MICROWAVE ASSISTED EXTRACTION OF BETACYANIN FROM PEEL RED DRAGON FRUIT (HYLOCEREUS POLYRHIZUS) (STUDY OF THE EXTRACTION TIME AND THE RATIO OF MATERIALS : SOLVENT)

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ABSTRACT

Dragon fruit peel has pigments other than anthocyanins, therefore this study aims to get the right combination of the best treatments and to find out the effect of extraction time and the ratio of ingredients: solvent on the yield and quality of red dragon fruit skin pigments using the MAE (Microwave Assisted Extraction) method using power 100 watts. In the betacyanin pigment collection stage, a randomized block design method was used with 2 treatment factors, namely extraction time (T), which consisted of 3 levels (T1, T2, and T3 5, 10, and 15 minutes respectively) and the second factor, namely the ratio of ingredients: solvent (R) consisting of 2 levels (R1 and R2) respectively 1:20 and 1:30 (w/v). Each treatment was repeated 3 times to obtain 18 experimental units. Followed by the DMRT test (Duncan Multiple Range Test) if there is an interaction between the two factors or a BNT further test is carried out. From the research that has been done, the best treatment was obtained with an extraction time of 5 minutes and a ratio of ingredients: solvent 1:30 (w/v) with a temperature range of ±60 oC resulting in the best characteristics with a total betacyanin value of 0.4120mg/100g, a pH value of 2.25 , the total yield was 15.11%, the brightness level (L) was 20.4, the intensity of red (a*) was 10.8, and the intensity of yellow (b*) was 6.5.*

KEYWORDS

Betacyanin, dragon fruit, microwave assisted extraction

INTRODUCTION

Red dragon fruit (*Hylocereus polyrhizus*) comes from the cactus group which is known to have high nutritional content, high economic value, and can be used for medicine. National production of dragon fruit in 2012 reached 6,696 tons (Anonymous, 2013). From that amount, a total of 2,008 - 2,343 tons of fruit skins can be obtained which unfortunately is only disposed of as waste. One of the suppliers of red dragon fruit on the island of Java is a red dragon fruit plantation in Banyuwangi Regency with production reaching 12,936 tons in 2012 and will continue to increase in the following year (BPS Banyuwangi Regency, 2013).

The growth of industrial centers for making fruit chips, such as the fruit chip SMEs of Bu Nur, which is located on Jalan Ciliwung II/2 Malang, produces 540 kg of red dragon fruit chips production process skin waste. Citramukti (2008) explained that part of dragon fruit 30-35% of the fruit weight in the form of skin has not been used optimally. On the other hand, this red dragon fruit peel waste still contains a number of bioactive components that have antioxidant properties as well as natural pigments, namely betalain as much as 5.7 mg/100g if extracted using acetone solvent (Saneto, 2010).

Betalains are polar pigments consisting of betacyanins and betaxanthins (Wybraniec et al., 2006). According to Cai et al. (2005) betalain is a nitrogen pigment and is water

soluble. Betalains also have antioxidant and radical scavenging properties. One of the ways to obtain betalain pigments in dragon fruit peels is the extraction process using the maceration method, using aquadest as a solvent which aims to facilitate the use of the extract in its processing into food (Cardoso-Ugarte et al., 2014). The use of the maceration method is more economical and easy to work with, the disadvantage is that it takes a long time to extract the material using this method. So to maximize and shorten the extraction time, the Microwave Assisted Extraction (MAE) method can be used. From the results of previous studies, the extraction of the red dragon fruit peel betacyanin pigment by maceration method using 95% ethanol solvent and distilled water with technical purity with an extraction temperature of 30°C produced the best characteristics with betacyanin content 45.81 mg/100g, yield 81.05%, ethanol residue 0.099 %, pH 6.68, brightness level 24.4, red intensity 4.7 and yellow color intensity 7.9 (Khuluq, 2007). Extraction of red beet betacyanin pigment by the MAE (Microwave Assisted Extraction) method using a combination of ethanol and water in a ratio of 1:1 resulted in a total betacyanin content of 189.02 mg/100g (Cardoso-Ugarte et al., 2014).

