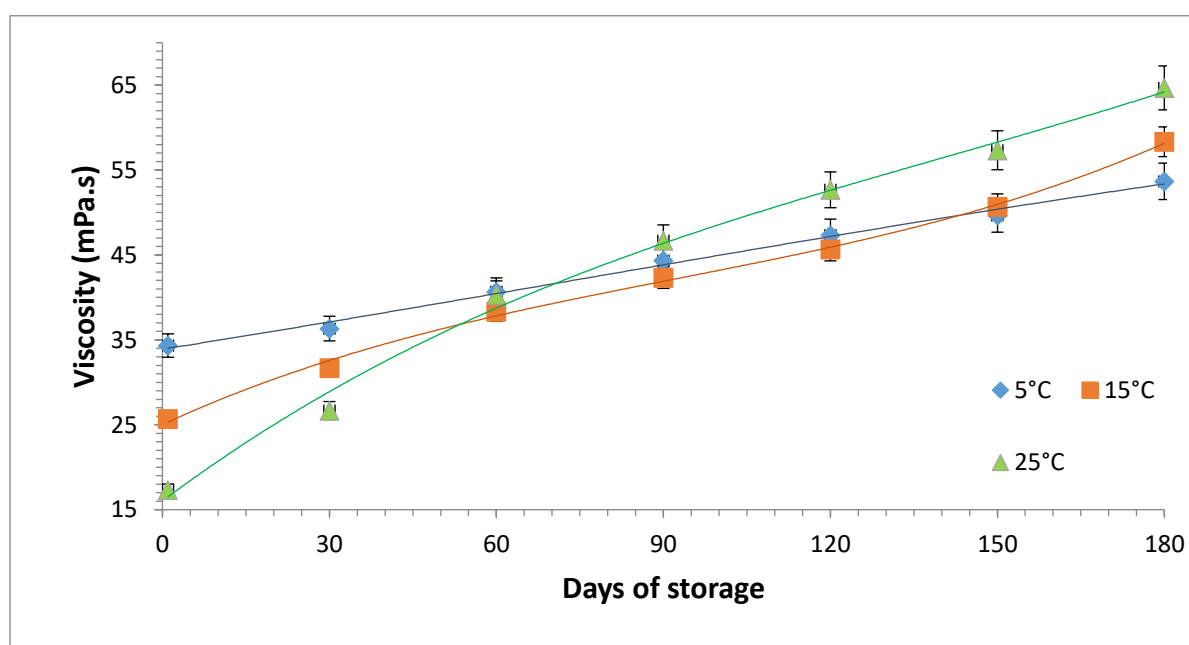


Figure 1: Effect Of Storage Time At Different Temperatures On Viscosity Of PGJD

According to a study conducted by Parquet et al. (2014), the viscosity of fruit juice beverages stabilised with xanthan gum stored at 20 °C for 16 weeks shows a significant viscosity increment when compared to the same samples stored at 4 °C. It is due to strong polysaccharide–polysaccharide interactions, resulting in increased aggregate size and formation of a semi-gel type network. A similar increment of viscosity values at various stabilisers and temperatures of storage was also reported by Szczepańska et al., (2021) and Aggarwal et al., (2020) on apple juice and kinnow juice, respectively. These phenomena were also found and explained by Bi et al. (2020) and Zhou et al., (2017) whereby leaching of natural soluble pectin and high molecular carbohydrate from cell structure due to processing and storage temperature played a role in viscosity escalation during storage of carrot juice and mango juice. Thus, a more concentrated pectin colloidal solution would result in a product with higher viscosity.

