Research Article

DOI: 10.22146/ifnp.65771 https://journal.ugm.ac.id/ifnp

ISSN 2597-9388

Indonesian Food and Nutrition Progress

Copigmentation of Anthocyanin Extract from Parijoto Fruit (*Medinilla speciosa*) and Its Stability at Different Temperatures and Heating Durations

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ABSTRACT: Parijoto fruit (Medinilla speciosa) has a red color and contains anthocyanins. Anthocyanins have low stability due to the effect of heating. Copigmentation can be applied to improve the stability of anthocyanins. This research aims to study the effect of the anthocyanin extract from parijoto fruit ratio and copigment on anthocyanin concentration and color stability during heating at different temperatures and durations. Anthocyanin copigmentation using tannic acid in a ratio of 1:20 and 1:40 with control without copigmentation (1:0) showed that increasing the tannic acid copigment would increase anthocyanin concentrations and reduce anthocyanin losses due to increased temperature and heating time. The use of 1:40 tannic acid copigment increased anthocyanin concentration by 38%. In addition, increasing the concentration of tannic acid increased the ability of anthocyanins to maintain color retention during heating and increased temperatures. The use of tannic acid at a ratio of 1:40 only decreased color retention by 16%, compared to control (54%) after heating at 75ºC heating for 120 minutes. Copigmentation with tannic acid up to a ratio of 1:40 was able to maintain the stability of the lightness (L*), the reddish value (a*), and the yellowish value (b*) of the anthocyanins. This study showed that this system is potential for food coloring application in the food industry.

Keywords: *Anthocyanins, Color stability, Copigmentation, Medinilla speciosa*

INTRODUCTION

The color of food products is one of the main quality parameters that determine consumer acceptance. It has led to the increasing use of dyes in food products (Catrien, 2009). Synthetic dyes are the most used food coloring in food industry because they have advantages over natural. They are attributed to better color stability in various food processing methods, and less concentration is required for their application. However, the use of artificial dyes has become a concern because it can interfere with health and cause damage to organs if consumed continuously (Fathinatul labibah et al., 2014), it can even cause death (Yuniwati et al., 2012). Therefore, the use of natural dyes can be an option in manufacturing food products.

Natural dyes can be produced from various natural materials such as plants, animals, and minerals (Koswara, 2009). Natural dyes are safer for health and have been widely used by the public (Catrien, 2009). One of the natural dyes is anthocyanin which can be extracted from plants. Anthocyanins are flavonoid derivatives that give red to purple colors (He & Monica, 2010). One source of anthocyanins that have potential as natural dyes come from parijoto fruit (*Medinilla speciosa*) (Hasbullah et al., 2018). Parijoto fruit has a characteristic red to purple color at full maturity (Nugroho et al., 2019). Parijoto fruit contains anthocyanins, which will be increased during fruit ripening (Ameliawati, 2018). Anthocyanin pigments produce a strong and sharp red color widely applied in various industries such as food and beverage and as dyes in DSSC (Dye-Sensitized Solar Cell) (Maulina et al., 2014). However, the application of anthocyanins as coloring agents has obstacles, especially in terms of color stability (Hidayah & Pratjojo, 2014). The use of anthocyanins as dyes is limited by their unstable properties to oxygen, light, pH, sugar, and temperature (Kokkaew et al. 2015; Pertiwi et al. 2019; Hasbullah et al. 2020)). Therefore, its stability needs to be improved (Nusantara et al., 2018).

One of the efforts to increase the stability of anthocyanins is using copigmentation (Sari, 2016). The interaction of anthocyanins with water molecules causes anthocyanins to degrade (González-Manzano et al., 2009). The addition of copigment compounds can increase the stability of anthocyanins by forming complex bonds with anthocyanins (Wahyuni et al., 2017). The copigmentation effect causes the anthocyanin color to become redder and more stable. It occurs because of the interaction between the anthocyanin structure and copigment molecules (Catrien, 2009). Copigments that match the anthocyanin chemical structure can form bonds between the free electrons of the copigment and the electron-deficient nucleus of the flavilium cation. This causes an electron balance that inhibits the rate of anthocyanin degradation (Castañeda et al., 2009). Copigmentation is influenced by several cofactors such as phenolic acid compounds, flavonoids, amino acids, alkaloids, and interactions between anthocyanin molecules (Bimpilas et al., 2016). Copigments that can be used include compounds derived from the flavonoids (catechins and epicatechins), alkaloids (caffeine), polymer flavonols (tannins), phenolics (catechols and methyl catechols), amino acids, organic acids, nucleotides, polysaccharides, metals, and even the anthocyanins themselves (Kopjar & Piližota, 2009). Tannic acid is a copigment that can be applied to anthocyanins.

Previous research has shown that copigmentation with tannic acid was able to stabilize the anthocyanin extract of Dutch eggplant skin (*Cyphomandra betacea* Sendtn) (Wahyuni et al., 2017). The effectiveness of tannic acid as a copigment was also shown by Wulandari et al. (2018) that the stability of tannic acid copigmented anthocyanins was higher than catechols. Tannic acid copigmentation was able to reduce the anthocyanin breakdown rate and the color retention rate to only 0.07 mM/days and 2.39%/days, respectively. Therefore, tannic acid is very potential to be used as an anthocyanin copigment. So far, research on anthocyanins copigmentation from parijoto fruit is still limited. Therefore, in this study, the

anthocyanin copigmentation of parijoto fruit was carried out using tannic acid copigment.

