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Application of response surface methodology in optimization condition of anthocyanin extraction process of cocoa peel waste with Microwave Assisted Extraction Method (MAE)

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Application of response surface methodology in optimization condition of anthocyanin extraction process of cocoa peel waste with Microwave Assisted Extraction Method (MAE)

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Abstract.Anthocyanins are present in high concentration in cocoa peels. A microwave-assisted extraction was used to extract the anthocyanins from cocoa peel by using aqueous ethanol solvent. Extraction process conditions such as particle size, material/solvent ratio (w/v), optimum extraction time, and microwave power in the extraction process will affect the obtained concentration of anthocyanin. The purpose of this study is to identify the relationship between each input variables and to develop predictive models used in optimizing the conditions of the anthocyanin extraction process. The Design Expert vs11 program with Response Surface Methodology (RSM) Box-Behnken Design was used for research and select process conditions from a combination of factors producing the optimal responses. Based on the Box-Behnken RSM Design, particle size, material/solvent ratio, extraction time, and microwave power in the extraction process are factors which mainly affect the response of produced anthocyanin concentration. Relationship between variables and the response of anthocyanin concentration was modeled by Y = 0.000178-4.17412E-07A + 0.012205B + 0.000022C + 7.75551R-08D + 0.000060AB – 2.96236E-08AC + 1.20818E-09AD – 0.001314BC – 0.000029BD + 1.84413E-07CD (A is particle size; B is the ratio of cocoa peel/ethanol; C is extraction time; and D is the power of microwave). The optimal response value of anthocyanin concentration is 1,435 mM, with the condition of the particle size at the extraction process of 60 mesh; the ratio of cocoa peel/ethanol of 0.0625 w/v; extraction time of 10 minutes; and the microwave of 450 watts.

Keywords: anthocyanin, microwave assisted extraction, response surface methodology

1. Introduction

Cocoa (*Theobroma cacao* L.), Theobroma is a genus of fruit trees planted in various tropical regions, including the family of Malvacae. Cocoa (*Theobroma cacao* L.) is a broadleaf plant with reddish-yellow fruit. Cocoa contains active compounds that can be used as an antiseptic, antioxidant, anti-inflammatory, and diuretic. Cocoa is also used as a traditional medicine that works to treat burns, snake bites, fever, dry lips, coughing, rheumatism, and fatigue[1-2].

The structure of the cocoa fruit is divided into four main parts namely the seeds, placenta, pulp, and skin. The cocoa husk is the biggest waste component of a cocoa bean processor. Cocoa skin mass reaches 75% of the total mass of fruit. Cocoa pod husk has not been used optimally because it is only used as animal feed, burned, or even thrown into agricultural waste. Cocoa skin contains many active substances namely phenolic compounds such as pyrogallol, tannin, quercetin, resorcinol, and lignin. Polyphenols contained in the skin of cocoa have the main components of catechins, proanthocyanidins, and anthocyanins. The content of anthocyanin in 600 grams of cocoa peel reaches 39.82% [3-4].

The extraction method that is developing both in the world of research and industry is Microwave-Assisted Extraction (MAE), which is extraction with the help of microwave energy. The MAE method can help increase the amount of crude extract yield in the extraction time and the number of solvents which is lower than the conventional extraction method [5-6,9].

Yusepa stated that the use of conventional methods is the simplest extraction method, but the weakness of the method is that it requires a longer extraction time[7]. The development of extraction methods to speed up extraction time is needed to reduce production costs. The extraction method that is developing both in the world of research and industry is Microwave-Assisted Extraction (MAE), which is extraction with the help of microwave energy. The MAE method can help increase the amount of crude extract yield in the extraction time and the number of solvents which is lower than the conventional extraction method [8-10].

In this study, design 11 vs Expert Design Program with Response Surface Methodology (RSM) Box-Behnken Design is used to research and select the process conditions from a combination of factors that produce an optimal response. Based on the Box-Behnken RSM Design it is known that the main effects of particle size, material/solvent ratio, optimal extraction time, and microwave power in the extraction process are factors that greatly influence the response of the resulting anthocyanin concentration.