Factors that can maximize efficiency and selectivity in conventional extraction processes include the combination of solvent, temperature and extraction time. The advantage of MAE is its wide application in extracting various compounds including heat-labile compounds. The MAE method is able to increase the efficiency and effectiveness of active ingredient extraction and MAE offers lower solvent consumption and a significant reduction in extraction time compared to conventional extraction (Farida and Nisa, 2014).

Several factors that affect the extraction with the help of microwaves include solvent volume and extraction time. The longer the extraction time, the higher the yield, while the larger the ratio of ingredients: solvent will also increase the yield of the extract. Yield has increased with increasing extraction time with microwave (Azmi, 2014). In general, with increasing extraction time, the amount of analyte extracted will be higher (Mandal, 2007). This is caused by friction between the material molecules with microwaves. This friction causes cell walls and tissue materials to be damaged and solutes can come out, so the longer the molecular friction occurs, the more energy is absorbed by the material so that a lot of solute will come out (Kurniasari, 2008). Therefore, it is necessary to do research on the extraction time and the ratio of material: solvent that affect the extraction using the MAE (Microwave Assisted Extraction) method on the quality of the pigment from the red dragon fruit peel and the total yield produced.

MATERIALS AND METHODS

The equipments used for the extraction of betacyanin include: cabinet dryer, blender (National), Desiccator, Digital Scales (Sartorius Instrument M-310), microwave oven (Electrolux, 2450 MHz, max 800 Watt), centrifuge (Universal PLC-012E), magnetic stirrer, rotary vacuum evaporator, glass bottles, aluminum foil, and various kinds of glassware (erlenmeyer, measuring cup, measuring flask, glass funnel). The tools used for analysis include a measuring flask, measuring cup, dropper, measuring pipette, erlenmeyer, spatula, suction ball, funnel, test tube, desiccator, color reader (Minolta CR-100), spectrophotometer (UV -2100), cuvette, and pH meter (Ezido).

The material used in this study was dragon fruit peel waste which was obtained from the business of selling fresh juice and SME's of Bu Nur's fruit chips located on Jalan Ciliwung II/2 Malang, East Java. The material used for the extraction of betacyanin is distilled water obtained from a chemical shop Makmur Sejati, Malang. Materials used for analysis with a purity of p.a. are citric acid monohydrate ($C_6H_8O_6.1H_2O$), disodium hydrogen phosphate dihydrate

($Na_2HPO_4.2H_2O$) (Malincroth) obtained from PT. Panadia Malang.

EXPERIMENTAL DESIGN

This study was arranged using a Randomized Block Design with 2 treatment factors, namely the extraction time (T) which consists of 3 levels and the second factor, namely the ratio of material: solvent (R) which consists of 2 levels. Each treatment was repeated 3 times to obtain 18 experimental units. Observational data were analyzed using analysis of variance or ANOVA (Analysis of Variance) using SPSS 16 software followed by DMRT (Duncan Multiple Range Test) test with a 5% confidence interval, if there is an interaction between the two factors and if there is no interaction, a further BNT test is carried out.

Factor 1 (T) : extraction time (minutes)
T1 = 5 minutes
T2 = 10 minutes
T3 = 15 minutes
Factor 2 (R) : Ratio of Ingredients and Solvent (Aquades – citric acid 2%) (w/v)
R1 = 1:20 (w/v) i.e. 5 grams of material is dissolved in 100 ml solvent
R2 = 1:30 (w/v) i.e. 5 grams of material is dissolved in 150 ml solvent

Observations observed included analysis of the water content of the starting material (Sudarmadji, 2007), total crude betacyanin (Eder, 1996), pH measurement with a pH meter (Sari, 2005), calculation of the yield of crude betacyanin extract (Tensiska and Natalia, 2007), and observations physical properties, namely color (Yuwono and Susanto, 1998).