Anthocyanin stability to temperature and heating duration is a concern in its application in food products. High temperatures cause anthocyanins to break down. Wahyuni (2017) explained the occurrence of anthocyanin structural damage due to an increase in temperature. It begins with the hydrolysis of the glycosidic bonds of anthocyanins at position 3 and produces labile aglycones. Then the aglycone ring opens to form a colorless carbinol and chalcone group. In addition, the duration of heating also causes an increase in the number of damaged anthocyanins (Sipahli et al., 2017). Copigment molar ratio affects the optimization of the copigmentation process. Copigment molar ratio that is too low causes the copigment complex to be not strong because the charge transfer from the copigment is insufficient. While the molar ratio is too large, the charge transfer can no longer occur because it has exceeded the charge required to form a strong copigmented complex (Wulandari, 2016). Copigmentation is expected to increase the stability of anthocyanins against heating. This study examined the stability of tannic acid copigmentation of anthocyanins from parijoto fruit against heating temperatures and durations.

MATERIALS AND METHODS

Material

Full ripe Parijoto fruits (*Medinilla speciosa*) with purplish-red color rind from Muria Mount, Kudus, Central Java, Indonesia were used. The samples were harvested in the morning, stored in cold storage boxes, and then delivered to the laboratory. Analytical materials include Ethanol 96% (PT. Brataco), Tannic Acid (Sigma Aldrich), Distilled water (PT. Multi Kimia Raya Nusantara), Formic acid (PT. Multi Kimia Raya Nusantara), Potassium chloride (PT. Brataco),

Preparation of Medinilla sp. Anthocyanins Extract

The selected parijoto fruit was a fruit with a purplish-red color. The extraction of parijoto fruit anthocyanin was conducted using the maceration method based on modified Juniarka et al. (2011). Parijoto fruits were mortared and finely grounded using a blender. Twenty-five grams of sample was added with 250 mL 96% ethanol (added 0.1M formic acid with ratio 97:3) in Erlenmeyer covered aluminum foil for 24 hours maceration on shaking water bath at 30 °C. The filtrate was obtained using filter paper and then collected and evaporated with a Rotary Evaporator (Lanphan RE-501) at a temperature of 40 °C for 3 hours to obtain crude anthocyanins. The anthocyanin concentrate was stored in cold storage until copigmentation.

Physicochemical, microbiological and sensory analyses were carried out on each fresh and stored pure juices and mixtures. Juice samples intended for storage were placed in plastic bottles (350 ml) and were kept in a refrigerator at 4 °C for one week or 14 days.

Copigmentation of Medinilla sp. Anthocyanin Extract

The sample segmentation was based on the method used by Wulandari et al. (2018) with modification. Parijoto anthocyanin concentrate (0.2 mg) was dissolved with an ammonium acetate buffer solution (pH 3.5) to 20 mL with a measuring flask. Then it was reacted with tannic acid copigment under the treatment of the molar ratio. Calculation of copigment ratio using the formula (Wahyuni et al., 2017):

Copigment = C x BM x V/1000 x R

Description:
C = Initial anthocyanin concentration (mM)
BM = Molecular weight of copigmen (BM Tannic acid = 1701.19 g/mol)
V = Extract volume (20 mL)
R = Ratio (0/20/40)

Tannic acid was prepared 20 and 40 times the number of moles compared to 0.2 mg of anthocyanin concentrated extract (1:20 and 1:40). Control was prepared without adding tannic acid (1:0). The anthocyanins concentrated were diluted in a 3.5 pH ammonium acetate buffer solution to 20 ml in a measuring flask. The homogenization process was performed using a magnetic stirrer for 5 minutes. A homogeneous solution was pipetted and inserted into a test tube of 5 mL each. Non-segmented anthocyanin was scanned at wavelengths of 250 - 700 nm and then analyzed with a specific time heating temperature based on He et al. (2018) modified by inserting anthocyanin extract into heated water and measuring anthocyanin absorption in each period time every 30 minutes to 2 hours with three replication. Measurements were performed at a maximum wavelength (λ) of 520nm.

Design of Experiments

The experiment was designed with Factorial Design with two factors. The first factor was the difference in the ratio of tannic acid added to parijoto anthocyanin concentrate which consisted of 3 levels. The second factor of heating temperature used after copigmentation consisted of 3 levels. The anthocyanin and tannic acid mol ratio for copigmentation were (1:0), (1:20), and (1:40). The heating temperature used after copigmentation consisted of 3 levels i.e. 30ºC, 60ºC, and 75ºC. Sampling analysis was taken every 30 minutes heating period with a total heating time of 2 hours.

