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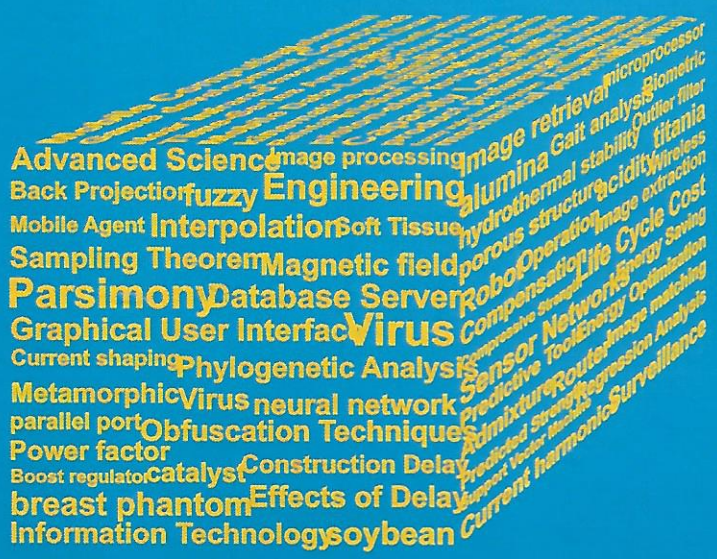

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Preface

It is our great pleasure to present the Vol. 5 No 3 of the International Journal on Advanced Science, Engineering and Information Technology (IJASEIT). This volume comprises articles which are selected from the 1st International Conference on Quality Improvement and Development of Food Product 2015 (QID-Food2015) held in Andalas University, Padang, Indonesia, 17-18 April 2015.

Article submissions came from different countries that cover varies topics in food sciences, food technology and food product development which consists of 23 articles. We would like to take this opportunity to thank all colleagues who had submitted their articles to the IJASEIT through the committee of the QID-Food2015. A lot of number of submissions indicates their high trustworthy to us to publish their current findings and spread to wide academic communities. We also send our appreciation to all reviewers who had dedicated their valuable time and comments to ensure articles significantly contribute to science and technology. In addition, we would like to acknowledge the organizing committee of QID-Food2015 for this great collaboration and the Editorial Board who had worked hard to prepare this volume.

We are pleased to inform you that the editorial boards of the journal have been trying to widen the journal indexing to main databases and to receive regular submissions for publication for the forthcoming issues. The process to be indexed by **Scopus** is in progress. Hope that IJASEIT will be indexed by **Scopus** this year.

We are committed to serve a fast publication and provide quick access to the recent articles for academic communities globally. Finally, we do hope that articles published in this volume might inspire a state of the art research and new findings for the advancement of science, engineering and technology.

June 2015

Sri Atmaja
Ifa Puspasari
Rahmat Hidayat
Ario Betha Juanssilfero
Slamet Riyadi
Huy Bich Nguyen

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Simple Characterization Of Betalain Compound From Red Pitaya (*Hylocereus Polyrhizus*) Peel Solution

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***Abstract*— The peel of red pitaya (Hylocereus polyrhizus) is often regarded as a waste hence. While the red pitaya peel contain betalain pigment which is useful as a natural colourant for functional food and can be applied to food products. The aim of this study was to simple characterization of betalain compound from red pitaya (Hylocereus polyrhizus) peel. The extraction method employed the use of distilled water, since betalain peel is water soluble. The compound of betalain in the solution was determined by HPLC analysis, spectrophotometrically at 200 nm to 800 nm with a UV–Vis spectrometer and Measurement stability, Antioxidant capacity and Antibacterial Activity. Concentration of the sample solution was 7,72 mg / 100 g. Absorption peak at a wavelength of 540 nm obtained from solution of samples showed the presence of betalain compound. The main compound present in a sample suspected of betanin, isobetanin and betanidin that compared to standard. Increasing betalain degradation rates resulting from increasing temperature. Sample solution has antioxidant activity, but weak, and do not to have antimicrobial activity.**

***Keywords*— betalain, red pitaya (Hylocereus polyrhizus) peel, antioxidant, antimicrobial.**

I. INTRODUCTION

Natural colourants from plant sources are receiving growing interest from both food manufacturers and consumers in the continuing replacement of synthetic dyes [1], [2]. Nature produces a variety of compounds adequate for food colouring, such as the water-soluble betalains, anthocyanins, and carminic acid, as well as the oil soluble carotenoids and chlorophylls [3]. However, replacing synthetic dyes with natural colorants offers a challenge because the colour and stability of plant pigments are dependent on several factors, including structure and concentration of the pigment, temperature, pH, light intensity, presence of, metallic ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products [4].