PROCEDURES

a. Process of making dragon fruit peel powder

The newly obtained dragon fruit peel waste is separated between the skin and the fruit that is still attached. Then weighed and cut into pieces. The leather waste was then dried in a cabinet dryer at 60°C for 20 hours. The dry skin is then blended until smooth. The sifted skin powder is stored at dry room temperature in a dark jar for further use.

b. Betacyanin Extraction by MAE Metode Method

1. Five grams of leather powder was weighed, put into an erlenmeyer, then aquades solvent and 2% (w/v) citric acid were added to the volume according to the experimental design (1:20 and 1:30 (w/v)).
2. Erlenmeyer is placed on a magnetic stirrer for 15 minutes to give the solvent penetration time into the material. Erlenmeyer was then put in a microwave oven with a power set of 100 Watt, the extraction time was adjusted according to the experimental design (5, 10, 15 minutes).
3. After the extraction process is complete, the sample is cooled at room temperature, then centrifuged at 4000 rpm for 15 minutes.
4. The supernatant obtained was passed on filter paper to obtain a dregs-free betacyanin filtrate.
5. The filtrate was concentrated with a rotary vacuum evaporator at a temperature of 60°C, a speed of 65 rpm to obtain a concentrated betacyanin concentrate. According to Giusti and Wrolstad (2001) the presence of betacyanin pigments in the solution until the pink color becomes red. During the evaporation process, do not evaporate the dry sample as this can complicate the subsequent dissolution process.
6. The concentrate was then stored in a dark bottle, closed and stored in a refrigerator at 4°C until ready for analysis.

ANALYSIS

To determine the best treatment combination, the multiple attribute method is used with the following weighting procedure:

1. Determine the ideal value for each parameter.
   The ideal value is the value that corresponds to the expectation, the

maximum or minimum value of each parameter. For parameters with a higher average the better, then the lowest value is the worst value and the highest value is the best value. Vice versa.

2. Density Degrees (dk).
   The degree of density is calculated based on the ideal value of each parameter. If the ideal value is minimal then:
   dk = (The value of reality that is close to the ideal)/(The ideal value of each reality). If the ideal value is maximum then:
   dk = (Ideal value of each alternative)/(Real value that is close to the result)
3. Density Distance (L).
   Assuming that all parameters are important, the density distance (λ) is calculated based on the number of parameters in each treatment.
   λ = 1/ number of parameters
   $L_1 = 1 - \sum(\lambda \times dk)$
   $L_2 = \sum(\lambda^2 \times (1 - dk)^2)$
   $L\infty = \max value\ (\lambda \times (1 - dk))$
   The best treatment was chosen from the treatment that had the lowest L1, L2, and L∞ values.

RESULTS AND DISCUSSION

Raw Material Characteristics

The following table is an analysis of the raw material for red dragon fruit peel used in the extraction of betacyanin.

Table 1. Analysis of red dragon fruit peel powder

Parameters	Result
Water content (%)	6.2
Yield (%)	23.91

Table 1 showed that the dragon fruit peel waste powder used in this study has a water content of 6.2%. The water content value was in accordance with the 6% moisture content of dragon fruit peel powder which was studied by the Center for Agricultural Postharvest Research and Development (2012). The yield of dragon fruit peel waste powder was obtained by 23.91%. The yield produced is quite large, due to the water content of the dragon fruit skin is lower than the flesh, so that after drying it will produce a higher amount of dry matter.

TOTAL CRUDE BETACYANIN

The results of the analysis of the total crude betacyanin of the red dragon fruit peel waste pigment extract using the microwave method due to the extraction time and the ratio of material: solvent ranged from 0.2504 to 0.4305mg/100g. The graph of the average total betacyanin of red dragon fruit peel e Analysis of the average total content of betacyanin crude pigment extract from red dragon fruit peel waste using the microwave method showed between 0.2504 and 0.4305 mg/100 g, depending on the length of the extraction process and the ratio of components and solvents. Graphs of the average total betacyanin of red dragon fruit peel extract with different extraction process times and component-solvent ratios are shown in Fig. 1.xtract with various treatments for extraction time and material : solvent ratio can be seen in Figure 1.

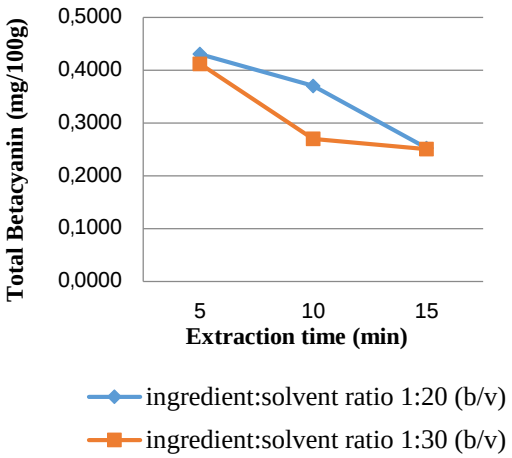

Fig. 1. Total Betacyanin of Red Dragon Fruit Skin Extract Due to the Effect of Extraction Time and Ratio of Ingredients: Solvent (w/v)

