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EFFECT OF SOLVENT RATIO TO ANTHOCYANIN CONTENT AS A NATURAL DYE ON SWEET PURPLE POTATO EXTRACTION

(*Ipomoea batatas L. Poir*)

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Abstract

Sweet Purple Potatoe (Ipomoea batatas L .Poir) has potential as one source of the anthocyanin that could serve as a natural dye, antioxidant, antimutagenic and anticarcinogen. Anthocyanin is water soluble pigment responsibles for blue, purple, violet, magenta, red and orange color. Anthocyanin itself is safe to be consumed, nontoxic and does not cause genetic mutation. This proves that a natural dye especially anthocyanin is safe to be used. Anthocyanin can be damaged at high temperatures (heating) which is commonly used in making some food products. The objective of this research are making a natural dye of the sweet purple potato, seeking the influence of the solvent ratio and the best ratio to its yield. Research was done with the maceration extraction methode (simple filtering by immersion sweet violet in 96 %ethanol and water for 24 hours at room temperature and protected from sunlight) by using the solvent ratio (1: 4, 1: 5, 1: 6, 1: 7, 1: 8). The extraction then filtered and concentrated with rotary vacuum evaporator and then analyzed using spectrofotometer uv-vis. The result was compared to standard billberry extract. The best yield was 4,6688 gr at 500C temperature with 2 atm pressure and its solvent ratio 1: 8. The trendline resulted y = 810,52x - 33,77 equation with R2 = 0,9693. The best anthocyanin content was obtained at 12.5 mg / l in 500C and 2 atm, 1: 8 ratio presented by y = 2156,4x -89,554 equation with R2 = 0,9638.

***Keywords**: sweet purple potato, anthocyanin, solvent ratio, natural dye.*

INTRODUCTION

The Color is an important factor for the quality of food. Color simultaneously extracted with smell, flavor and texture plays an important role in the reception of food (Man , 1997). Recognizing the importance of color, food manufacturers often add dyes in their food products, neither in the form of dye (pigment) or synthetic dyes . The dyes can be classified into four categories, namely dye synthesis, dyes similar to natural dyes , dyes inorganic and natural dyes for food are mostly made from plant extracts, but also from other sources such as insects , algae , cyanobacteria , and fungi (Mortensen , 2006). Synthetic dyes more preferable to use with because its more economical, practical and have a stable and uniform coloring properties. But its has negative impactspecifically in carcinogenic and toxic nature (Winarno , 1997) . Concerns about the safety of use of synthetic coloring encourage the development of natural dyes as a food coloring.

Natural dyes can be obtained in several plants like *Taget erecta* L flower extract as textile dyes (Jothi, 2008), anthocyanin from Redcabbage extract (Xavier et al, 2008), *Indigoferatinctoria* L and *Baphicacanthuscusia* Brem leaves extract (Chanayath, et. al , 2002). Sweet Purple Potato can also be used as natural dyes because they contain anthocyanins. Reason to use natural dye because they are safe to used in a long term. Production of Sweet Potato have an increase trend during 5 years, 6.78 % per year from 1.8 million tonnes in 2008 to 2.4 million tonnes in 2012. However, its application is still relatively small so mostly exported to other countries. The purple color from purple sweet potato is derived from natural pigments contained in it . Purple sweet potato (*Ipomoea batatas L. Poir*) not only tastes good but also has pretty colors (purple) and usually used as a natural food coloring . The difference in Anthocyanin content in potato causing the different in purple color level. Anthocyanins in purple sweet potato have another advantage as an health antioxidant.

Nowadays, the demand for healthy and tasty food start to rise. Many kinds of processed food made from simple materials have a high value like Purple Sweet Potato (*Ipomoea batatas L. Poir*). Purple Sweet Potato (*Ipomoea batatas L. Poir*) is not only used as a food substitute for rice but also, can be use as processed foods such as ice cream, pudding, flour, cake, meat vegetarian burger. Furthermore, sweet potato starch can be used as raw material for pharmaceutical chemical products, manufacture of alcohol and fructose (sweetener) in the beverage industry and quickly decompose plastics. Sweet potato starch is also one ingredient in the manufacture of textiles and paper as well as substitute fuel (bioethanol) after first processed into alcohol (Yusuf and Widodo, 2002). So the need for research on the effect of solvent ratio on the anthocyanins levels as a natural dye from purple sweet potato (Ipomoea batatas L. Poir).