The advantages of this program can be used for analysis and modeling of a problem with one or more treatments in research [11-13]. According to Radojkovic, RSM is a collection of statistical and mathematical techniques that are useful for developing, improving, and optimizing processes, where the response is influenced by several factors (independent variables) [14]. The Surface Response Methodology (RSM) not only defines the influence of independent variables but also produces mathematical models, which explain chemical or biochemical processes. The main idea of this method is to know the effect of independent variables on responses, get a model of the relationship between independent variables and responses and get the conditions of the process that produces the best response [15-16]. In addition, the advantages of the RSM method include not requiring large amounts of trial data and does not require a long time [17-18].

The purpose of this study is to identify the relationship between input variables and to develop predictive models used in optimization of the conditions of the anthocyanin extraction process.

2. Materials and Methods

2.1. Material

Materials used in this study include cocoa peels from the Indonesian Coffee and Cocoa Research Center, ethanol (pa) from Merck, and aquades. microwaves (MAE) is done using a microwave accelerated reaction system (SAMSUNG MS23H3125FK-SE) with a power of 1150 W, equipped with a digital timer and UV-VIS Spectrophotometer for analysis.

2.2. *Methods*

This research method consists of 3 stages, 1) creation of the formulation and response design, 2) response analysis, 3) optimization. Then proceed to the verification stage, as proof of the prediction of the optimum formula solution response value.

2.2.1 Optimization Conditions of anthocyanin extraction on the cocoa peel.

There are three stages in applying RSM [11]. The research formulation design and response analysis were carried out using the Design-Expert v11 program. In this study, the dependent variable was the concentration of anthocyanin extracted while the independent variable was A. particle size (60, 80, 100, 120, dan 140 mesh), B. ratio of cocoa: solvent (1: 2; 1: 4; 1: 6; 1: 8 w / v), C. maceration time (2, 4, 6, 8, and 10 minutes for MAE extraction method; 1,2,3 days for maceration method), D. microwave power (100; 300; 450; 600 watt). The results of the research formulation design from the expert design program were applied in several types of solvents (water; distilled water: 10% citric acid (1: 6 v / v); ethanol) are listed in Table 1. for the MAE method.

Table 1. The design of anthocyanin extraction research formulation using MAE method

Formulation	Particle size (Mesh)	Ratio (b/v)	Time (minutes)	Power (W)
1	100	0.01875	6	100
2	100	0.01875	6	275
3	140	0.00625	10	100
4	100	0.01875	6	275
5	100	0.01875	2	275
6	100	0.01875	6	275
7	100	0.01875	6	275
8	140	0.00625	10	450
9	100	0.01875	6	275
10	100	0.04375	6	275

11	60	0.03125	2	100
12	60	0.00625	10	100
13	140	0.03125	2	100
14	60	0.03125	10	100
15	140	0.00625	2	100
16	60	0.01875	6	275
17	60	0.03125	10	450
18	60	0.00625	10	450
19	140	0.00625	2	450
20	140	0.03125	2	450
21	60	0.03125	2	450
22	100	0.00625	6	275
23	140	0.03125	10	100
24	180	0.01875	6	275
25	100	0.01875	6	625
26	100	0.01875	6	275
27	100	0.01875	14	275
28	140	0.03125	10	450
29	60	0.00625	2	100
30	60	0.00625	2	450

Minimum and maximum limit values are entered to the Box-Behnken Design Design-Expert v11 Response Surface Methodology (RSM) program for randomization. After randomization of the combination, 30 treatments were analyzed [16].

2.2.2 Anthocyanin Extraction

The initial stages of cocoa peel dye extraction is Cocoa peel was finely macerated in ethanol solution [3]. The sample was placed in the microwave according to the study design in Table 1. The resulting product was filtered and then centrifuged. The obtained supernatant was concentrated with a rotary evaporator at 40 ºC. The supernatant was characterized by UV-Vis spectrophotometer (the supernatant is stored in a closed and translucent container in the freezer).