The extraction time of 5 minutes to 10 minutes decreased as the extraction time with the microwave increased. This is due to

the longer the extraction time, the longer the material will be exposed to microwave radiation from the microwave oven which results in the breakdown of the material tissue so that it releases the solute into the solvent (Navas et al., 2012). At the extraction time of 10 to 15 minutes the total betacyanin decreased which indicated that the increase in total betacyanin stopped until the extraction time reached a certain point. This is in accordance with the literature of Chan et al. (2011) which states that too long exposure times need to be avoided because it can increase the risk of degradation of the extracted compounds.

In the 1:20 (w/v) material : solvent ratio treatment, the highest total betacyanin average was 0.4305, while the lowest total betacyanin average was obtained in the 1:30 (w/v) material:solvent ratio treatment of 0.2504. This is due to the use of the volume of solvent used is too large as a result the time used for evaporation using a rotary vacuum evaporator is getting longer so that it can lead to degradation of betacyanin compounds. It is assumed that the material : solvent ratio used has reached the optimum point or saturation point, namely the material:solvent ratio of 1:20 (w/v), so the material:solvent ratio is 1:30 (w/v) at the extraction time factor of 10 to 15 minutes was not able to give the effect of increasing the total betacyanin from red dragon fruit peel extract.

pH

Microwave analysis The average pH of red dragon fruit peel pigment extract with extraction process time and material:solvent ratio ranged from 2.25 to 2.62. The graph of the average pH of red dragon fruit peel extract and the ratio of components to solvents for each time of the extraction process is shown in Fig. 2.

The average pH value of each treatment has a tendency that the longer the extraction time will increase the pH value, on the contrary, the larger the ratio of material: solvent will decrease the pH value. The existence of an evaporation process in the concentrate which causes a decrease in water in the material can increase the concentration of acid so that it triggers a decrease in pH.

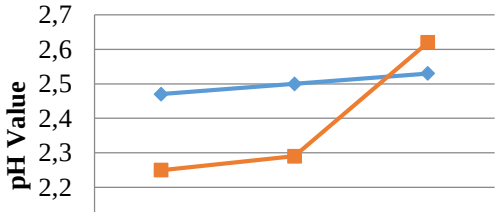

Fig. 2. Average pH Value of Red Dragon Fruit Skin Extract Due to the Effect of Extraction Time and Ratio of Ingredients: Solvent (w/v)

According to Sykes (1998) that the properties of pigments similar to anthocyanins, which are generally acidic, are more stable under acidic conditions. The pH value of a solution is strongly influenced by the concentration of H+ ions. If the concentration of H+ ions is high, the pH value will be lower.

The greater the ratio of material : solvent used, the pH value tends to decrease because the larger volume of solvent is expected to increase the solubility of the acid. The organic acids contained in the dragon fruit peel are also extracted, the more the level of ionization will also increase. If the acid solubility is getting better, then the tendency to release protons (H^+ ions) is also getting bigger. Corlett and Brown (1980) literature states that the higher the acidity of an acid in the solution, the greater the tendency to release protons (H^+) so that it can lower the pH value.

YIELD

Microwave-assisted analysis of the average yield and component:solvent ratio of pigment extract from red dragon fruit peel waste from a long-term extraction process ranged from 12-36%. The graph of the average yield of red dragon fruit peel extract and the ratio of components to solvents for each time of the extraction process is shown in Fig. 3.

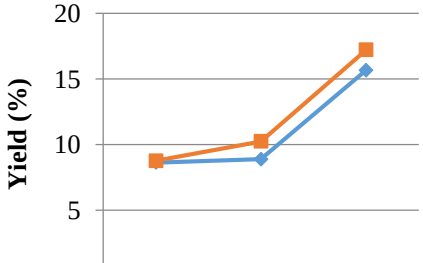

Fig. 3. Average Yield of Red Dragon Fruit Peel Extract Due to the Effect of Extraction Time and Ratio of Ingredients: Solvent (w/v)

The yield of dragon fruit peel extract tends to increase with the longer extraction time, while the larger the ratio of ingredients: solvent will also increase the yield of the extract. At the extraction time of 10 minutes, the yield increased from the extraction time of 5 minutes, the longer the extraction time used, the temperature obtained would increase so that it would increase the amount of yield. According to Routary and Orsat (2012), in general, the yield of the extraction is directly proportional to the time of irradiation with microwaves to a certain extent.