According to Suprapti (2003), Potato plant have a characteristic : composition of the main body consists of stems, leaves, flowers, fruits, seeds , and tubers. Plant stem is round, not woody and jointed. Have an upright growth mode and vine or creeper . Upright type rod length : 1 m - 2 m , whereas the type of vines : 2 m - 3m .

Meanwhile the chemical properties of Sweet Potato were highly dependent on the variety and level of maturity and its storage time. Carbohydrates forms consisting of monosaccharides , oligosaccharides , and polysaccharides . It contains about 16-40% dry matter and about 70-90 % of the dry matter are Carbohydrates consisting of starch, sugar, cellulose, hemicellulose and pectin (Meyer , 1982).

Table 1. Carbohydrate content in Sweet Purple Potate (dry weight)

Component	*Weight (%)*
Starch	*46,2*
Glucose	*22,4*
Hemicellulose	*3,6*
Cellulose	*2,7*
Pectin	*0,47*

Source(s): Meyer, 1982

Purple sweet potato has a high number of calories and other nutritional values are not much different from other types of sweet potato . The amount of the nutrient content in 100 gram sweet potato can be seen in the table 2. Below.

Table 2 Nutrient content on 100 Gram Sweet Purple Potato.

Nutrient	Content
Calori (kal)	123
Protein (g)	1.80
Fat (g)	0.70
Carbohydrate (g)	27.90
Calsium (mg)	30
Phosphor (mg)	49
Iron (mg)	0.70
Natrium (mg)	-
Kalium (mg)	-
Niacin (mg)	-
Vitamin A (SI)	7,700
Vitamin B1(mg)	0.90
Vitamin B2(mg)	-
Vitamin C (mg)	22
Water (g)	68.50
Potato content (%)	86

Source(s): Suprapti, 2003

Chemical and physical composition on Sweet Purple Potato can be seen on tabel 3 below.

Table 3 Chemical and Physical composition of Sweet Purple Potato (% db)

Chemical & Physical property	MSU 03028-10
Water (%)	60,18
Ash (%)	2,82
Starch (%)	57,66
Reduction Glucose (%)	0,82
Fat (%)	0,13
Anthocyanin (%)	1419,40
antioxidan activity(%)	89,06

Source: Widjanarko, 2008.

Anthocyanins, secondary metabolites from the flavonoid family, in large quantities are found in fruits and vegetables (Talavera, et al., 2004) . Anthocyanins are a class of flavonoids, which are widely divided into plant polyphenols. Flavonols, flavan-3-ol, flavones, flavanones, and flavanonol is an additional class of flavonoids that differ in the oxidation of anthocyanin. Flavonoids solution are colorless or pale yellow (Wrolstad, 2001). Anthocyanin is a water-soluble pigments that responsible for blue, purple, violet, magenta, red and orange color.

Hydrophilicantocyannin is a group of flavonoid pigments in most plants, in form of blue, purple and red color. The concentration of Anthocyanin that causes some kind of purple sweet potato has different shades of purple (Hardoko et al. , 2010).

According to Jackman & Smith (1996), Anthocyanin is safe to consumed, non toxic and does not cause genetic mutation. This proves that a natural dye especially anthocyanin is safe to be used. Source of Anthocyanin can be find in strawberry, chery, plum, cabbage, grape, blackcurrant, choke berry, red bean, black bean and red paprika (Jackman dan Smith, 1996).

Anthocyanins is a amphoteric compounds, have the ability to react either with acids or in bases. In an acidic medium, anthocyanin have red color as well as when the cell vacuole and turned purple and blue if change to alkaline. This color changes due to the groups attached to the basic structure of the bond position (Charley, 1970) .

Anthocyanin have a molecule structure as figure 1 :

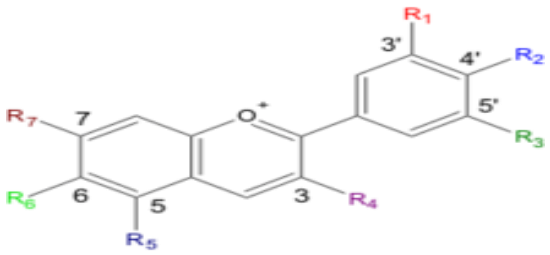

Figure 1. Anthocyanin molecular structure (Anonymous, 2007)

Anthocyanin concentration level causes some kind of purple sweet potato has different shades of purple (Hardoko et al. , 2010) . Different cultivars sweet purple potato have different anthocyanin content. Anthocyanins provide excellent health effect as an antioxidant and anticancer due to deficiency of electrons in its chemical structure that can counteract free radicals (Jiao et al . , 2012) .