Colour Changes in PGJD During Storage

Lightness Value (L^*)

Figure 2 shows the effect of PGJD's L^* values on storage time at various temperatures. During 180 days of storage, the lowest and highest L^* values of PGJD that were stored at 5°C, 15°C, and 25°C were recorded at storage days 0 and 180. PGJD stored at all temperatures recorded a similar L^* value of 33.45 ± 0.05 . At storage day 180, the highest L^* value of PGJD that was stored at 5°C, 15°C and 25°C was 45.03 ± 0.03 , 52.35 ± 0.03 , and 57.51 ± 0.02 , respectively. At storage day 0, there were no significant differences in L^* value between PGJD stored at 5°C, 15°C, and 25°C. However, there were significant differences in L^* value between PGJD that were stored at 5°C, 15°C and 25°C starting from 30 to 180 days of storage. The results also revealed that the L^* value for all samples increased over the course of 180 days of storage, with the red colour from PGJD gradually fading over time. Varasteh et al. (2012), Aguiló-Aguayo et al. (2010), Cortes et al. (2008) and Choi et al. (2002) observed a similar increasing pattern of L^* value during storage in pomegranate, watermelon, red grapefruit, orange, and blood orange juice due to partial precipitation of unstable suspended particles in the juice. The L^* value of PGJD stored at 25°C increased by 171.9% from its initial value (storage day 0), while it increased by 156.5% and 134.6% in PGJD stored at 15°C and 5°C, respectively. These findings revealed that higher storage temperatures resulted in a faster-increasing pattern of the L^* value. The result also indicated that low-temperature storage could slow the increment of the L^* value in PGJD. Similarly, Esteve et al. (2005) found that storing orange juice at low temperatures resulted in slower changes in the L^* value. In addition, research conducted by Wibowo et al., (2015), Alighourchi & Barzegar (2009) found that storing orange and pomegranate juice at high temperatures for an extended period of time or processing may result in a faster loss of colour, a fading of the red colour, an increase in the browning effect, and alterations in the L^* values.

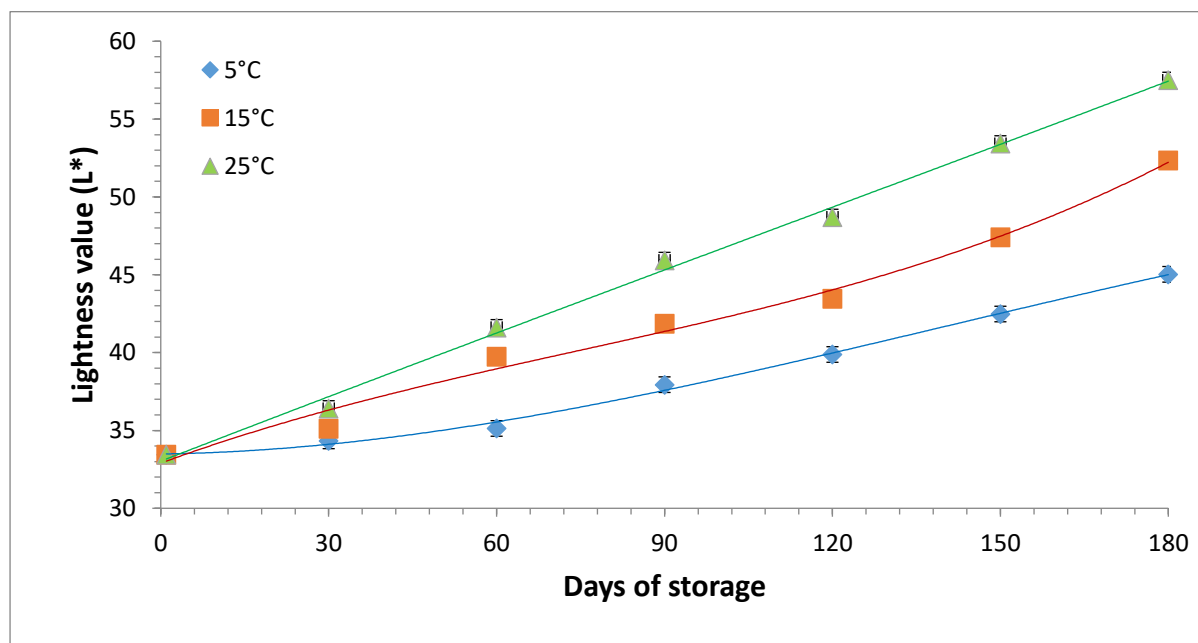


Figure 2: Effect Of Storage Time At Different Temperatures On Lightness (L^*) Value Of PGJD