Determination of Total Monomeric Anthocyanin

Determination of anthocyanin levels was performed using modified differential pH method according to Inggrid & Iskandar (2016) a 0.2 mg anthocyanin concentrate was added KCl buffer solution at pH 1 and Na-Acetate buffer at pH 4.5 in 20mL measure flask (DF= 100). Then it was added tannic acid copigment with a specified ratio. The absorbent value of each sample was measured by a Spectrophotometer UV-Vis at λ 525 nm and λ 700 nm. The total anthocyanins in the sample were calculated using the formula:

$A=(A_{520}-A_{700})_{pH1.0}$ - $(A520-A700)_{pH4.5}$

Total Anthocyanins (mg/L)= (A x MW x DF x 1000) / (ϵ x l)

Description:
ε = molar absorptivity of cyanidin-3-glucoside (26900 L/mol.cm)
l = width of cuvette (1 cm)
MW = molecular mass of cyanidin-3-glucoside (449.2 g/mol)
DF = dilution factor

Measurement of Color Retention

The color stability of parijoto anthocyanin concentrate was conducted based on Sipahli *et al.* (2017) with modification. The absorbent color of anthocyanin concentrate that was not coffeed or segmented during heating at a certain time was read on a buffer solution of pH 3.5 at λ 520 nm. The color of anthocyanin concentrate was a combination of the color sourced from anthocyanins and the color of other flavonoid compounds (λ700). Anthocyanins and other flavonoids with a pH of 3.5 were in a stable structure so that color retention observations were read at pH 3.5.

Color Analysis

Color stability was based on Catrien's (2009) measurements using Precision colorimeter through color intensity parameters with color notation systems L*, a*, b*. An L* for light levels and a* and b* for green-red and blue-yellow components, respectively (Sinaga, 2019). 20 mL samples were placed in the glass cells. Observations with a Precision colorimeter (NR20XE) described the degradation of anthocyanins in terms of the appearance of the color of parijoto anthocyanin.

Statistical Analysis

Data were analyzed by one-way ANOVA using the SPSS program. If there was a difference continued with DMRT ($P<0.05$).

RESULT AND DISCUSSION

Determination of Anthocyanin Levels in Heating Treatment and Different Copigmen Ratios

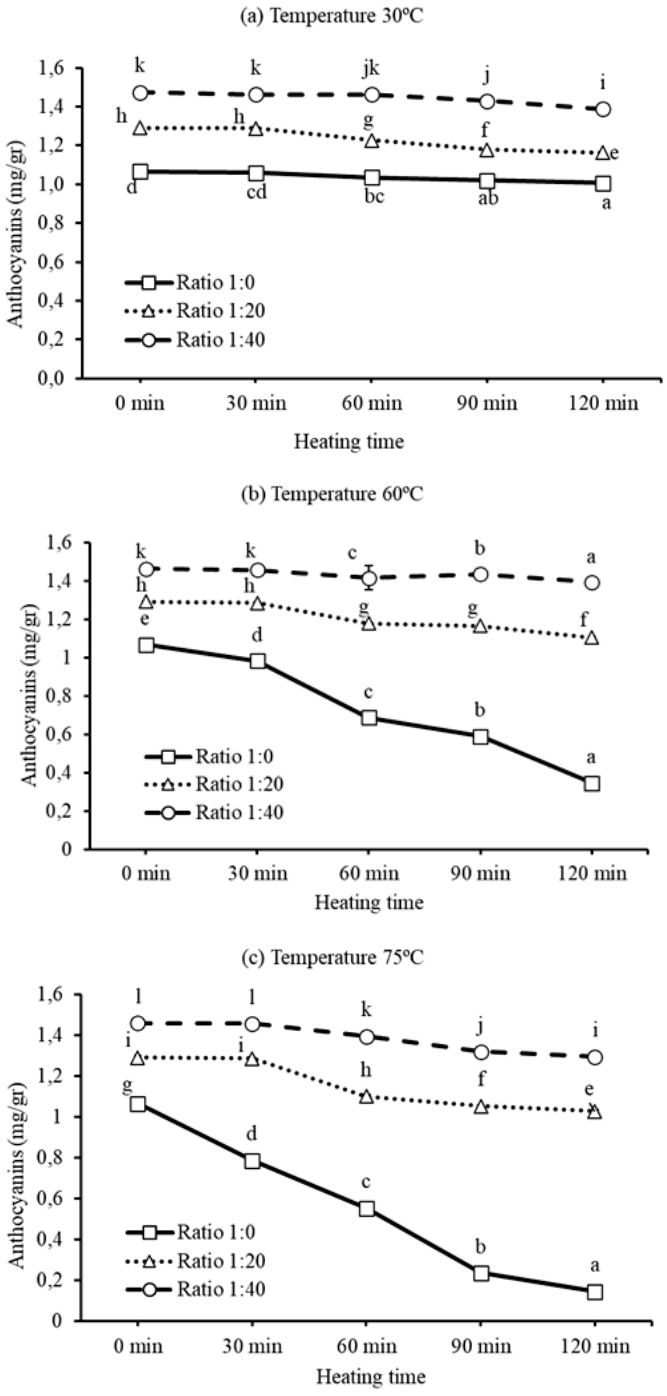


Figure 1. Anthocyanins concentration (a) under 30ºC heating treatments, (b) under 60ºC heating treatments, (c) under 75ºC heating treatments. The different notation shows significantly different ($p<0.05$). The data are presented with the standard deviation of 3 sample replications.