Anthocyanins extracted from sweet purple potato can also significantly counteract the formation of fat peroxide. In previously studies, have been found as many as 16 types of Anthocyanins using HPLCDAD techniques (Jiao et al., 2012) . Anthocyanins can be degraded due to several factors: pH , temperature , structure , light , oxygen , solvents , enzymes and metal ions. Shan et al. (2009) reported that Anthocyanin on sweet purple potato serves as a natural antioxidant .

Physical and chemical properties of anthocyanins can be view from of its solubility in polar solvents such as methanol, acetone , or chloroform , especially with water and acidified with hydrochloric acid or formic acid (Socaciu , 2007). Anthocyanin is stable at 3.5 pH and 50 °C temperature has a molecular weight of 207.08 g/mol and a molecular formula $C_{15}H_{11}0$ (Fennema, 1996). Anthocyanins appearance in red, purple and blue has a maximum wavelength of 515-545 nm, moving with BAA eluent (nbutanol - acetic acid - water) on paper (Harborne , 1996).

The purpose of this research : make a natural dye from sweet purple potato, find the best ratio of solvent to the maceration process of sweet purple potato to get maximum yield and to obtain maximum levels of anthocyanins.

Factors that affect the stability of anthocyanin is transformation of Structure, pH, temperature, light, oxygen and co-pigmentation.

In general, the addition of hydroxylation decrease stability, while the addition of methylation increases stability. The color in foods contain anthocyanin-rich in pelargonidin, cyanidin , or delpinidin aglycone less stable than a diet rich in the aglycone petunidin or malvidin (Fennema , 1996) . The pH is not only affect the color of anthocyanins but also affecting its stability. Anthocyanins are more stable in acidic solution than in alkaline solution (Markakis , 1982). In liquid

forms, anthocyanin have four possibilities structure depends on pH. The structure is a base quinoidal (A), flavilium cation (AH +), a colorless carbinol base (B), and khalkon colorless (C) (von Elbe and Schwartz , 1996 in Arthey and Ashurst , 2001) .

Temperature. Heating is "irreversible" in affecting the stability of the pigment which colorless cation can not go back into the cation flavilium red. Degradation of anthocyanins is influenced by the temperature. Hydroxylated anthocyanins are less stable in hot conditions than methylated anthocyanin glycosylated or methylated (Arthey and Ashurst, 2001).

Light. Anthocyanin is not stable in neutral or alkaline solution and even in an acid solution the color can fade slowly if exposure to light, so the solution should be kept in the dark and cold temperatures (Harborne, 1996). It is generally known that light accelerates the degradation of anthocyanins. The effect can be seen in grape juice and red wine. In winemethylation of diglicoside, the acylated and methylation monoglicoside (Fennema, 1996). Anthocyanins are also unstable when exposed to ultraviolet and visible light and other nuclei of ionizing radiation. Decomposition largely looked into photooxidation as p-hydroxybenzoic acid were identified as minor degradation products (Arthey and Ashurst, 2001). The ability of light to make anthocyanins excited through the transfer of electrons that can affect the pigment to photochemical decomposition.

Oxygen. Oxidative resulting molecular oxygen in anthocyanin. Oxygen and the temperature seemed to accelerate the destruction of anthocyanin. Anthocyanin color stability during processing of fruit juices become damaged by oxygen (Arthey and Ashurst2001).

Copigmentation. Copigmentation (anthocyanin merging with anthocyanin or other organic components) can speed up or slow down the degradation process, depending on environmental conditions. Complex shapes will be related with their proteins, tannins, flavonoids other, and polysaccharides. Although some components are colorless, they can increase the anthocyanin color with a bathochromic shift, and improve color absorption at a wavelength of maximum absorption of color. The complex is likely to stabilize during processing and storage. Stable color of wine is believed to result from the compound anthocyanin itself (Fennema, 1996).

Anthocyanins have been widely used as a colorant, especially drinks. Since many synthetic dyes are known to be toxic and carsinogenetic, the usage have decresead significantly (Francis, 1999). According to Clifford et al. (2000), JEFCA (Joint FAO / WHO Expert Committee on Food Additives) has stated that the extracts that contain anthocyanins low toxic effects. Attention to the anthocyanin pigments intensified in recent years because its benefit for health, including reducing the risk of coronary heart disease, the risk of stroke, the activity of anti-carcinogenic, anti-inflammatory effects, improve eye acuity and improve cognitive behavior.