Generally, increasing temperature in food processing will decrease betalain pigment stability. Characterization stable aqueous colorant solution (e.g. juices or solution of fruit or vegetable) is attractive because their GRAS (Generally Recognized As Safe) status makes them easily commercialized. Betacyanins are the group of reddish to violet betalain pigments that are common in many flowers and fruits. Betacyanin can be classified into four kinds: betalain, amaranthin, gomphrenin and bouginvillein. They are water-soluble betalain pigments derived by glycosylation of betanidin, [5], [6]. They have antioxidant, antimicrobial, anti-inflammatory and anticancerous property which can be better studied as the natural source of food colorant [7], [8]. [9] reported that betalain pigments can be applied to food products such as Indonesia cakes, drinks, ice cream, jelly, pudding, jam and lunkhead. It is presumed that betalain pigment concentration in these products is quite low. The low concentration of betalain characteristic that applied in food products is very important to study. The objective of this research is to know the simple characterization (stability, antioxidant and antibacterial activity) of betalain from red pitaya (*Hylocereus polyrhizus*) peel. Distilled water is used as the extraction medium since betalain is water soluble. The compound of betalain in the solution was determined by using HPLC analysis, spectrophotometrically at 200 nm to 800 nm with a UV–Vis spectrometer to measure its stability. Its antioxidant capacity and its antibacterial activity were also measured.

II. MATERIALS AND METHODS

A. Material, Solvents and Reagents

Fruits were obtained from certain sources in Padang, West Sumatra, Indonesia and stored for 5 days. Solvents and

reagents used were betalain standard (sigma), distilled water, citrate-phosphate, formic acid, acetonitrile, DPPH (2,2-diphenyl-1-picrylhydrazyl), ethanol, acetic acid, and eritrosin (commercial synthetic red dye), bacteria Staphylococcus and Salmonella typhimurium.

B. Sample Preparation

Red pitaya peel that has been stored for 5 days with the water solvent were extracted using solvent ratio of 4: 1, extraction temperature 36 ^{o}C, and the extraction of 9 hours. The solution was filtered and centrifuged solution and then prepared for analysis.

C. Betalain Determination

The compound of betalain in the solution was identified by spectrophotometrically at 200 nm to 800 nm with a UV–Vis spectrophotometer. Betasianin analysis was performed by taking samples of 1 ml, diluted with citrate-phosphate buffer pH 5 and measured absorbance at λ537 and λ500. Value absorbance was calculated with A = 1.095 (λ537-λ500) [10]. Betalain concentration calculation based on the formula:

$$\frac{A \times FP \times BM \times 1000}{\varepsilon \times l}$$

(A: absorbance; FP: dilution factor; BM: 550g/ mol; ε: 60000 L/mol cm, l: cuvette of 1 cm thick)

D. HPLC Analyses

HPLC analyses were performed on HPLC (UFLC Shimadzu HPLC-System). The analytical column Shimpack 250×4 RP-18 (5 µm) was operated at 30°C. Solvents were 0.2% (v/v) formic acid in water (A) and acetonitrile (B). At a flow rate of 1 mL/min, simultaneous monitoring was performed at 535 nm

E. Measurement of the Stability

Measurement of the color intensity of betalain solution was performed on four temperature treatment (30, 50, 70 and 100^{0}C). Each solution was measured with a maximum wavelength double beam spectrophotometer Shimadzu UV-1800 with an area measuring wavelengths between 200 - 800 nm

F. Determination of Antioxidant Capacities

The ability to scavenge DPPH free radical was determined based on the method of Brand Williams with minor modification [11]. Briefly, reaction mixtures that containing 20, 40, 60 and 80 µL solutions and 2 mL 6.25 × 10-5 M DPPH solution were prepared, mixed, and then reacted in the dark for 30 min. A control sample containing the same volume of solvent in place of solution was used to measure the maximum 2,2-diphenyl-1-picrylhydrazyl (DPPH) absorption. The absorbance at 517 nm was recorded to determine the concentration of the remaining DPPH.