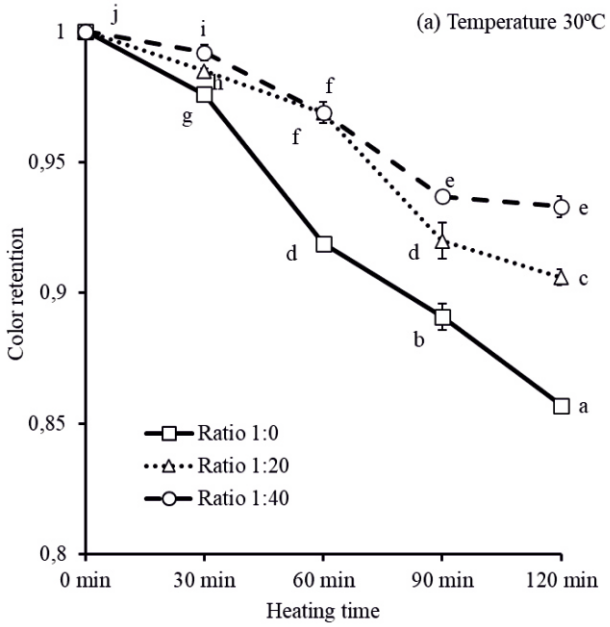
The increase in heating temperature caused a decrease in the anthocyanin content in the concentrated anthocyanin extract (Figure 1). The longer the heating, the lower the anthocyanin content. The total anthocyanin content in Parijoto fruits extracts before copigmentation was 1.068 mg/g. Heating at room temperature (30ºC) for 120 minutes only decreased the anthocyanin content by 6.3% in the non-copigmentation (Figure 1a). Meanwhile, the copigmentation of parijoto anthocyanins with tannic acid at a ratio of 1:20 and 1:40 reduced the anthocyanin content by only 9.8% and 5.8%, respectively. Heating at a common temperature for drying (60°C) for 120 minutes reduced the anthocyanin content by 67.6% in the non-copigmentation treatment (Figure 1b). Meanwhile, the copigmentation treatment with tannic acid at a ratio of 1:20 and 1:40 reduced the anthocyanin content by only 14.3% and 4.8%, respectively. Heating at a pasteurization temperature (75ºC) for 120 minutes reduced the anthocyanin content by 86.3% in the non-copigmentation (Figure 1c). Meanwhile, the copigmentation treatment with tannic acid at a ratio of 1:20 and 1:40 reduced the anthocyanin content by only 20.2% and 11.3%, respectively.

The decrease in anthocyanin content was caused by heat-induced damage to anthocyanins. The higher the temperature and the longer the heating causes anthocyanin damage to increase (Ingrath et al., 2015). Anthocyanin damage occurs due to anthocyanin degradation due to the loss of glycosyl bonds due to glycosidic hydrolysis. The heating causes the pyramid ring to open, resulting in further degradation which results in a decrease in the anthocyanin content (He et al. 2015; Brouillard et al. 2003). Heating at a temperature of more than 70ºC causes significant degradation of anthocyanins (Dai & Mumper, 2010).

The rate of anthocyanin damage during heating at 30ºC was 16.8 µg/g per 30 minutes in the non-copigmentation treatment can be seen in Figure 1a. Meanwhile, parijoto anthocyanin copigmentation with tannic acid at a ratio of 1:20 and 1:40 had anthocyanin damage rates of 31.8 µg/g per 30 minutes and 21.5 µg/g per 30 minutes, respectively. The rate of anthocyanin damage during heating at 60°C was 180.5 µg/g per 30 minutes in the non-copigmentation treatment (Figure 1b). Meanwhile, the tannic acid copigmentation treatment at a ratio of 1:20 and 1:40 decreased the rate of anthocyanin damage to 46.3 µg/g per 30 minutes and 17.5 µg/g per 30 minutes, respectively. The rate of anthocyanin damage during heating at 75ºC was 230.5 µg/g per 30 minutes in the non-copigmentation (Figure 1c). Meanwhile, the tannic acid copigmentation treatment at a ratio of 1:20 and 1:40 decreased the rate of anthocyanin damage to 65.3 µg/g per 30 minutes and 41.3 µg/g per 30 minutes, respectively.

Copigmentation of parijoto anthocyanins with tannic acid causes an increase in anthocyanin stability due to an increase in temperature and heating time (Figure 1). The increasing of the tannic acid copigment ratio (1:0, 1:20, and 1:40) when heating at 30ºC for 120 minutes kept the anthocyanin stability up to 93.7%, 90.2%, and 94.2%, respectively. Increasing the tannic acid copigment ratio (1:0, 1:20, and 1:40) on heating at 60 °C for 120 minutes increased the stability of anthocyanins from 32.4% to 85.7% and 95.2%, respectively. Increasing the tannic acid copigment ratio (1: 0; 1:20 and 1:40) on heating at 75ºC for 120 minutes increased the stability of anthocyanins from 38.9% to 79.8% and 88.7%, respectively. This was due to the formation of bonds between anthocyanins and copigments to protect anthocyanins from water nucleophilic attacks on the pyruium ring, the anthocyanin structure (Escribano-Bailon & Celestino, 2012). Previous research on the anthocyanins of Berberis fruit extract showed a decrease with increasing temperature, but the addition of copigments reduced anthocyanin degradation and increased anthocyanin stability (Santoso & Estiasih, 2014). The addition of tannic acid as a copigment of Dutch eggplant anthocyanins led to increased anthocyanin concentrations (Wahyuni et al., 2017).