Clinical study in Italy showed that 79% of patients with diabetes who ate bilberry extract (160 mg twice daily for 1 month) showed an increase in diabetic retinopathy at the end of the experiment (Wrolstad, 2004). Anthocyanins are believed to provide benefits for human health. Anthocyanins can be absorbed in the form of the molecule intact in the stomach (Passamonti et al., 2003). Although it absorbtion far below 1%, anthocyanin after transported to a place that has the activity of metabolic high activity show a systematic such as antineoplastic, antikarsinogenetik, antiatherogenik, antiviral and effects anti-inflammatory, lowers the permeability and fragility kapilerdan inhibition of platelet aggregation as well as immunity. All of this activity is based on its role as an antioxidant (Clifford et al. 2000; Middleton et al., 2000). Anthocyanins are not adsorbed provide protection against colon cancer (Halliwell et al., 2000).

MATERIALS AND METHODS

This research was conducted at the Chemical Engineering Laboratory, Engineering Faculty, Universitas Muhammadiyah Jakarta and TIAP BPPT laboratory. The tools used in this research is Uv-vis spectrophotometry and rotary vacuum evaporator. Raw materials used in this research are purple sweet potato, ethanol 96%, water, concentrated HCl, metahol, and NaOH 10%.

Purple sweet potato peeled and blended, so it will expand the surface area and will increase it reaction rate. Put 25 grams of purple sweet potato into erlenmeyer. For a ratio of 1: 4 ethanol 96% , used 100 ml and 100 ml of distilled water and so on and then stand it for 24 hours in 30 ^{0}C and 1 atm. Filtered the samples then produce a filtrate.

Anthocyanin identification tests conducted qualitatively with the filtrate by taking as 7ml of filtrate obtained and 2 drops of NaOH 10%. This will resulting in a change of color, from green becomes red. Inserted The filtrate into a vacuum rotary evaporator to evaporate the solvent. To calculate the yield, dried the filtrate added tool rotary vacuum and then weighed. Formula for calculating the percentage yield as follows:

$$Yield = \frac{wieght\ anthocyanin\ extraction}{Sweet\ Purple\ Potato\ Weight}\ x\ 100\%$$

As for calculating the levels of anthocyanin, the filtrate obtained was added to the rotary vacuum evaporator and the solvent is evaporated until dark, then concentrated result spectrophotometry instrument with billberry extract standarized 1000 ml. And then inserted
to uv-vis spectrophotometry instrument with billberry extract standarized was diluted with HCl 1% solvent in metahol 1000 ml. And then inserted to uv-vis To make the standard solution, weighed 20.20 mg bilberry extract (anthocyanin content 41.8%) Entered it into a 100 mL flask, dissolved with 60 mL of ethanol 96%, shake it until a clear solution (anthocyanin perfect solution) and tera until get 100mL volume. Drop 12 mL solution and added with 100mL HCL 1% till enough. Analyse it using UV-Visible spectrophotometer at 528 nm wavelength. The concentration of standard solutions obtained from equation:

$$Standard\ Concentration: \frac{20{,}20}{100}\ X\ \frac{`12}{100} = 0{,}02424\ \frac{mg}{ml} = 24{,}240\ \frac{mg}{L}$$

Samples standard solution added to uv-vis spectrophotometry tool to determine the absorbance of each sample with a wavelength of 528 nm, and equation for calculating the anthocyanins levels as follows:

% anthocyanin : $\frac{sample\ of\ Absorbant}{Standard\ Absorban}\ x\ Standard\ concentration\ x\ FP\ x\ Volume\ Ekstrak\ x\ 0{,}418$

Notes :
Extract volume ekstrak is concentrated volume gained on spectrophotometry.
FP: dilution volume.
Standard concentration is 24,240 mg/L.
0,418 is a standard anthocyianin content on extract billberry.

RESULTS AND DISCUSSION

Anthocyanin Identification

This research was conducted with a 1: 4, 1: 5, 1: 6, 1: 7, 1: 8 variable ratio of solvent ethanol to water, with 24 hours maceration and 25 grams purple sweet potato at room temperature, 1 atm pressure and a wavelength of 528 nm. The results are anthocyanin identification, colors, Alkaline Color and Acid Color in table 4.

