ANTHOCYANIN STABILITY OF ROBUSTA COFFEE CHERRIES DURING STORAGE

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Abstract

The overripe Robusta cherries had potential as a source of anthocyanin. The aim of this reseach was to investigated anthocyanin stability which extracted from cherries. Anthocyanin was extracted used ethanol and aquadest with ratio 1:1. Extraction was repeated 3 times untill the residue was colorless. After that this extract was kept at different temperature 5, 25 and 45 °C for 4 weeks. The stability was evaluated by content and lost anthocyanin, Hue color, pH and antioxidant activity. The result showed that during storage anthocyanin stability of all sample decreased. Sample which kept at 5 °C had the best stability with anthocyanin content 3.26 %,lost anthocyanin 26.55 %, Hue color 146.12, pH 5.01 and antioxidant activity 16.26 % after 4 weeks. The least stability was obtained at samlple which kept at 45 °C with anthocyanin content 1.30 %, lost anthocyanin 29.02 % Hue color 124.92 ,pH 5.03 and antixidant activity 6.60 %.

Keywords : *Robusta cherries, anthocyanin stability, antioxidant activity, temperature*

Introduction

Anthocyanins mainly contribute to the bright red color of fruits, vegetables, and grains [3]. Epidemiological data associate anthocyanins with prevention of various disease such as visual and vascular diseases such and vascular diseases,obesity and some cancers [18; 8; 17; 1; 13; 6].

Recently, as consumers are incresingly concerned about the safety of synthetic colorant sed in food, the trend of using natural colorants in food products has also increased intensively. The potential health benefits of anthocyanins enhance consumer interest in using it in food to replace artificial red or purple colorant [12]. Anthocyanin colorant could be used in many solid foods such as extruded snacks and baked cakes as well as in drinks and beverages [11].

The degradation of the anthocyanins in friuits and vegetables during processing and storage has been reported in some studies [9; 14; 11]. Those studies were carried out at a temperature below 100^0C and a high moisture condition. The degradation of the anthocyanins was significantly accelerated by various glycosidase activities and severe pH in the sample, beside heating temperature. Thus, the liability of the anthocyanins observed in those studies could be different from that under dry heating condition.

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The overripe Robusta coffee cherries containing anthocyanin 8.34 % , pH 5 showed the highes stability during storage [16].

At prereseach, have been tried to get Robusta coffee ectract by using aquadest: ethanol (1:1) as a solvent .Robusta coffee cherries extract solution preparation . In this study, robusta coffee cheries extract was used to evaluate the stabilities of anthocyanin at different temperature during storage. Also antioxidant activities of anthocyanin was evaluated using DPPH (2,2 ´-diphenyl-1-picrylhydrazyl) free radical scavenging method as well.

Material and Methodes

Raw material which used in the making of extract of robusta coffee is the fruit of overripe Robusta coffee with red colour from Durjo estate , Jember ,East Java Indonesia. We also use glicerol and Citric acid

Chemicals and Equipment

Aquades, ethanol 97% , Reagent DPPH , acetic acid, buffer pH 1 and pH 4.5, NaOH. Equipment needed in this researh were rotavapor, blender, cloth filter, glassware, Eppendorf micropipete, pH metre, oven, centrifuge, cooler.

Statitical Analysis

Design research were consist of 2 factors . Three times replication to every treatment. D= temperature of storage D1= 5°C, D2 = 25 °C and D3= 45°C and length of storage M: M1= 1 week, M2= 2 weeks, M3=3 weeks and M4= 4 weeks. The data was analyzed by Descriptif test and showed the histogram.

Overripe Robusta coffee cherries extract:

After washing the fruit, separated the pulp and seed. 15 grams the pulp blend 75 ml solvent (contained of aquadest : ethanol 97% 1:1 and 15% citric acid v/v), maceration during 15 minute and filtered the solution. The residue replicated extracted 2 times until colorless. Then centrigugated on 4000 rpm during 20 minutes. After that the filtrate to consetrated by rotary evaporator on 40°C until the volume of extract 75 ml.The extract maked pH 5 and then added with 10% gliserol . The sample packing on the bottle and then kept in 5°C, 25°C , 45°C and storage for 4 weeks.

Total Anthocyanin with pH differential method (Giusti and Wrolstad, 2001)

Total Anthocyanin concentration : Dissolved 1 ml sample with 4 ml KCl (pH 1)) for 20 min and Na-acetate (pH 4,5) for 20 min. Measured every solution in 520 nm and 700 nm with buffer pH 1 and buffer pH 4,5 as standard.