Effect of Copigmentation on Color Retention on Heating Treatment



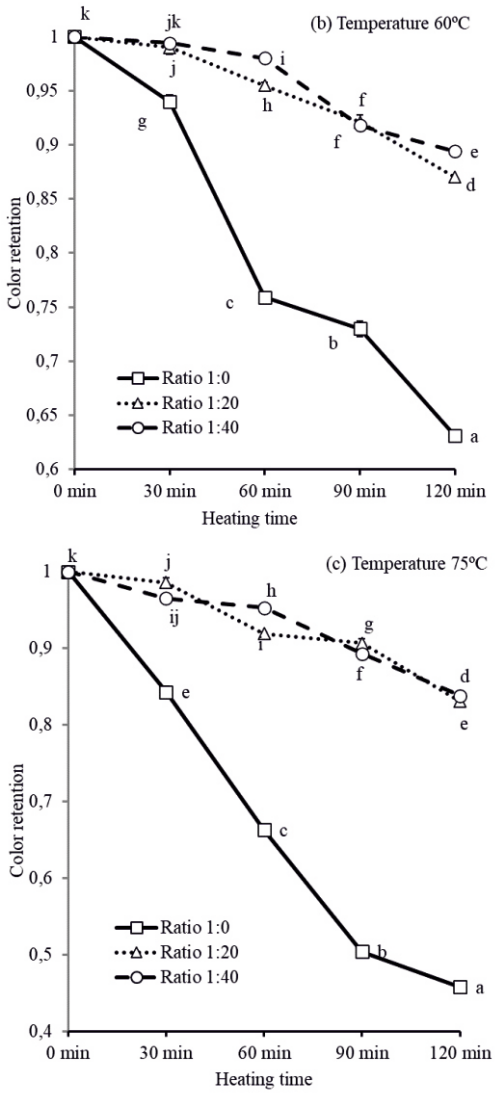


Figure 2. Color retention (a) under 30ºC heating treatments, (b) under 60ºC heating treatments, (c) under 75ºC heating treatments. The different notation shows significantly different ($p<0.05$). The data are presented with the standard deviation of 3 sample replications.

Increasing heating temperature and longer heating decreased color retention of parijoto anthocyanins (Figure 2). Heating at 30ºC for 120 minutes resulted in decreased color retention by 14.3% in the non-copigmentation (Figure 2a). Whereas 1:20 and 1:40 copigmentation only caused a decrease in color retention of 9.4% and 6.7%, respectively. Heating at 60ºC for 120 minutes caused color retention to decrease by 36.9% in the non-copigmentation (Figure 2b). Whereas 1:20 and 1:40 copigmentation only caused a decrease in color retention by 13% and 10.6%, respectively. Heating at 75ºC for 120 minutes caused color retention to decrease by 54.2% in the non-copigmentation (Figure 2c). Whereas 1:20 and 1:40 copigmentation only caused a decrease in color retention by 16.9% and 16.2%, respectively.

Heating greatly affected the reduction of color retention due to anthocyanin degradation. This corresponds to the anthocyanin damage due to heating (Figure 1). Anthocyanin copigmentation was able to maintain color retention. It is because anthocyanin copigmentation stabilized the anthocyanin content during heating (Saati et al., 2012). The stability of the anthocyanin content causes stable color retention. The use of a copigment ratio that was too low causes ineffective copigmentation, while a copigment ratio that was too high was not efficient for the use of copigments. (Wahyuni et al., 2017). Pigment strengthening was shown by the increase in the absorbance value of the sample and the increasing ratio of the added copigments. Copigment compounds have hydroxyl groups that bind to flavilium cations to form anthocyanin-copigment complexes through charge transfer (Castañeda-Ovando et al., 2009). Meutia et al. (2019) stated that in general, the color retention value of pigments that were copigmented with an increase in temperature tended to decrease since the beginning of heating. This indicated that the pigment was degraded. Lestario & Andini (2016) also explained that the extract which was added with the tannic acid copigment showed changes with an increased color retention value.