Table 4. Anthocyanin identification result

Ratio	*Initial Color*	*Alkaline Color*	*Acid Color*
1:4	*Brown*	*Green*	*Red*
1:5	*Brown*	*Green*	*Red*
1:6	*Brown*	*Green*	*Red*
1:7	*Brown*	*Green*	*Red*
1:8	*Brown*	*Green*	*Red*

In table 1 above, the researchers wanted to prove to other benefit in natural coloring of anthocyanins substance. At the time of the identification of anthocyanin, obtain extract from maceration was filtered, and then input 1 ml into a reaction tube to get brown color with a pH of 5, then the extract was dropped with NaOH 10% as much as 2 drops, the anthocyanin substance will changes to green in pH of 10, then give another 2 drops of concentrated HCl and anthocyanin substance color will changes to red with a pH of 3. Thus it can be concluded that anthocyanins can also be used as an acid-base indicator in addition to natural dyes.

Anthocyanin yield calculation.

The yield is done by weighting the results divided by the initial weight, by using a rotary evaporator at a temperature of 45 ^{0}C with a variable ratio of solvent 1: 4, 1: 5, 1: 6, 1: 7, 1: 8 with 24 hours maceration served in table 5.

Table 5 Anthocyanin yield calculation

Solvent ratio	*Yield(%)*
1:4	*2.3923*
1:5	*2.69461*
1:6	*3.3692*
1:7	*3.750996*
1:8	*4.6688*

After the screening process, purple sweet potato extract inserted into rotary vacuum evaporator to vaporized the solvent, with a temperature below the solvent boiling point by raising the pressure, because the anthocyanins can be damaged at 50 ^{0}C. The extracts dry rotarized. After getting dry extract, the yield can be calculated with the formula described in Appendices. At a ratio of 1: 4 gained an average yield of 2.39%, ratio 1: 5 obtained an average yield of 2.69%, the ratio of 1: 6

obtained an average yield of 3.36%, solvent ratio of 1: 7 obtained average - average yield of 3.75% and a ratio of 1: 8 obtained an average yield of 4.67%. From the data obtained the best yield of the ratio of 1: 8 amounted to 4.67%. The complete results of the yield at various solvent ratios are presented in the figure 1.

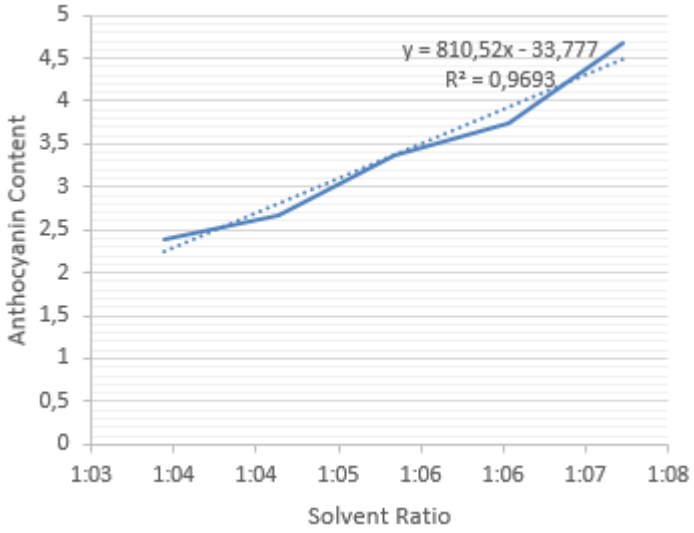

Figure 1. Effect of solvent ratio to Anthocyanin yield

The equation obtained on the relationship between the time of maceration with the results of the yield is as follows y = 810.52x - 33 777 with R^2 = 0.9693, where y as the yield and x as a ratio of solvent. From the graph above shows that the greater the ratio of the solvent, will increase the yield. Because the more solvent used it will enlarge the amount of dissolved compounds and increase the rate of extraction.

Analysis of Anthocyanin content

Anthocyanins levels performed by input the sample to Spectro UV-Vis absorbance and compare with its standard. Sample solvent ratio of 1: 4, 1: 5, 1: 6, 1: 7, 1: 8 with 24 hours maceration time at 30^0C and 1 atm, that presented in table 6.