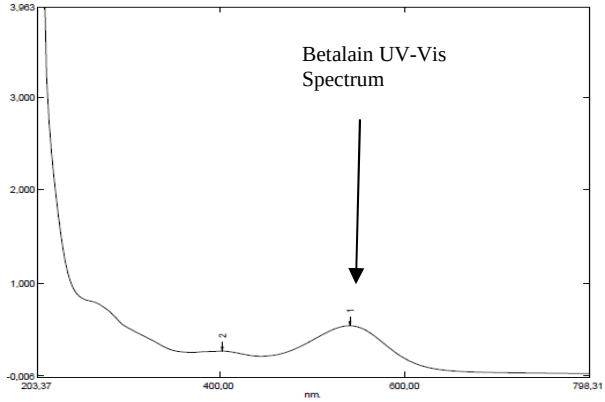
G. Determination of Antibacterial Activities

The solution was investigated for its antimicrobial activity. Sterile nutrient agar was inoculated with the test organism under sterile condition and then poured into sterile petri dishes. A sterile cork borer was used to remove five plugs from each agar plates to produce 8 mm diameter wells. Then each disc is dropped by 25 µL, 50 µL, 100 µL, 200 µL, 400 µL of solution solution and allowed to diffuse at room temperature for 20 minutes and the plates were incubated overnight at 37 ^{o}C. Test sample was tested against each organism in duplicate. The calculated of agar diffusion technique was used as zone of inhibition. The recorded diameter of inhibition zones of growth measured in millimetres will be reported [12].

III. Result and Discussion

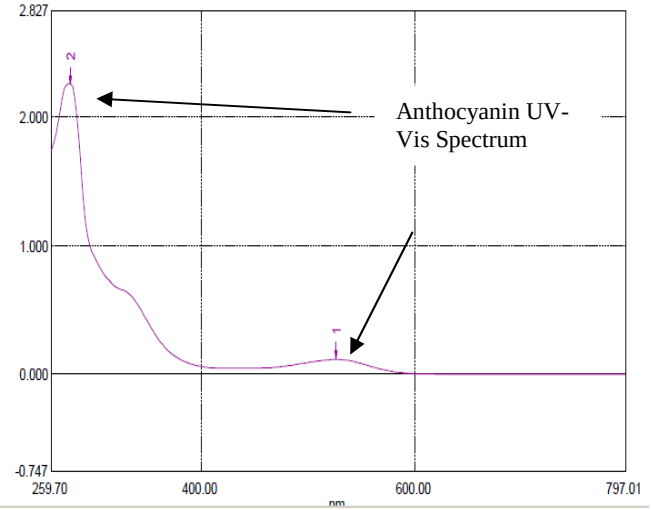
A. Characterization of Betalain with the Spectrophotometer

The measurement of UV-Vis spectra of the sample solution that obtained from the peak wavelength range of visible light was at 540 nm wavelength showed in figure 1.



Explanation :

Wavelength	Abs
540,00	0,542
402,50	0,272



Explanation :

Wavelength	Abs
526,00	0,115
278,00	2,221

Fig 1. UV-Vis spectra of the sample solution at a wavelength of 540 nm

Spectrophotometric data from aqueous solution of red pitaya peel recognized by the absorbancy of the sample

solution, of the data it can be calculated betalain content in the solution. It was found that the level of aqueous solution of red pitaya peel of 7,72 mg / 100 g. Concentration obtained was very small due to the small amount of betalain content in the solution.

The presence of a dominant absorption peak at a wavelength of visible spectra obtained from solutions of samples showed the presence of the compound betalain. This confirm the differences between the betalain compound and antosianin compound. The emergence of a dominant peak indicated that there was a class of compound betalain which was not a group of anthocyanin. One peak that appears was given by the uptake of conjugated double bonds contained in betalain structure, whereas anthocyanin which have a more complex structure in which containing the benzoil and the cinnamoyl skeleton that will give two observations main absorption peak in the wavelength range of UV and visible light. Anthocyanin has two characteristic absorption at the wavelength region, UV (260-280 nm) and visible (490-550 nm).