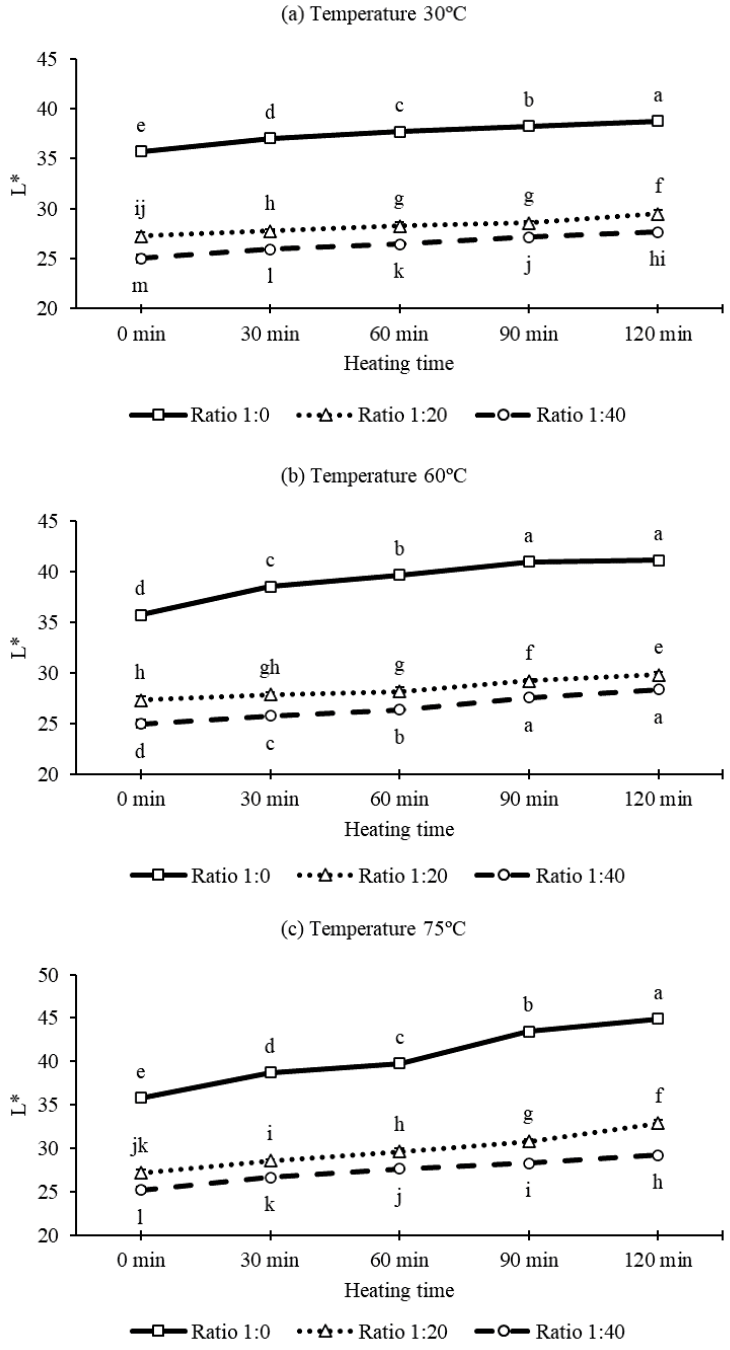


Figure 3. The value of L* (Lightness) (a) under 30ºC heating treatments, (b) under 60ºC heating treatments, (c) under 75ºC heating treatments. The different notation shows significantly different ($p<0.05$). The data are presented with the standard deviation of 3 sample replications.

Increasing the heating temperature and time increased the lightness (L*) of the parijoto anthocyanin color (Figure 3). Copigmentation of parijoto anthocyanins with tannic acid reduced lightness (L*) by up to 30%. Heating to 30ºC for 120 minutes increased the L* value by 8.5% in the non-copigmentation (Figure 3a). Whereas the 1:20 and 1:40 co-pigmentation increased L* by 8.3% and 10.5%, respectively. Heating to 60ºC for 120 minutes increased the L* value by 15.2% in the non-copigmentation (Figure 3b). Whereas the 1:20 and 1:40 co-pigmentation increased L* by 9.2% and 13.6%, respectively. Heating at 75ºC for 120 minutes increased the L* value by 25.4% in the non-copigmentation (Figure 3c). While in copigmentation treatment 1:20 and 1:40 increased L* by 21.1% and 15.9%, respectively.

An increase in L* value indicated an increase in color lightness which indicates anthocyanin degradation during heating. The process of anthocyanin degradation occurred due to heat which makes anthocyanins turned into colorless chalcone (Satyatama, 2008). The higher the copigment ratio the lower the L* value. This showed that the copigmentation prevents the color fading which was indicated by the decreasing L*. This also occurred in the anthocyanin drink model copigmented by rasmarinic acid in which the L* value

lower compared to the control without copigmentation. The interaction between anthocyanins and copigments was exothermic (Catrien, 2009). Another study on copigmentation of grape juice anthocyanins with rosemary extract copigments led to a decrease in L* values (Brenes et al., 2005).