2.2.3 Response Analysis

The response value of the dependent variable was analyzed by ANOVA. The ANOVA model used can be chosen as suggested by the program, the model that has the highest level and produces a significant ANOVA value. ANOVA models contained in this design are Linear, Quadratic, Special Cubic, and Cubic. Models that give significance to ANOVA and non-significance to the Lack of fit are chosen to analyze the variables. In addition, the DX v11 program also provides a normal plot of residual facility that indicates whether the residual (the difference between the actual response and the predicted response value) follows the normality line (straight line). Data points that are getting closer to the normal line show a normal spread of data, which means that the actual results will be close to the results predicted by the program [20].

2.2.4 Optimization stage

At this stage, the response (antioxidant concentration) is determined by the optimization goal in the DX v11 program. This program will optimize according to variable data and response measurement data entered. The output of the optimization stage is the recomme ndation of several new optimal formulas according to the program. The most optimal formula is a formula with a maximum desirability value. Desirability value is the value of the optimization objective function that shows the ability of the program to fulfill desires based on the criteria set on the final product. The range of values is from 0 to 1.0. The desirability value which is getting closer to the value of 1.0 indicates the ability of the program to produce the desired product more perfect. The purpose of optimization is not to obtain a desirability value of 1.0, but to find the best conditions that bring together all the objective functions [13].

3. Result and Discussion

3.1 Determination of Optimum Wavelengths

Anthocyanins are polyphenol compounds that give plants a red-blue color. The color is formed due to the absorption of light at certain wavelengths. Light absorption with certain wavelengths of organic compounds occurs because of the transition π→π * and n→π*. The structure of organic compounds that can produce colors must have unsaturated groups that can undergo transitions π→π * and n→π* or called chromophores. The type of anthocyanin contained in Cacao peel is Pelargonidin (Pg) with a brownish yellow color. The chemical structure of Pelargonidin (Pg) is shown in Figure 1.

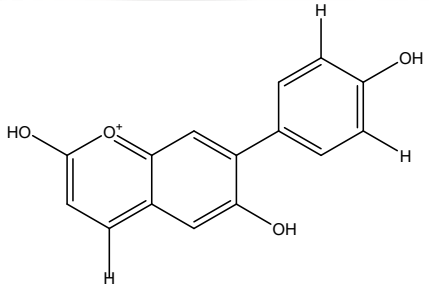

Figure 1. Chemical structure of Pelargonidin (Pg)

Wavelengths used in the range of 400-800 nm. The maximum wavelength of anthocyanin extracted from the skin of cacao is 494 nm. This result is in accordance with the color of the cocoa peel extract which is brownish yellow (complementary color) with the absorbed color analysis being blue-green. The results of the optimum wavelength spectrum analysis of the anthocyanins of cocoa peel extract are shown in Figure 2.

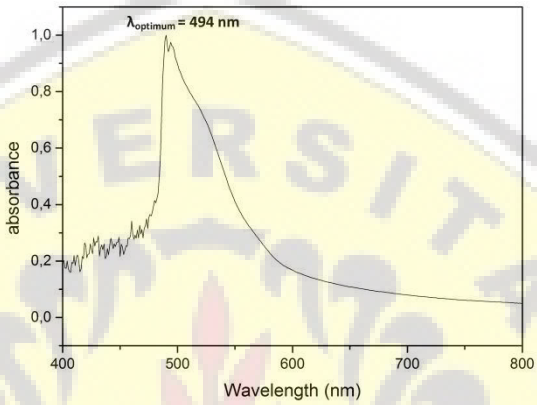

Figure 2. Determination of the optimum wavelength of anthocyanin extract of cocoa peel

Determination of pigment concentrations equivalent to anthocyanins from cocoa peel is carried out using the Lambert-Beer equation, namely:

$$A = \varepsilon\, b\, c \qquad \text{(i)}$$

A: Absorbance
ε: molar absorptivity coefficient (26900 $Lmol^{-1}cm^{-1}$)
b: cuvette thickness (cm)
c: concentration (M)

3.2 Process Condition Optimization with RSM

The analysis result of the concentration of cocoa bean extraction to the response can be seen in Table 2. The results of measurements of the effect of the extraction conditions on the response of anthocyanin concentration are listed. Based on Table 2, it can be seen that the response value of anthocyanin concentration for the MAE method, the anthocyanin concentration is 0.000412-0.001407 M for ethanol solvents.