Table 6. Anthocyanin content calculation

Solvent Ratio	Anthocyanin level (mg/L)
1:4	6.85
1:5	7.22
1:6	8.91
1:7	10.9
1:8	12.5

Extracts anthocyanin has been obtained from the maceration, inserted into a rotary vacuum evaporator to evaporate the solvent. The result then diluted with HCl 1% in 500 ml methanol, then analyzed it with UV-VIS spectrophotometric.

Then the next sample is billberry extract that concentration was known and average absorbance 0.5597. The samples were diluted added spectrophotometry tool, then compared with the standard of comparison is billberry extract. For sample 1: 4 absorbance average is 0.445, sample 1: 5 absorbance average is 0.469, sample 1: 6 absorbance average is 0.615, sample 1: 7 absorbance an average of 0.682 and the sample 1: 8 absorbance Average is 0.7397.

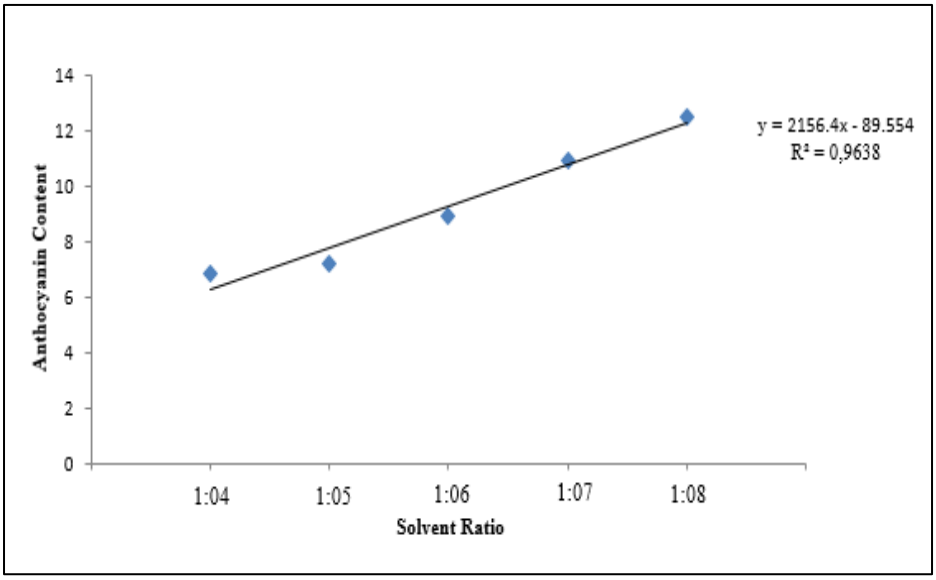

Figure 2. Effect of Solvent ratio on Anthocyanin content

From the figure 2 is obtained in the form of the equation y = 2156.4x - 89 554 with R^2 = 0.9638 where y and x as the anthocyanins. levels and solvent ratio. It can be seen that the more solvent used, the greater the levels of anthocyanins obtained. The results obtained for each ratio of solvent is also not differ much from one point to another point because of anthocyanin can dissolve well in ethanol and water due to the polarity of the two agents approached, the levels of dissolved substances grew, and the more the ratio of solvent used the greater the concentration of the substance.

CONCLUCIONS

Anthocyanins can be obtained by extracting purple sweet potato using the ratio of solvent to produce a concentrated extract of brown. Anthocyanins from purple sweet potato can be used as an acid-base indicator because can reacted with acidic or alkaline. Extract anthocyanins from purple sweet potato is influenced by the ratio of the solvent, the more the ratio of solvent used, the greater the yield and content of anthocyanins obtained. On solvent ratio of 1: 8 showed the best yield of 4.6688% with the equation y = 810,52x - 33.77. R2 = 0.9693 where y as the yield and x as the ratio of the solvent. And extracts obtained more than the other solvent ratio. In the solvent ratio is obtained anthocyanin content of 12.5 mg / L by the equation y = 2156,4x -89.554. R2 = 0.9638 where the 974 y as the anthocyanins levels and x as a variable ratio of solvents.

Based on the overall results, we can suggest a few things : use more adequate and up to date tools, neither for process and analysis; complete analysis on all alternative fuels specifications quality according to Indonesian National Standard (SNI). This research can be developed by examining some variables such as concentration, pH, solvents, the maximum temperature that can be done so as not to

damage the anthocyanin content or using raw materials in a dry state like sweet purple potato dried beforehand and the extract can continue to manufacture powder extract antosinian antosianian by mixing with a binder such as maltodextrin and dried by spray drying.

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