Chroma Value (C^*)

Figure 3 depicts the C^* value results, which show that at storage day 0, the C^* values for PGJD stored at 5°C, 15°C, and 25°C were 31.22 ± 0.04 , 31.47 ± 0.02 , and 31.69 ± 0.01 , respectively. On the other hand, the C^* value for PGJD stored for 180 days at 5°C, 15°C and 25°C were 10.64 ± 0.02 , 6.87 ± 0.04 , and 4.21 ± 0.01 , respectively. It demonstrated that C^* values gradually decreased after 180 days of storage and the intensity of the red colour decreased during storage. Wibowo et al. (2015) and Choi et al. (2002) reported similar findings on orange juice, where significant degradation of C^* values was observed due to anthocyanin degradation during storage. Furthermore, C^* value loss during storage was explained by Krebbers et al. (2003) and Rodrigo et al. (2007) who found that colour deterioration in red-coloured fruit juice may be related to a loss of anthocyanin and lycopene pigments during storage. Furthermore, Kong et al. (2010) stated that lycopene is the pigment that gives pink guava its red colour and that it is easily degraded during storage in an oil-containing emulsion. Meanwhile, Sharma (1996) discovered that lycopene auto oxidises during storage and where exposure to temperature, air, and light has a significant impact on lycopene degradation. Through 180 days of storage, PGJD stored at 25°C showed the greatest reduction in C^* value (86.7% decrease) compared to PGJD stored at 15°C (78.2% decrease) and PGJD stored at 5°C (66.2% decrease). These results revealed that by using a lower temperature of storage, C^* values in PGJD decreased slower compared to samples that were stored at a higher temperature. Similar findings were reported by Habibi et al., (2021), Wibowo et al., (2015), Alighourchi & Barzegar (2009), who found that orange and pomegranate juice stored at lower temperatures showed a slower loss of C^* value, whereas Kong et al. (2010) found that lycopene in food and beverages had low stability and its loss rate varied depending on temperature, pH, and the presence of oxygen and light.

