THE COMBINATION OF DRAGON FRUITS SKIN AND TEAK LEAVES ANTHOCYANIN EXTRACT AS SOYMILK'S NATURAL DYE

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ABSTRACT

Natural dye is easier to dissolve in water and to be absorbed by our body than artificial pigment. Anthocyanin pigment combination from different sources is able to improve functional food quality. Soymilk is a white liquid vegetarian functional food which hasn't adorable appearance. The dragon fruit skin and teak leaves anthocyanin extract combination can be used as alternative natural pigment to improve soymilk's quality. Dragon fruit skins bright red, while the teak leaves is dark red with higher anthocyanin content. This study aimed to determine the dragon fruit skin and teak leaves anthocyanin extract combination to Argomulyo var. soymilk's quality. Completely Randomized Design (CRD) one factor (dragon fruit skin and teak leaves extract combination) with 3 levels (5%, 10%, and 15%) were applied. The experiments consisted of two main process, the first step was anthocyanin extraction which followed by soymilk's production. Dragon fruit skin and teak leaves anthocyanin extraction used maceration method with water as solvent through blanching process at 80oC. Soymilk's color intensity measured by color reader, organoleptic (hedonic-test), pH (pH-meter), anthocyanin (pH differential and UV-Vis spectrophotometer), and antioxidants activity (DPPH). The result showed that 15% anthocyanin addition gave the best results with redness (8.39), yellowness (3.24), brightness (41.33), organoleptic color (4.15), pH (6.54), anthocyanin (4.007 mg/L), and antioxidant (69.13%).

KEYWORDS: Anthocyanin, dragon fruit skin, teak leaves, soymilk

استخلاص صبغة الانثوسيانين للحليب من قشور الفواكه

وايهيني ساتي واناريش سوزي تاياريني روكماه
استاذ مشارك استاذ استاذ مشارك استاذ مساعد مدرس

المستخلص

هدفت هذه الدراسة إلى تحديد قشور فاكهة التنين وأوراق من خشب الساج مزيج مستخلص الأنثوسيانين إلى أرغومولیو فار. جودة حليب الصويا. تم تطبيق التصميم العشوائي بالكامل (CRD) على عامل واحد (تركيبة خلاصة أوراق فاكهة التنين وخشب الساج) مع 3 مستويات (5٪ و 10٪ و 15٪). تتكون التجارب من عمليتين رئيسيتين، كانت الخطوة الأولى استخراج الأنثوسيانين الذي تبعه إنتاج حليب الصويا. استعمل قشور فاكهة التنين وخشب الساج استخراج الأنثوسيانين طريقة التعقيم بالماء كمذيب من خلال عملية التبييض عند 80 درجة مئوية. تم قياس كثافة ألوان حليب الصويا بواسطة قارئ الألوان، الحسي (اختبار المتعة)، الرقم الهيدروجيني (متر الرقم الهيدروجيني)، الأنثوسيانين (تفاضل الأس الهيدروجيني ومقياس الطيف الضوئي للأشعة فوق البنفسجية – فيس)، ونشاط مضادات الأكسدة .(DPPH) أظهرت النتائج أن إضافة 15٪ من الأنثوسيانين أعطت أفضل النتائج مع الاحمرار (8.39)، والأصفر (3.24)، والسطوع (41.33)، واللون الحسي (4.15)، ودرجة الحموضة (6.54)، والأنثوسيانين (4.007 مجم / لتر)، ومضادات الأكسدة (69.13٪).