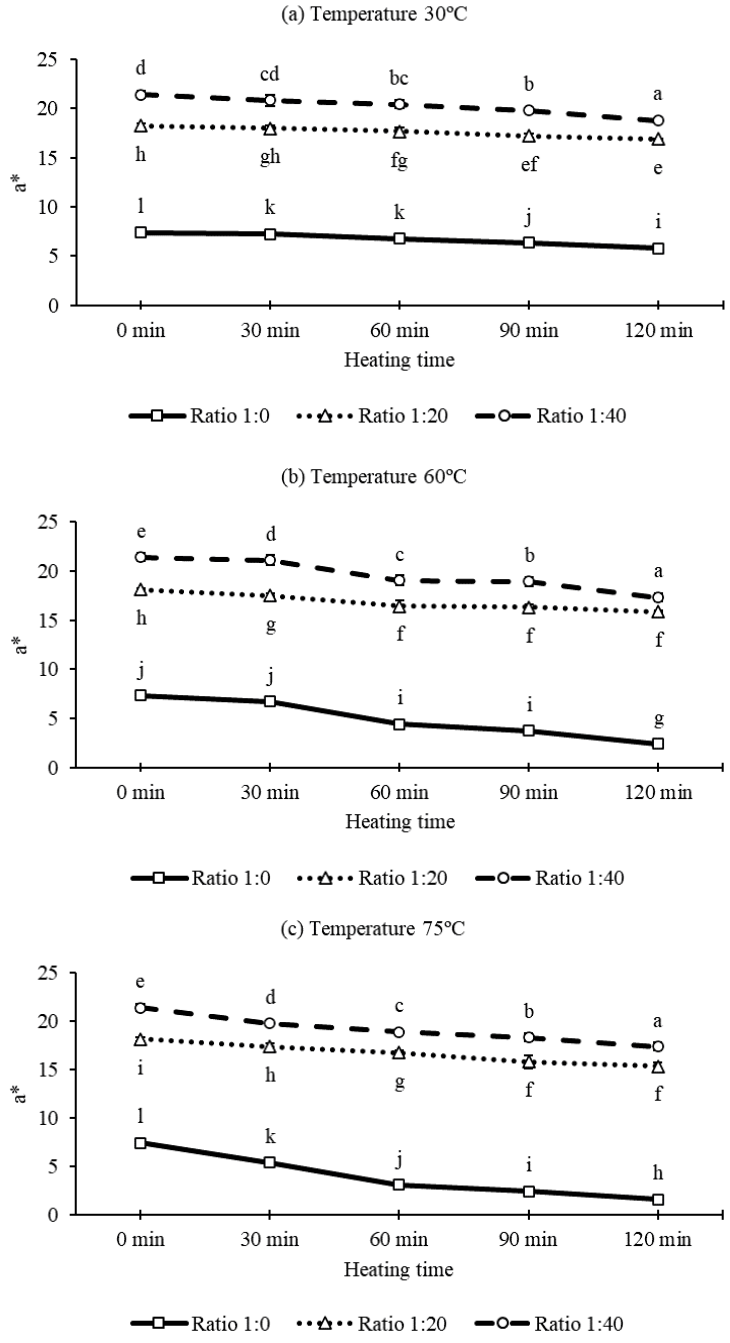


Figure 4. The value of a* (redness) (a) under 30ºC heating treatments, (b) under 60ºC heating treatments, (c) under 75ºC heating treatments. The different notation shows significantly different ($p<0.05$). The data are presented with the standard deviation of 3 sample replications.

Copigmentation of parijoto anthocyanins with tannic acid increased the red color (a* value) by 189%. Heating to 30ºC for 120 minutes reduced the a* value by 21.6% without copigmentation (Figure 4a). Whereas in 1:20 and 1:40 copigmentation decreased a* by 7.5% and 12.3%, respectively. Heating to 60°C for 120 minutes reduced the a* value by 67.3% without copigmentation (Figure 4b). Whereas the 1:20 and 1:40 co-pigmentation reduced a* by 12.6% and 19.1%, respectively. Heating to 75ºC for 120 minutes reduced the a* value by 78.3% without copigmentation (Figure 4c). Whereas in 1:20 and 1:40 copigmentation decreased a* by 15.3% and 18.7%, respectively. A decrease in the value of a* indicated the fading of the red color. This gave a sign of anthocyanin degradation. The decrease in the value of a * was caused by an increase in the speed of the structural transformation of the flavilium cation which was red-colored to become colorless chalcone. The decrease in the core concentration of flavilium cations can reduce the degree of redness of food models containing anthocyanins (García-Viguera & Bridle, 1999). Heating can cause a loss of red color from anthocyanins and increase brown color as a result of pigment degradation and

polymerization (Lindriati et al., 2005).

Increasing the tannic acid copigment ratio increased the a * value. This showed that the red color was getting stronger. Increased on the value of a* was associated with an increase in hyperchromic which increases with the increased in the copigment ratio. Hyperchromic increases indicate the increased color intensity of anthocyanins (Satyatama, 2008). Previous studies have also shown that the addition of copigments to anthocyanins increased the a * value (Catrien, 2009). Copigmentation can naturally improve the color of anthocyanins in food products. The stability and color strength of anthocyanins can be increased by the addition of extracts from different plants rich in copigments (Saati et al., 2012). The copigmentation process reacted between flavinium cations that were positively charged and electron-deficient with copigment compounds with excess electrons. Therefore, there was an electron transfer which causes an electron equilibrium (Castañeda-Ovando et al., 2009).