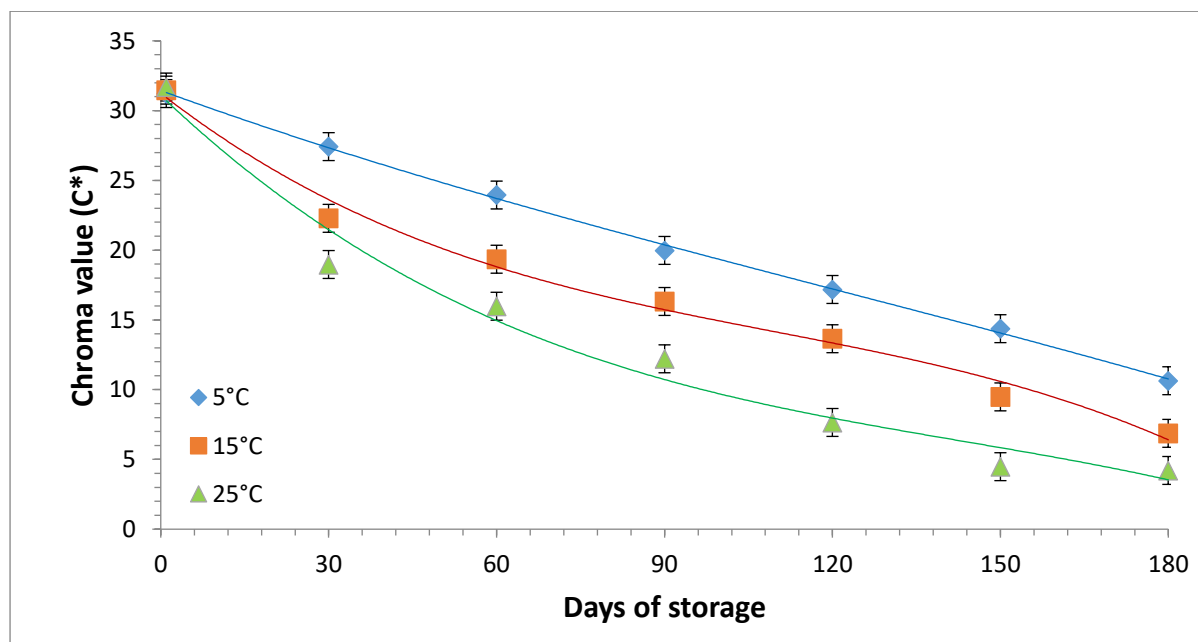


Figure 3: Effect Of Storage Time At Different Temperatures On Chroma (C^*) Value Of PGJD

Hue Angle Value (H°)

Meanwhile, the result for H° value was illustrated in Figure 4 where it showed that on day 1, there was no significant difference ($p < 0.05$) between H° value of PGJD stored at 25°C (23.44 ± 0.02), 15°C (23.42 ± 0.03) and 5°C (23.43 ± 0.04). After 180 days of storage, there was

a significant difference in H° value, with the value for PGJD stored at 25°C, 15°C, and 5°C being 60.27 ± 0.01 , 53.35 ± 0.06 , and 48.41 ± 0.03 , respectively. It also confirmed that after 30 days of storage, all PGJD samples showed a gradually increasing pattern in H° value. Furthermore, the results showed that the H° value of PGJD was affected equally by storage period and storage conditions. According to Varasteh et al. (2012) and Choi et al. (2002) the H° value of blood orange and pomegranate juice increased significantly during storage. The H° value for PGJD stored at 25°C increased by 257.1% from its initial value (storage day 0), while PGJD stored at 15°C and 5°C increased by 227.7% and 206.6%, respectively. This result indicated that higher temperature of storage resulted in a faster-increasing pattern on H° value. Mart et al. (2002) found that the H° value for pomegranate juice stored at 25°C increased significantly faster than juice stored at 5°C. Furthermore, the value changes in colour parameters such as L^* , C^* , and H° were slower in fruit juice stored at low temperatures rather than in higher temperatures, which was most likely due to lower enzyme activity (Varasteh et al., 2012).