كلمات مفتاحية حليب الصويا، اللون الحسي، السطوع، درجة الحموضة

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INTRODUCTION

Food safety is a critical and an important issue in food productivity (home or industry scale), because it's closely related to the product quality which affected to the human health (29, 33). Food safety also concerns about additives usage (artificial sweeteners, colorants, flavoring, and preservative), production process, genetic manipulation, food radiation, packaging, food quality, laboratory analysis hygiene (microbial pathogen contaminant), and sanitation (14, 18, 30). Based on the data from USFDA Year 1999, 10% Indonesian products were rejected because of food additives usage, 11% pesticide residue and 2% heavy metal (19, 31). Food safety definition is the condition and effort which needed to prevent from biological and chemical contaminant or from other's that can disturb, disadvantageous, and harms human health. Consumer and industry need to concern especially about food additives effects to the human health (4, 16). As technology development, the natural dye production is decrease, while synthetic dye production and usage is increase which harms human health alike carcinogenic and toxic (35). Natural dye comprise colorants that obtained from vegetable or animal substances without any chemical processing (1), which can be found in carotenoid, curcuma, anthocyanin, and other pigment in fruit, flower, leaves or root tissue and it is safer to be applied than synthetic (36). The advantages of natural dyes are mostly eco-friendly, biodegradable, less toxic, and less allergenic as compared to synthetic dyes (1, 34, 36). Natural dye extraction process by crushing the ingredients which consisted natural dye and soak it in solvent. The usage of natural dye is very limited due to non-availability standard application procedures (1).Anthocyanin is a pigment with hydrophilic characteristic that dissolves in polar solvent such as alcohol and water. Anthocyanin is one of natural dye that can be applied to the food and beverage (11), which can be found in plants with red to blue identity (32). Bioactive compound such as flavonoid pigment or anthocyanin is a molecule that has function as antioxidant (23). Dragon fruit and teak leaves contain high anthocyanin that produce red color which is able to prevent free radical and high of antioxidant activities.Dragon fruit (*Hylocereus polyrhizus*) is originated from Latin America (21, 26) has unique color characteristics. The pulp has deep purple color which indicated the betalains availability that always found together with anthocyanin (21, 26). Even though the flesh contains higher anthocyanin than the peel but it is applicable for improve the food quality and appearance (27). According to other research, dragon fruit skin extract with 40% concentration was able to produce good quality soymilk with stabile natural color. While, for the best organoleptic quality was dragon fruit skin extract 20% (8). Teak (*Tectona grandis* L.) is one of tropical native herbs from Indonesia, which tubers development has been focused and the leaves become wastes. Nowadays, the teak leaves compound has been investigated alike its phenolic compound for coloring agent, mosquito larvicidal, and organic fertilizer composite. The result was consistent with Siskawardani (25) which showed that the effect of teak leaves extract addition was very significant ($p>0.01$) on the pH, color (L and a), viscosity, and sugar content of syrup. While it was also significant ($p>0.05$) on anthocyanin content and insignificant on color (b) of syrup. Based on Hermawati (12), teak leaf anthocyanin extract gave significant effect to the ice cream color stability. Other researcher (15) explained that pH and temperature also affect teak leaves extract stability (anthocyanin content, and antioxidant activity). Teak leaves antioxidant is higher about 643100 mg/L than dragon fruit skin's138000 mg/L. Soymilk is a beverage from soy extract which has a lot of advantages such as high nutrition, suitable for lactose intolerance consumer, and doesn't contain cholesterol. Soymilk protein quality is equal to 80% regular milks (8, 11). Meanwhile, soymilk consumption level in Indonesia is lower than other country like China, Philippine, or Thailand. It may be caused by the unpleasant beany aroma (9). This beany aroma can be reduced with right processing technology and right variety choice alike the yellow bean that has high protein, and less beany flavor. Superior varieties such as Argomulyo var. that has similar characteristic as Bromo var. is suitable as

soymilk material. The main problem of soymilk product expansion is unattractive appearance. Hence, synthetic food color was commonly used for improve consumer interest, even though it affect to human health (22, 23). There is commonly commercial brand soymilk that used, synthetic red color Alura CI 16035, which is known it improved kid hyperactivity (24). Nowadays, people start to realize that natural food color is more efficient than artificial color to be applied (3, 10). Therefore, according to that occasion, this study aimed to investigate the effect of anthocyanin extract (dragon fruit skin and teak leaves combination) to the local variety (Argomulyo-East Java) soymilk quality alike color intensity (brightness, redness, and yellowness), pH, organoleptic, and total anthocyanin.