Table 2. Results of MAE Extraction Response

Form	Particle size (Mesh)	Ratio (b/v)	Time (minutes)	Power (W)	Concentration (M)
ME 1	100	0.01875	6	100	0.000538
ME 2	100	0.01875	6	275	0.000567
ME 3	140	0.00625	10	100	0.000464
ME 4	100	0.01875	6	275	0.000630
ME 5	100	0.01875	2	275	0.000556
ME 6	100	0.01875	6	275	0.000727
ME 7	100	0.01875	6	275	0.000553
ME 8	140	0.00625	10	450	0.001374
ME 9	100	0.01875	6	275	0.000635
ME 10	100	0.04375	6	275	0.000801
ME 11	60	0.03125	2	100	0.000449
ME 12	60	0.00625	10	100	0.000412
ME 13	140	0.03125	2	100	0.000532
ME 14	60	0.03125	10	100	0.000617
ME 15	140	0.00625	2	100	0.000443
ME 16	60	0.01875	6	275	0.000617
ME 17	60	0.03125	10	450	0.000846
ME 18	60	0.00625	10	450	0.001407
ME 19	140	0.00625	2	450	0.000457
ME 20	140	0.03125	2	450	0.000778
ME 21	60	0.03125	2	450	0.000564
ME 22	100	0.00625	6	275	0.000468
ME 23	140	0.03125	10	100	0.000696
ME 24	180	0.01875	6	275	0.000678
ME 25	100	0.01875	6	625	0.000550
ME 26	100	0.01875	6	275	0.000604

ME 27	100	0.01875	14	275	0.001109
ME 28	140	0.03125	10	450	0.001029
ME 29	60	0.00625	2	100	0.000406
ME 30	60	0.00625	2	450	0.000434

The results of the analysis of variance (ANOVA) are listed in Table 3. Mathematical equation models to predict significant anthocyanin concentrations with p values less than 0.05. ANOVA results also showed that each component, namely the particle size, the ratio of cocoa: solvent, maceration time, and microwave power had a significant effect on the response of anthocyanin concentration. Lack of Fit F-value yield response with a p-value greater than 0.05 indicates that Lack of fit is not significant. The insignificant Lack of fit value is a prerequisite for a good model because it shows the suitability of anthocyanin concentration-response data with the model [21].

Table 3. Analysis of the model for anthocyanin concentration responses

Response	**Mathematical Equations**	**Significant (p<0,05)**	**Lack of fit (p<0,05)**	**R^2**
Concentration of anthocyanin extraction	Y=0.000178-4.17412E-07A+0,012205B+0.000022C+7.75551E-08D+0.000060AB-2.96236E-08AC+1.20818E-09AD-0.001314BC-0.000029BD+1.84413E-07CD	0.0033	0.0074	0.7703

3.3 Analysis of Anthocyanin Concentration Response

The RSM equation for optimizing the conditions of the anthocyanin extraction process with the MAE method using ethanol solvent to the anthocyanin concentration response is as follows:

Y=0.000178 - 4.17412E-07A + 0.012205B + 0.000022C + 7.75551E-08D + 0.000060AB – 2.96236E-08AC + 1.20818E-09AD – 0.001314BC – 0.000029BD + 1.84413E-07CD

The equation shows that the response of anthocyanin concentration will increase directly proportional to the increase in the ratio of cocoa: a solvent; maceration time; power; particle size and ratio; particle size and power; and maceration time and power. The normal plot of the residual graph that indicates the relationship between the actual value and the value predicted in Figure 3, approaches

the normal line that shows data for the normal spread yield response. This means that the actual results will be close to the results predicted by the DX vs11 Program.