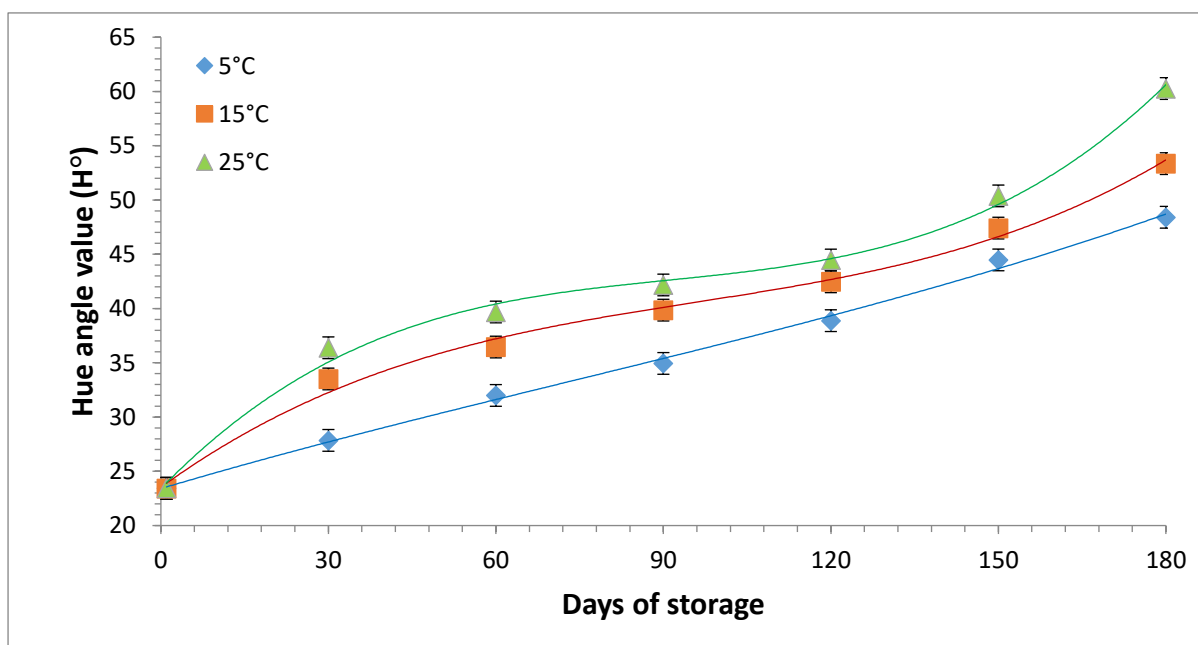


Figure 4: Effect Of Storage Time At Different Temperatures On Hue Angle (H°) Value Of PGJD

Emulsion Stability in PGJD During Storage

Figure 5 shows several emulsion stability values of PGJD that were stored for 180 days at 5°C, 15°C, and 25°C. The PGJD's emulsion stability was assessed using the ESI equation, with a higher percentage indicating a better result. According to the findings, the ESI of all PGJD samples decreased over the course of 180 days of storage. It also indicated that PGJD samples from all storage temperatures destabilised slowly and phase separation occurred where precipitation formed at the bottom of glass bottles due to gravitational separation (McClements, 1999). Mirhosseini et al. (2008) discovered a similar decreasing pattern in orange juice after 180 days of storage.

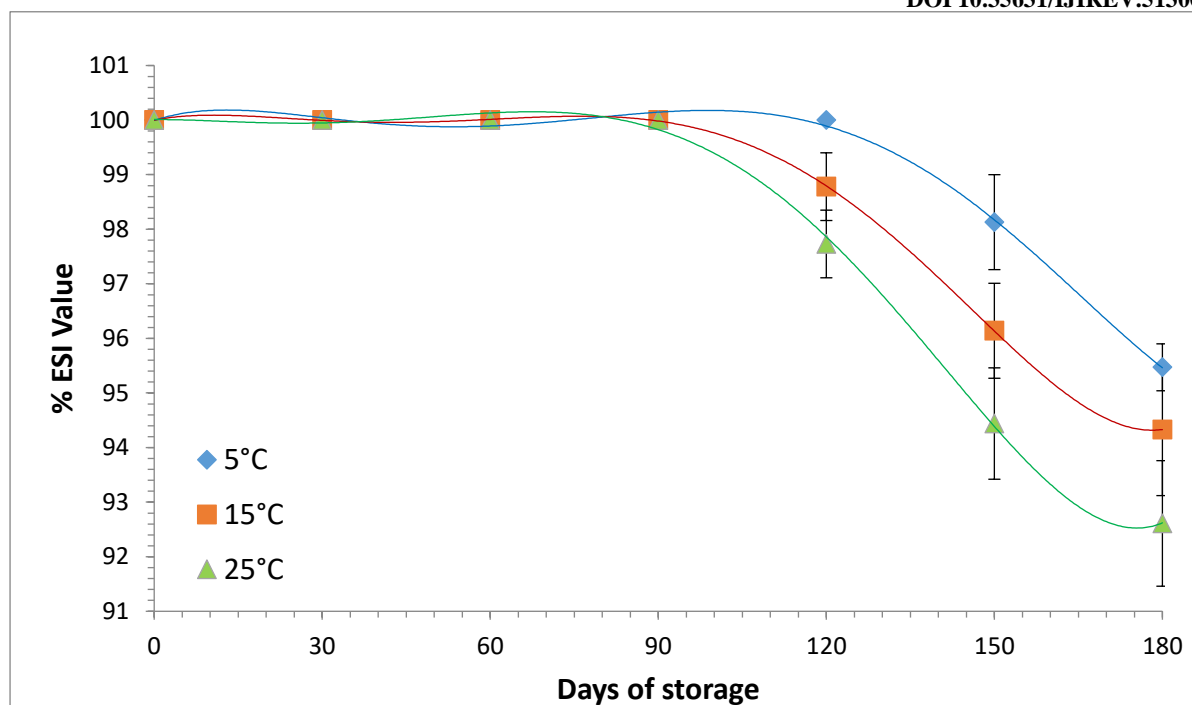


Figure 5: Effect Of Storage Time At Different Temperatures On Phase Separation Of PGJD