The spectrum observation of sample solution found an absorption as a shoulder at 310-340 nm, showed the possibility of the compound contained in betalain solution has aslilasi aromatic organic acid or glycosylation with sugar molecules. Betalain existing group consist of several compound that bind to molecules of sugars. Based on the observation of the UV-Vis spectrum of possibilities which was detected only compound total betalain. If the composition of betalain compound in a sample solution has no dominant or relative as much it will be difficult to predict the type of a betalain based on observation of the UV.

For comparison the spectral data which was obtained from spectrophotometer measurement with the data from the literature for the determination of compound in a sample solution showed betalain was not good enough. Then to minimize confusion you should use a standard material, but because of many variaties of betalain compound that exist so little is also likely to be able to get a betalain standards in accordance with the existing betalain compounds in the solution of the sample being tested, based on this is required a more in-depth analysis Reviews such as analysis by HPLC system.

B. Characterization of Betalain by HPLC Analysis

Based on the results of HPLC analysis, it can be seen betalain separation on HPLC chromatogram obtained from a sample solution compared with standard betalain chromatogram (Figure 2). At the peak of the chromatogram allegedly identified several classes of betalain compound. Compared with standard peak of betalain that the first peak appeared at a retention time of 8,459 minutes, the second peak appeared at 10,707 minutes and the third peak appeared at 11,725. Each peaks had the same retention time that derived from standard, this indicated that the second chromatogram were betanin, isobetanin and betanidin [13]. Based on the retention time of betalain emergence peak, only a few of betalain compound having different polarity compared to the previous betalain, this may be due to the difference in sugar or acyl bound on betalain aglycone compounds. Analysis of the temperature treatment.

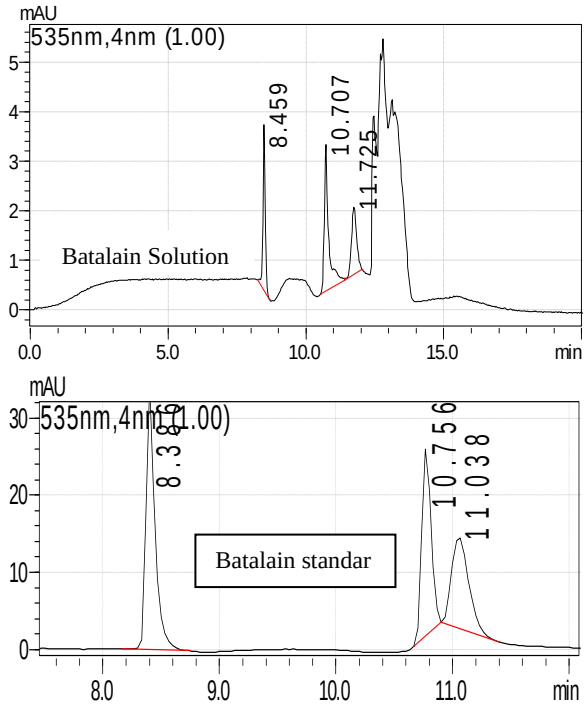


Fig 2. Betalain separation on HPLC chromatogram obtained from a sample solution compared with standard betalain chromatogram

C. Measurement of the Stability of Betalain Solution from Temperature Treatment

Red pitaya peel water's solution treated by heat at various of temperature such as 30 (as a control), 50, 70 and 100°C. Heat treatment carried out for 15 minutes in the oven, a point to see the degradation or damage betalain with the treatment given temperature. Reason takes over the temperature variation to the application as a dye for food and beverage processing, because in general the food and beverage processing using a water system that using the temperature of the boiling point of water is 100°C [14]. Observation on the treatment given to the effect of temperature in the solution which has been diluted with a water solvent, where the water was the basis of a solvent commonly used in food processing.