MATERIALS AND METHODS

This research was conducted at Food Science and Technology Laboratory, Universitas Muhammadiyah Malang, East Java-Indonesia. Independent variable was anthocyanin extract addition (dragon fruit skin and teak leaves combination) in soymilk. It consisted of 3 (three) levels P1 (5%), P2 (10%), and P3 (15%). Dependent variables were color intensity, pH, total anthocyanin, antioxidant activity, and organoleptic. While the control variables were maceration time, extraction temperature, and solvent varieties. The equipment's used were analytical scale, measuring cylinder, filter paper, trimulus dye meter/color reader CR 10, and UV spectrophotometer. Materials which needed were teak leaves, dragon fruit skin, soy bean, distillated water, and citric acid.

Anthocyanin Extraction

The first step for anthocyanin extraction was preparing dragon fruit skin and teak leaves (sorted, cleaned, and cut) which followed by weighing 150 gr, then blanching it at 800C for 5 minutes. Maceration process was 1 hour at 360 mL water and 3 gr citric acid as solvent. Then the extract strained with filter paper and saved in glass bottle. The anthocyanin extract combination, consisted of dragon fruit skin and teak leaves which applied was 50:50.

Soymilk Production

The 500 grams soy bean (Argomulyo var.) from BALITKABI Malang, East Java was weighed and cleaned, then soaked in water for 10 hours. Then, its skin was separated from the bean, and blended with water (1: 8) later. The slurry was strained with filter paper and boiled to sterilize from bacterial, reduce beany flavor, and improve digestion function. The last step was anthocyanin extract mixing to the soymilk according to the treatment (5%, 10%, and 15%) in heat condition.

Total Anthocyanin Analysis

The pH differential was used for total anthocyanin analysis (9). 0.5 mL concentrated anthocyanin combination diluted in 4.5 mL methanol PA and leaved it for an hour. Then 0.5 mL sample was taking and filled to 2 different atom cuvettes (pH 1 and pH 4.5) which already filled by 4.5 mL buffer solution. Both cuvettes were measured in spectrophotometer with 520 nm and 700 nm (15 minutes), then calculated absorbance value.

Diluted sample anthocyanin formulation (formula 1):

$$A=(A_{500}-A_{700})pH_1-(A_{500}-A_{700})pH_{4.5} \quad (1)$$

Anthocyanin value (Formula 2):

$$total\ Anthocyanin = \frac{mg}{L} - \frac{A*BM*FP*1000}{\varepsilon*l} \quad (2)$$

where, ε is Cyanidin 3-glucoside molar; A is absorbace = 269000L/(mol.cm); l is cuvette width = 1 cm; BM is Cyanidin 3-glucoside molecule = 449.2 g/mol; and FP is dilution factor = 10.

Soymilk's Color Analysis

Soymilk sample was token, put in plastic tube, and closed tight. The sample was token closer to color reader, then target button was pushed ("L" for lightness, "a" for redness, and "b" for yellowness). Make sure whenever start, it was in zero scale.

Statistical Analysis

All data were analyzed with statistical analysis (One-way ANOVA 5%). Then, the data which gave significantly effects was analyzed using DMRT (Duncan's Multiple Range Test) 5%.

RESULTS AND DISCUSSION

The Soymilk Color

The anthocyanin extract (dragon fruit skin and teak leaves combination) effect to the soymilk color intensity can be seen on Figure 1. The highest soymilk Lightness (L) was 52.51 which 5% addition, and the lowest was 41.33 (15% anthocyanin extract). The highest redness (a) was 15% and the lowest was 5%

anthocyanin extract (8.39 and 5.43). The highest yellowness (b) was soymilk with 10% anthocyanin extract (4.21) and the lowest was soymilk with 15% anthocyanin extract (3.24). Based on analysis of variance, the results was significant ($p< 0.05$) for all color intensity (L, a, b). The decreasing of lightness and yellowness but increasing of redness is caused by the anthocyanin extract application enhancement to the soymilk. In addition, the anthocyanin extract contains anthocyanin that make red intensity increase. This result was consistent with the research of anthocyanin effect to the yoghurt (5), syrup (25), and ice cream (12).