According to the results in Figure 5, ESI for all samples was initially (at storage day 0) 100%. The highest significant ESI value was obtained after 180 days of storage on PGJD stored at 5°C (95.47 ± 0.43). Meanwhile, there are no significant differences in all storage temperatures between PGDJ stored at 15°C (94.33 ± 1.21) and 25°C (92.61 ± 1.15). The result also showed that PGJD samples that were stored at 15°C and 25°C started to destabilise after day 120 and PGJD samples that were stored at 5°C destabilised after day 150. It was discovered that when emulsions are stored at low temperatures, samples remain stable for approximately 30 days longer than when stored at higher temperatures. According to Zulueta et al. (2010), orange juice stored at 4°C showed 7 days slower phase separation compared to orange juice stored at 10°C. Furthermore, Salvia-Trujillo et al. (2011) observed a similar pattern in orange juice stored at a refrigerated temperature (4°C) and found no evidence of phase separation even after 56 days. The results also indicated that the application of lower storage temperature resulted in no significant reduction of ESI percentage.

Vitamin E Degradation in PGJD During Storage

Figure 6 depicts the vitamin E stability of PGJD stored at 5°C, 15°C, and 25°C over a 180-day period. The results showed that processing PGJD at 100°C for 5 minutes significantly reduced the vitamin E content by up to 88.4%. Vitamin E was found to be less stable to heat and light after processing. After 180 days of storage at 25°C, the content of vitamin E in the PGJD was reduced to 9.9 ± 0.78 mg/l, but then, the content deterioration was slower compared to the colour deterioration. According to Öztürk (2017), Eitenmiller and Lee (2004), natural vitamin E is relatively unstable to air, heat, light, alkali, and metal ions.