Zhang et al. (2011) predicted that the increase in the yield of the extraction results is due to the greater contact between the material matrix and the solvent when a larger volume of solvent is used, making it easier for the solvent to penetrate into the cell matrix of the material and dissolve the target compound. In this case, it does not mean that the increasing yield can produce betacyanin which is also increasing because of the presence of other impurities that contribute to the content of the yield.

BRIGHTNESS LEVEL (L*)

The L* value is expressed in lightness. Analysis of the average brightness (L) of red dragon fruit peel pigment extract after long-term microwave extraction showed that the ratio of components to solvents ranged from 19.87 to 22.27. The graph of the average brightness of red dragon fruit peel extract for different extraction process times and component-solvent ratios is shown in Fig. 4.

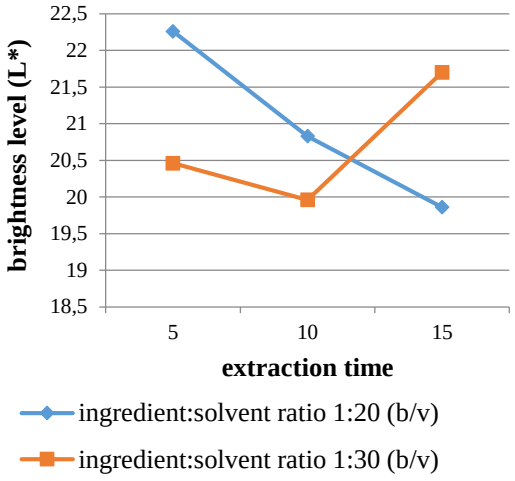

Fig. 4. Average Brightness Level of Red Dragon Fruit Skin Extract Due to the Effect of Extraction Time and Ratio of Ingredients : Solvent (w/v)

The brightness level (L*) of the red dragon fruit peel betacyanin extract tends to decrease at 10 minutes of extraction time and 15 minutes of extraction time with a material:solvent ratio of 1:20 (w/v). Meanwhile, the ratio of material: solvent 1:30 (w/v) decreased at 10 minutes of extraction and increased again to 15 minutes. The brightness level decreases as the amount of betacyanin extracted decreases. The more betacyanin extracted, the darker the color of the extract. Betacyanin pigments are red or red-violet pigments belonging to the betalain pigment group. According to Cavalcanti et al. (2011) increasing the concentration of betacyanin in the extract causes the stability of betacyanin to increase so that the color becomes more concentrated and dark.

Similar to the effect of extraction time, the decrease in brightness level in the material:solvent ratio was caused by the increase in the concentration of betacyanin extracted. The higher the betacyanin concentration, the higher the L value and vice versa. This is in accordance with Delgado-Vargas et al. (2000), where the higher the pigment concentration, the greater the number of chromophores, which causes the color to darken.

RED COLOR INTENSITY (a*)

The average reddish yield (a*) of betacyanin filtrate from red dragon fruit skin ranged from 9.2 to 14.7. The graph of the average red color intensity of red dragon fruit peel extract and the ratio of components to solvents at different extraction process times is shown in Fig. 5.

The intensity of the red color of the red dragon fruit peel extract tends to decrease the longer the extraction time. The extraction time of 5 minutes is the best treatment for betacyanin extraction on dragon fruit skin so that in the filtrate there is a high betacyanin content which means it contributes a better red color. According to Francis (1985), the red color of the concentrate is an indicator of the higher levels of anthocyanin-like compounds. Gross (1991) argues that carotene is a natural yellow or orange pigment. Therefore, the more carotene extracted, the higher the concentration, this caused the intensity of the red color (a*) of the carotene extract to increase.