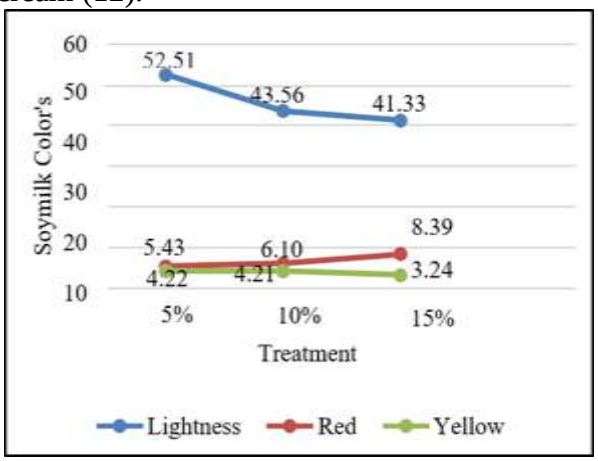

Figure 1. Anthocyanin (dragon fruit skin and teak leaves combination) effects to soymilk's color

The Soymilk Ph

The result showed that anthocyanin extract addition was insignificant different to the soymilk's pH. The pH range was 6.36 – 6.54, which indicated that increasing anthocyanin extract application gave enhancement effect to the pH value (Figure 2).Even though, soymilk still had the pH standard. This result was related to the total anthocyanin (Table 1) which explained that increasing pH is possible to enhance anthocyanin. Torskangerpoll (28) also reported that in acidic solutions, increment in pH produced bathochromic shifts of anthocyanin occurs.

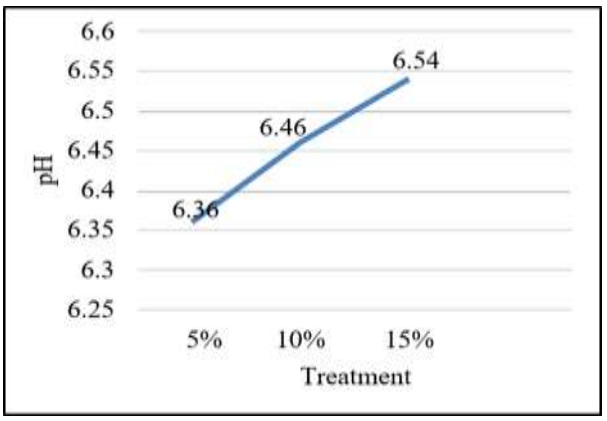

Figure 2. Soymilk pH with anthocyanin extract addition

The Soymilk Organoleptic

Organoleptic test was done by 10 different panelists who were understand and familiar to the soymilk characteristic. The soymilk organoleptic score trend was decrease (aroma, taste, and texture) based on increasing anthocyanin extract addition, except color which was increase (Figure 3). The best treatment was 10% anthocyanin extract addition, because score was the highest, alike aroma (3.37), taste (3.57), and texture (3.69). While the lowest was 5% anthocyanin extract, aroma (3.01) taste and color score (3.28 and 3.14).According to (9), soymilk's production process would give different organoleptic result and soy varieties also affected soymilk flavor.

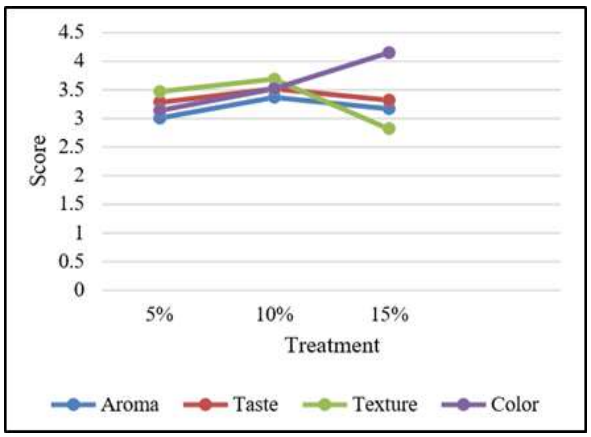

Figure 3. Soymilk Organoleptic with anthocyanin extract addition

Meanwhile, 15% addition was the highest color and the lowest texture score (4.15 and 2.82).It was appropriate with (8), soymilk and coconut milk 35 organoleptic score was increase during anthocyanin application enhancement. In other word, the anthocyanin extract 10% and 15% addition were more acceptable and improved soymilk's organoleptic than 5%.