A = (A 520 – A700)pH 1 – (A520 – A 700)pH4,5

Total anthocyanin formula :

$$\text{otal anthocyanin} = \frac{A}{\varepsilon x L} \text{ x MW x DF x } \frac{V}{Wt} \text{ x } 100\%$$

Explanation

ε = Absorptivity molar Sianidin-3-glukoside = 26900/mol

L = Kuvet width =1 cm

M MW=Molecule weight of Sianidin-3-glukoside = 449.2 g/mol

DF = Dilluted factor

V = Volume (L)

Wt = Sample weight (g)

Detemination Lost Anthocyanin

$$\text{Lost Anthocyanin (\%)} = \frac{\text{mg/l anthocyanin (a-b)}}{\text{Mg/l anthocyanin a}} \text{ x } 100\%$$

Explanation :

a = concentration anthocyanin before storage

b = concentration anthocyanin after storage

Determination of color (Color reader, Fardiaz et al, 1992)

The initial color used ceramic as standard had L. A.b value sequently 94.35; -5,75 and 6.51

$$H = \tan^{-1} \frac{b^*}{a^*}$$

Explanation :

a * = range value -80 – (+ 100), showed green until red

b * = range value – 50 – (+70), showed blue until yellow

H = angel of color (0°= neutral color.90° = yellow. 180° = green.270° = blue)

Determination pH (pH metre)

Investigated pH using pH meter, the electrode immersed on buffer pH 7, after that immersed on the sample until stable then noticed.

Determination of DPPH free radical scavenging capability (Gadow, 1997)

The DPPH free radical capability in the robusta coffee extract was determined using the spectrophotometer method. The robusta coffee extract 10 µl in the test tube was redissolved in 1 ml DPPH for 20 min, added with ethanol 97% until 5 ml, vortex measured the absorbans on 517 nm .The concentration of DPPH solution was 0.0394 g in 250 ml ethanol 97% (the concentration 400 µ mol/l). Kurva standard DPPH Y= 1.791 x + 0.014

$$\text{Aktivitas antioxidant} = \frac{A\ (\text{blanko} - \text{sample})}{A\ \text{blanko}} \times 100\ \%$$

Explanation

Blanko maked from DPPH solution without sample

Y = absorbans

X = konsentrasi (µ mol DPPH)

Result and Discussion

Anthocyanin content

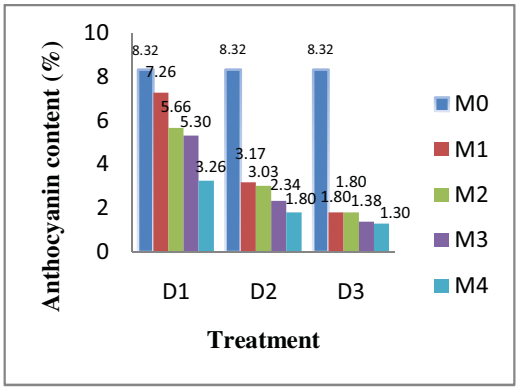

Figure 1.Anthocyanin Content of Robusta Coffee Cherries Extract

Histogram of anthocyanin Robusta Coffee Cherries extract is shown in Figure 1. The total anthocyanin content initially is 8.32 %.The highest content anthocyanin in the extract was D1 (7.26 % this extract kept on 5 0C the first week, 5.66 % on the second weeks, 5.30 % on the three and 3.26 the forth weeks).In this study shown the anthocyanin

more stabil kept on 5 0C .(6), investigated the characteristic of anthocyanin which kept on various temperature, the result shown the anthcyanin degradation more so much with the temperature hight . The high temperature accelerated the degradation of anthocyanin.(8;13).The range of total anthocyanin content was from 1.298 % to 7.26 %.

Lost anthocyanin

Lost anthocyanin Robusta Coffee Cherries extract shown in Figure 2. The highest lost anthocyanin shown on D3 it was 29.02 %.This shown the temperature storage high so much, the degradation anthocyanin also so much. The range of degradation is was 27.05 % to 29.02 %

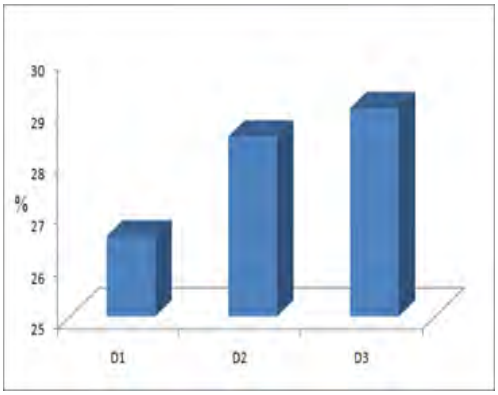

Figure 2.Lost Anthocyanin of Robusta Coffee Cherries Extract

Color of Robusta Coffee Cherries.

The color of robusta coffee cherries shown in Figure 3 and Figure 4. Figure 3 shown robusta coffee cherries extract kept on 5^0C (D1) had bright red then extract which kept on 25^0C had purple red and extract kept on 45^0C had dark red. The finished observation extract kept on 45^0C the color so much dark beside extract kept on 27^0C and 5^0C so much purple.