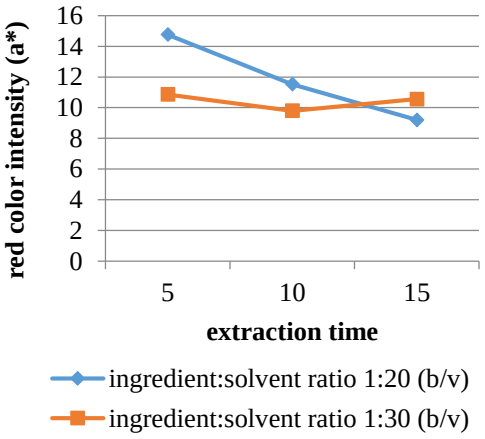

Fig. 5. Average Red Color Intensity of Red Dragon Fruit Skin Extract Due to the Effect of Extraction Time and the Ratio of Ingredients: Solvent (w/v)

The brightness of the betacyanin filtrate was obtained which was low, presumably because the extraction results obtained a low concentration of betacyanin, which means that the intensity of the red color is low so that the filtrate is brighter in color. This happens because there is no damage to betacyanin by heat in the evaporation process of solvent separation. It is suspected that the extraction with a lower concentration of betacyanin causes the intensity of the red color to be low so that the color of the extract is not too dark. In addition, it is suspected that betacyanin damage occurs due to decomposition of the pigment structure by heat in the evaporation process of solvent separation so that bleaching occurs and causes the color to get lighter.

YELLOW COLOR INTENSITY (b*)

The average yield of yellow color (b*) of betacyanin extract from red dragon fruit skin ranged from 6.4-7.6. The graph of the average yellow color intensity of red dragon fruit peel extract at different extraction process times and the comparison of materials and solvents is shown in Fig. 6.

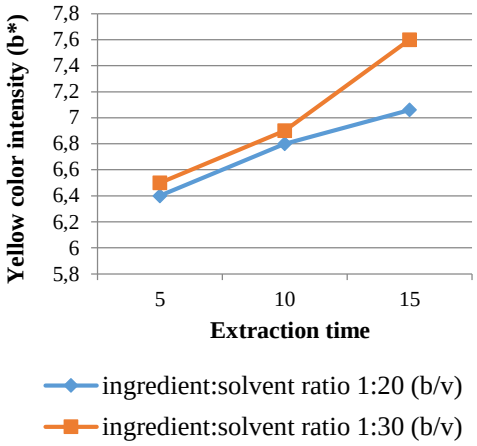

Fig. 6. Average Yellow Color Intensity of Red Dragon Fruit Peel Extract Due to the Effect of Extraction Time and Ratio of Ingredients: Solvent (w/v)

The effect of extraction time on the intensity of the yellow color of the red dragon fruit peel extract tends to increase at the extraction time of 10 minutes to 15 minutes. According to Herbach et al. (2006) a decrease in the intensity of the yellow color (b*) indicates an increase in betacyanin concentration because betacyanin gives a greater effect of red color than the yellow color in the filtrate, in addition to showing an increase in betacyanin damage. This is

presumably because increasing the heating temperature can decrease the stability of betacyanin in the extract which in turn causes damage to betacyanin. This statement is supported by the opinion of Havlikova et al. (1983) which states that the stability of betacyanin decreases at 70 and 80^{0}C heating temperatures. Coultate (1996) stated that betalains were divided into two groups, namely betacyanins with purplish red pigment (λ_{max} 534-537 nm) and betaxanthins with yellow pigment (λ_{max} 480 nm) yellow color on product damage.

CONCLUSIONS

The results showed that the analysis of the pH value with the length of the microwave extraction and the ratio of the material: solvent gave a significant effect (α 5%) and there were interactions. In the analysis of total betacyanin, yield, red color intensity and yellow color intensity showed a significant average difference (α 5%) on the T factor (extraction time) but there was no interaction on these parameters. The results of the analysis of the best treatment were obtained from the red dragon fruit peel betacyanin extraction treatment at a combination of 5 minutes extraction time and a material: solvent ratio of 1:30 (w/v) with a temperature range of ±60^{0}C producing the best characteristics with a total value of betacyanin 0.4120mg/100g, the pH value is 2.25, the total yield is 15.11%, the brightness level (L*) is 20.4, the red color intensity (a*) is 10.8, and the yellow color intensity (b*) is 6.5.

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