Soymilk's Total Anthocyanin

Table 1 showed that the highest soymilk' total anthocyanin was 15% (4.007 ± 0.05 mg/L) and the lowest 5% (0.501 ± 0.01 mg/L). It can be concluded that increasing anthocyanin extract addition (dragon fruit skin and teak leaves combination) enhance the soymilk's total anthocyanin content. Anthocyanin color is unstable and susceptible to changes due to several factors, such as: anthocyanin structure and concentration present in main materials (fruits or vegetables), pH, light, acids presence, temperature, sugars, phenols, metallic ions, in addition to substances called co-pigments (6, 7), enzyme, oxygen, oxidation and storage (13).

Table 1. Soymilk's Total Anthocyanin with anthocyanin extract addition

Anthocyanin Extract	Antioxidant Activity
P1 (5%)	**0.501 ± 0.01 a**
P2 (10%)	**2.671 ± 0.08 b**
P3 (15%)	**4.007 ± 0.05 c**

Note: The same letter on the same column indicated insignificant result according to DMRT 5%

According to the ANOVA test, the result was significant difference. It is caused by anthocyanin color come from long conjugated double bond, so it can absorb light in visible light range. Solvent used in this study was water and citric acid combination, which is anthocyanin and water have same polar characteristic. Also citric acid had cell tissue destructive character (13). Anthocyanin dye characteristic in plant tissue affected by pigment content, also hydroxyl and methoxylocation. High anthocyanin concentration in plan tissue will produce red color, medium concentration will produce orange to purple color, and low concentration will produce blue color (23, 35). Teak leaf also consists antimicrobials such as flavonoids, alkaloids, naphthoquinone, anthraquinone, and tannins that obstruct bacteria growth. Altogether teak leaf is promoting for food coloring and food preservation (2, 20).

Soymilk's Antioxidant Activity

Analysis of variance test showed that anthocyanin extract effect was significant difference (Table 2). The highest soymilk antioxidant activity was 69.31 ± 0.2% (15%), and the lowest was 63.89 ± 0.1% (5%). The result indicated that increasing anthocyanin extract enhanced the antioxidant activity which same as experiment in jabuticaba (*Myriciaria cauliflora*) (17). The antioxidant activity can be due to the presence of large amount of anthocyanin and the synergistic effect of other substances (17). Young teak leaves and dragon fruit skin antioxidant combination come from high anthocyanin content (10). Meanwhile, nuts are main isoflavone resource which is polyphenol that can show role as estrogen which has ability as antioxidant. Commonly, local soy bean has average antioxidant activity about 22.49%, but after made as soymilk's the antioxidant activity was increase about (63.89-69.31%).

Table 2. Soymilk's antioxidant activity with anthocyanin extract addition

Anthocyanin Extract	Antioxidant Activity
P1 (5%)	**6389 ± 0.1 a**
P2 (10%)	**68.45 ± 0.1 b**
P3 (15%)	**69.31 ± 0.2 b**

Note: The same letter on the same column indicated insignificant result according to DMRT 5%.

CONCLUSION

The best treatment is 15% anthocyanin extract (dragon fruit skin and teak leaves) addition to the soymilk's with redness (8.39), yellowness (3.24), brightness (41.33), pH (6.54), anthocyanin (4.007 mg/L), antioxidant (69.13%), and organoleptic score (color (4.15), texture (2.82), taste (3.32), and aroma (3.17)). This formulation is recommended for producing acceptable soymilk.

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