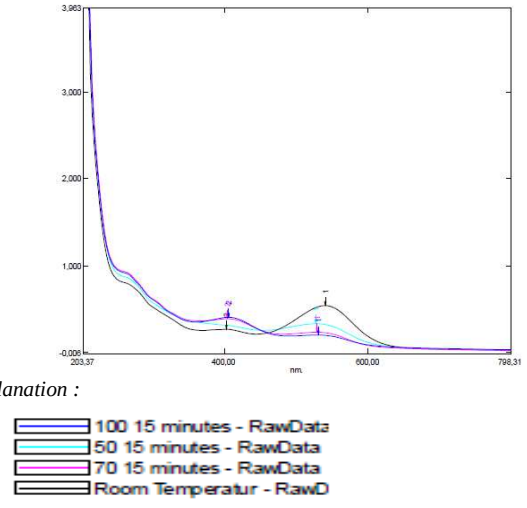


Fig 3. Betalain Solution Stability Influenced by Temperature

TABLE I
INFLUENCED OF TEMPERATURE TO THE SPECTRUM OF EXISTING BETALAIN COMPOUND

Treatment	Wavelength	
	530	400
RT	0,542	0,272
50	0,335	-
70	0,238	0,392
100	0,205	0,409

The treatment temperature in the sample solution can be seen from the data that was attached to the table that the treatment temperature influenced the spectrum of existing betalain compound. From the data it can be observed that the treatment temperature of the sample solution provides observation impairment absorbance of the sample solution was not given temperature treatment. At wavelength of maximum 530 nm was increased betalain degradation rates resulting from increasing temperature, this was based on the research [15], [16]. [17] reported that betacyanin pigmentation decrease at 40, 50, 60^{o}C but increased at 0, 10, 20^{o}C. The absorbance at the maximum wavelength of betalain compound in the sample solution decreased by the rise of temperature. This showed the presence of betalaian compound degradation contained in the sample solution. But there were interesting things can be seen from the data of existing spectrum, with decreasing values at a wavelength of maximum absorbance at ± 530 nm betalain, an increase in the value of the absorbance at a wavelength of 400 nm. This showed along with the degradation of betalain form other compound with maximum wavelength of about ± 400 nm which was the region of the yellow color spectrum. This can be examined more deeply by HPLC-MS analysis.

D. Antioxidant Activity

The antioxidant activity of the sample solution quantitatively determined by DPPH (1,1-diphenyl-2-picrylhydrazyl), betalain in reducing or capturing DPPH radical. These capabilities can be seen from the decrease in the intensity of the purple color of the DPPH solution was added to the sample. Reduced intensity of the color of DPPH solution may indicated that the test material reaction with DPPH radical molecules form the compound 1,1-diphenyl-2-pikrilhidrazin yellow. The greater the concentration of the test material, yellow color will be stronger. Reduction of the intensity of the color purple is quantitatively DPPH solution can be calculated from the decrease in the absorbance of the solution. The greater the concentration of the test substance absorbance read smaller, which means that the activity of the test material in capturing greater DPPH radical. The measured absorbance of DPPH absorbance rest that did not react with the test solution.

DPPH free radical scavenging activity of the solution samples can be expressed by parameters EC50 (efective consentration) is the concentration of test compound that led to the capture of the free radicals by 50%. EC50 values determined from the linear regression equation between the concentration of the test material with the free radical scavenging percentage of the average of each concentration.

In particular, the compounds can be said to be a very powerful antioxidant if EC50 values of less than 50 ug / ml, strong for EC50 worth 50-100 ug / ml and the medium for EC50 worth 151-200 ug / ml (Widyaningsih, 2010). The antioxidant activity of the solution samples, EC50 value was more than 200 ug / ml, this indicates that the sample has a weak antioxidant activity. This might be due to the concentration of active substances in the solution low as solution solvent removal process has not been carried out, because the solutionion was done using water solvent so that the solvent removal process is quite difficult to remove because of the high boiling point of water

E. Antimicrobial Activity

The result showed that the solution has no significantly comparable antimicrobial activity against the well-known microorganisms. This may relate to the same conditions as the test antioxidant activity where the concentrations of the solution was not high enough because there were many existing water solvent, thus making the bioactivity of the active compounds in the solution was not too optimal. it is very interesting to study further the concentration of the sample solution or by solvent removal process by means of freeze drying.

IV. CONCLUSIONS

Compounds contained in the solution of red pitaya peel was betalain group confirmed their absorption maximum wavelength of 540. The solution of red pitaya peel contains the main compound were betanin, isobetanin and betanidin. Stability of solution in red pitaya peel was increasing betalain degradation rates resulting from increasing temperature. The antioxidant activity of solution in red pitaya peel, EC50 value was more than 200 ug/ml, this indicates that the sample has a weak antioxidant activity. And the antimicrobial test, the result showed that the solution has no significantly.

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