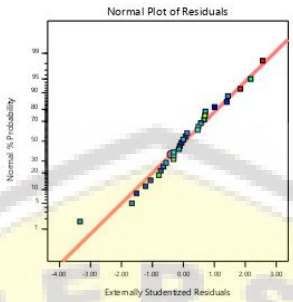

Figure 3. Normal plot of the residual anthocyanin concentration response

The surface shape of the interaction between the various components can be seen more clearly on the three-dimensional graph shown in Figure 4.

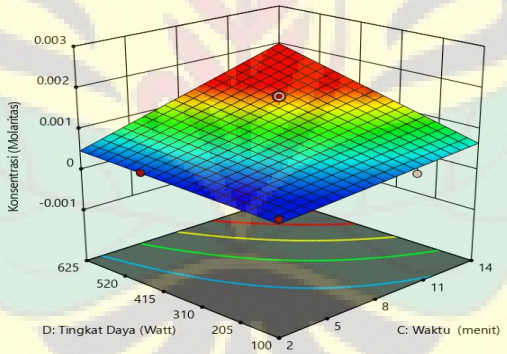

Figure 4. Three-dimensional graph of anthocyanin concentration response test results

Figures 4 and 5, explain the results of optimization in three dimensions (3D) and contour (2D). A contour plot is a two-dimensional picture of the response presented using a prediction model for the concentration value. The lines consisting of points on the contour plot graph show the combination of three components with different amounts that produce the same concentration value. The prediction point in the figure shows a combination of 450 W power level and 10 minutes time which results in a concentration value of 0.001407M. Three-dimensional graph (3-D) shows projections from contour plot graphs. A low area on a three-dimensional graph shows a low concentration value, while a high area shows a high concentration value.

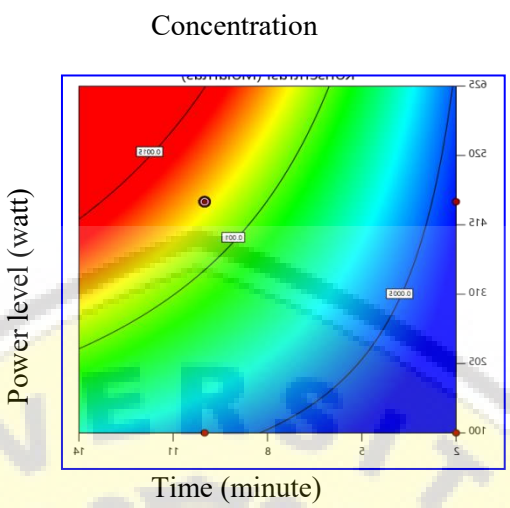

Figure 5. Contour plot graph for optimum formula desirable value

The results showed that the influence of the ratio could increase the extraction result allegedly due to the greater amount of cocoa husk in the extraction media indicating the source of active substances that could be extracted was also higher, so the anthocyanin yield obtained was also greater with increasing the b/v ratio of the cocoa peel /solvent. However, when the amount of cocoa peel is significantly increased the saturation condition will be formed so that the reverse extraction process occurs which causes the concentration of anthocyanin in the product to decrease.

Extraction time also shows the effect on anthocyanin concentration where the longer the extraction time, the higher the product concentration. This is analyzed because the length of the process of contact between anthocyanin and the solvent causes the number of active substances produced to increase.

Another factor analyzed in the anthocyanin extraction process is the particle size of the cocoa shell. The smaller particle size of the cocoa shell (the larger the mesh size) indicates an increase in anthocyanin concentration. The decrease in particle size will cause the surface area of cocoa shells to be higher so that the probability of contact between anthocyanins contained in the cocoa shell and the solvent increases, this leads to an increase in the extraction rate so that the quantity of anthocyanin produced becomes greater. but an increase in reaction rate causes side reactions that produce impurity products.