The observation on H value shown on Figure 4. Range of H value were 129.962 to 155.028.There were Yellow Green (YG) , beside Mo had value 144.382 (data not presented).There were shown on the same range. Maybe the sample contained the other substance as ascorbic acid, polifenol, carotenoid and the others so that color combined shown Yellow Green.

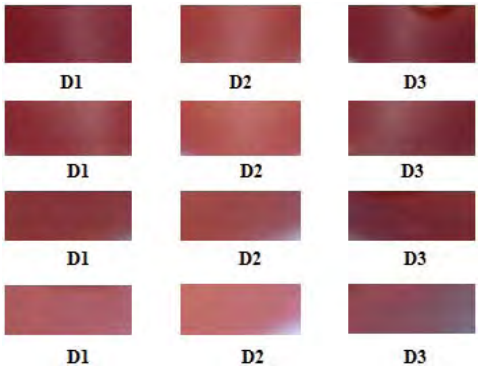

Figure 3.Color of Robusta Coffee Cherries Extract

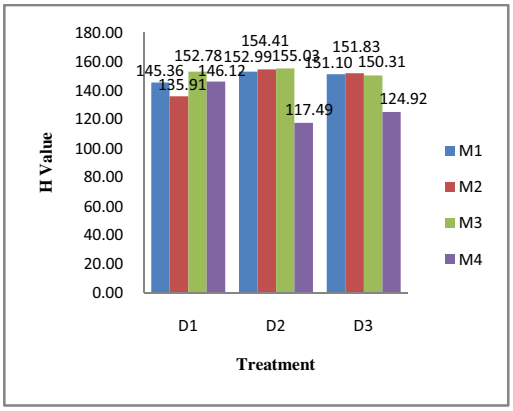

Figure 4.Color (Hue) of Robusta Coffee Cherries Extract

pH

Figure 5 shown pH robusta coffee cherries extract on various treatment. Storaged treatment on 5^0C; 25^0C; 45^0C shown the pH increased. Range of pH 4.73 to 5.04. The Initial pH 4.81 (data not presented), there were shown during storage pH extract trend increased on all the treatment. There were shown degradation anthocyanins also happened on all sample.Degradation of anthocyanin result increased pH

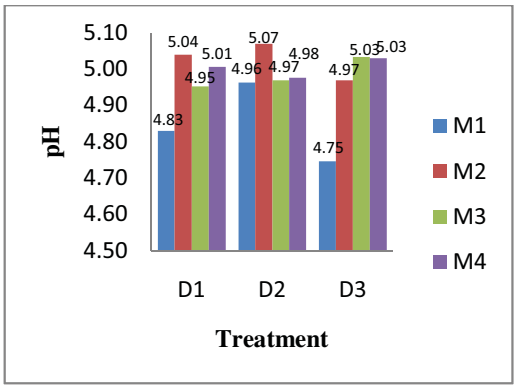

Figure 5.pH of Robusta Coffee Cherries Extract

Antioxidant Activity

The changes of DPPH free radical scavenging capability of the robusta coffee cherries extract shown on Figure 6. The highest antioxidant activity shown on D1 treatment (extract kept on 5^{0C}).The range of antioxidant activity 6.60 % to 24.39 %. M0 (before storage) had antioxidant activity 78.16 %. There were shown antioxidant activity had trend decreased during storage all the treatment. But the decreased highest was the sample wich kept on 45 ^{0}C. There were shown high temperature accelerated the degradation antioxidant substance include anthocyanin. It this as regard [10] reported radical scavenging activity of acylated anthocyanin in the red radish extract. Also, some degradation product of anthocyanins were reported to have antioxidant capability [15]

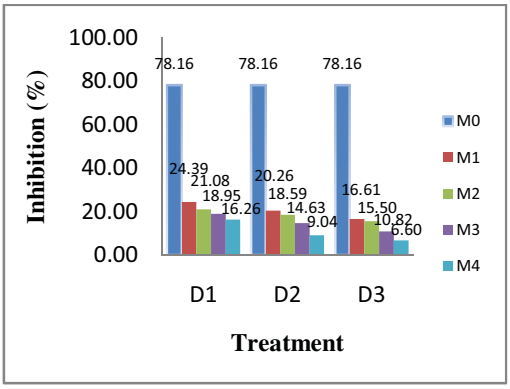

Figure 6.Antioxidant Activity (Inhibition %) of Robusta Coffee Cherries Extract

Conclusions

From this study, stabilities of anthocyanin during storage were revealed. The anthocyanin trend decreased during storage , there were not stable on storage in low temperature ,the decreased it small but on high temperature was as high as the temperature. The optimal temperature storage it 5^{0}C , anthocyanin content was 3,34 %, beside on 25^{0}C and 45^{0}C there were 1.80 % and 1.30 % after 4 weeks.

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