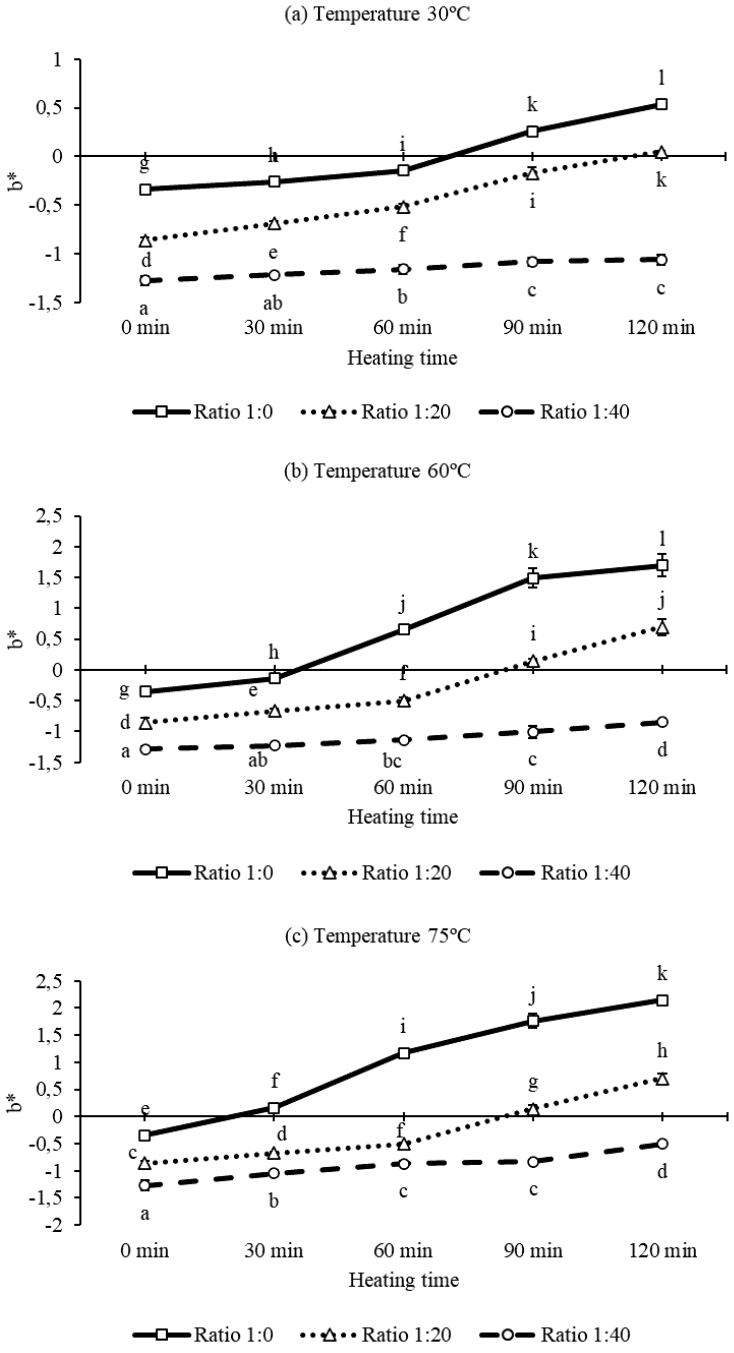


Figure 5. The value of b* (a) under 30ºC heating treatments, (b) under 60ºC heating treatments, (c) under 75ºC heating treatments. The different notation shows significantly different ($p<0.05$). The data are presented with the standard deviation of 3 sample replications.

Copigmentation of parijoto anthocyanins with tannic acid decreased the b* value by 278%. Heating to 30ºC for 120 minutes increased the b* value by 257.2% in the absence of copigmentation (Figure 5a). Whereas at 1:20 and 1:40 copigmentation decreased b* by 105.6% and 99.9%, respectively. Heating to 60ºC for 120 minutes increased the b* value by 581% without copigmentation (Figure 5b). Whereas in 1:20 and 1:40 copigmentation decreased b* by 180.9% and 33%, respectively. Heating to 75ºC for 120 minutes increased the b* value by 723% without copigmentation (Figure 5c). Whereas in 1:20 and 1:40 copigmentation decreased b* by 180.9% and 60%, respectively.

Increasing the copigment ratio decreases the b* value. This showed that the intensity of the bluish color was getting bigger. Increasing the heating temperature increases the b * value. In addition, the longer heating increases the b* value and causes the blue color to disappear which was replaced by yellow which was indicated by the b* value turning positive. Satyatama (2008) reported on anthocyanin copigmentation with copigment of ferulic acid and gallic acid, shifting the value of b* to positive, which indicates an increasing degree of yellowness. Another study showed that the increase in the value of b* in the anthocyanin-rosmarinic acid copigmentation drink model was caused by the dissociation process of the copigmentation complex between anthocyanins and copigments which increased the production of carbinol/ hemiacetal/ pseudo-alkaline bases which were pale to colorless (Catrien, 2009).

CONCLUSION

The addition of copigment tannic acid to parijoto anthocyanins significantly affected the stability of anthocyanin content, color retention, and color intensity during heating at 60°C and 75°C. The increasing of the copigment ratio resulted in increased color intensity and anthocyanin stability and decreased color degradation during heating. Tannic acid copigmentation on Medinilla sp. anthocyanin succeed to elevate the stability of anthocyanins during drying temperature (60°C) and pasteurization temperature (75°C) for 120 minutes. Tannic acid copigmentation of Medinilla sp. anthocyanin has beneficial applications for the food industry such as coloring dairy products.

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