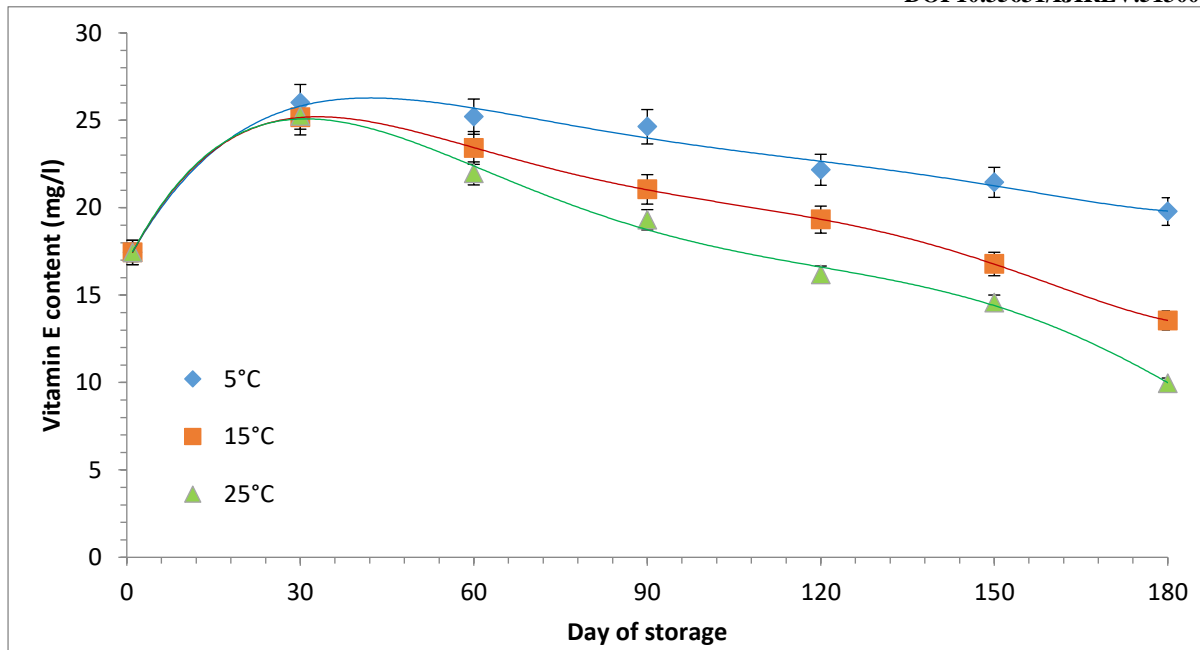


Figure 6: Effect Of Storage Time At Different Temperatures On Vitamin E Content Of PGJD

At storage day 0, PGJD stored at 5°C, 15°C, and 25°C had similar vitamin E content of 17.44 ± 1.63 mg/l. Vitamin E content increased drastically during the first 30 days of storage and then gradually decreased until the end of the storage period. PGJD stored at 5°C (19.78 ± 1.13 mg/l) had the highest vitamin E content at 180 days, followed by 15°C (13.55 ± 1.32 mg/l) and 25°C (9.96 ± 0.78 mg/l). By comparing to the initial vitamin E fortification amount which was 225 mg/l, the loss of vitamin E in PGJD that was recorded after 180 days of storage were 91.2%, 93.9%, and 95.6% for 5°C, 15°C and 25°C respectively. As a result of exposure to environmental factors such as light, oxygen, temperature, moisture content, water activity, and lipid oxidation, vitamin E naturally produced a consistent degradation pattern during storage (Ferguson et al., 2014, Behery et al., 2010; Hurtado et al., 2000). Correspondingly, the highest vitamin E content recorded for PGJD stored at 5°C, 15°C, and 25°C after 30 days was 26.01 ± 1.03 mg/l, 25.17 ± 0.54 mg/l, and 25.25 ± 0.98 mg/l, respectively. The vitamin E content increased during the first 30 days of storage, which could be attributed to the degradation of the P80 (polysorbate 80) emulsifier. P80 acted by encapsulating and dissolving vitamin E oil particles in PGJD emulsion. P80's emulsifying ability weakened over time due to its own degradation, and vitamin E particles were gradually released into the juice emulsion. As a result, vitamin E concentration increased during the first 30 days and then began to decline. According to Hvattum et al. (2012), polysorbate 80 auto-oxidizes due to the formation of peroxides and hydrolysis of the fatty acid ester bond. According to Kerwin et al. (2008), auto-oxidation and hydrolysis of polysorbates were highly dependent on the sample, pH, oxygen, peroxides, heat, UV light, and metal ions such as copper. According to Whitney et al. (2018), the recommended dietary allowances (RDA) for vitamin E for adults and children (4-8 years) are 15 I.U/day (10.005mg/day) and 10.4 I.U/day (7mg/day), respectively. Figure 6 showed that the lowest vitamin E content in PGJD after 180 days of storage was 9.96 ± 0.78 mg/l at 25°C storage temperature. One litre of juice contains 2.49 ± 0.2 mg of vitamin E per serving. The study also concluded that consuming four servings of PGJD that had been stored for six months could still provide nearly enough vitamin E for an adult in a day.

Conclusion

The results show that PGJD viscosity increased significantly slower when stored at a lower temperature. Furthermore, low-temperature storage will keep emulsions stable for approximately 30 days longer than higher-temperature storage. The colour's L* value for all samples demonstrated an increasing pattern during storage where the C* values indicated that the red colour intensity from PGJD was gradually fading over time. Furthermore, during storage, H° value for all PGJD samples produced a gradually increasing pattern, whereas vitamin E produced a consistent degradation pattern. Based on the research findings, it can be concluded that all the objectives were successfully achieved. It also reinforces the fact that lower storage temperatures can significantly slow down the degradation process of pink guava juice as well as its vitamin E stability. The results of this study can assist beverage manufacturers and retailers in determining the temperature range in which fruit juice products should be stored for the best possible quality. Similar research can be conducted in the future on the various types of fruit juice that are available in Malaysia.

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