The last factor that influences the extraction of anthocyanin in cocoa skin is the power supply to the MAE method. Giving power to the MAE method shows an increase in energy possessed by the solvent molecule. The increase in energy in the solvent molecule will accelerate the initiation of the contact process between the active substance and the solvent. This condition increases the amount of anthocyanin extract obtained during extraction using MAE. The provision of high power does not only affect the amount of anthocyanin that increases but also the presence of side reactions that actually interfere with the extraction process. This is because the high power causes the energy possessed by the molecules also increases until the chemical bond is broken. So that at high power conditions, the resulting anthocyanin concentration has decreased.

4. Conclusions

Based on the Box-Behnken RSM Design it is known that the particle size, material/solvent ratio, extraction time, and microwave power in the extraction process are factors that greatly affect the response of anthocyanin concentration produced. Relationship between variables to the response of anthocyanin concentration was modeled by Y = 0.000178-4.17412E-07A + 0.012205B + 0.000022C + 7.75551R-08D + 0.000060AB – 2.96236E-08AC + 1.20818E-09AD – 0.001314BC – 0.000029BD + 1.84413E-07CD

5. References

[1] Albertini, Schoubben, Guarnaccia, Pinelli, Vecchia, Ricci, Renzo and Blasi 2015 Effect of Fermentation and Drying on Cocoa Polyphenols *J.of Agricultural and Food Chemistry* **63 (45)** 9948-9953

[2] Arlorio M, Coïsson J D, Travaglia F, Varsaldi F, Miglio G, Lombardi G and Martelli A 2005 Antioxidant and biological activity of phenolic pigments from *Theobroma cacao* hulls extracted with supercritical CO 2 *Food Research International* **38** 1009–1014

[3] Lubis S S, Sulastri and Zubair MS 2018 Mikroenkapsulasi Antosianin Kulit Buah Kakao (*Theobroma cacao*L.) dengan Metode Koaservasi Kompleks *Jurnal Farmasi Galenika* **4(2)** 106-112.

[4] Corcuera L A S, Amézqueta L, Arbillaga A, Vettorazzi S, Touriño J L, Torres and López de Cerain A 2012 A Polyphenol-Enriched Cocoa Extract Reduces Free Radicals Produced by Mycotoxins *Food and Chem Toxicology* **50 (3–4)**989– 95

[5] Vázquez-Espinosa M, Ferreiro-González M, Espada-Bellido E, González de Peredo A F, Barbero G F, Carrera C, Palm M, Barroso C G 2018 Optimization of Microwave-Assisted Extraction for the Recovery of Bioactive Compounds from the Chilean Superfruit (*Aristotelia chilensis* (Mol.) Stuntz) *J. of Agronomy* **2018(8)** 240

[6] Dahmoune F, Nayak B, Moussi K, Remini H, Madani K 2015 Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves *Food Chem.* **166** 585–595

[7] Yusepa B 2016 *Analisis Kesulitan Siswa dalam Menyelesaikan Soal Kemampuan Representasi Matematis* (Widya Sari Press[online]. Tersedia: http://widyasaripress.com/index.php?option=com_content&view=article&id=962:analisiskesulitan-siswa-dalam-menyelesaikan-soal-kemampuan representasimatematis&catid=107:vol-18-no-6-desember-2016-seri-ii [13 Juni 2017])

[8] Pavli B, Vidovi S,Vladi J, Zekovi Z 2017 Optimization of Microwave-Assisted Extraction of Polyphenolic Compounds from *Ocimum basilicum* by Response Surface Methodology *Food Anal. Methods* **10** 2270–2280

[9] Langat M K 2011 *Chemical Constituents of East European Forest Species In A. f. Standards Book of Extended Extracts* (Kenya: Napreca) 77-78

[10] Mandal V 2007 Microwave Assisted Extraction – An Innovative and Promising Extraction

Tool for Medicinal Plant Research *Pharmacognosy Reviews* **1(1)** 7-18

[11] Septiyaningsih L, dkk (2012) *Ekstraksi Minyak Nabati Dari Scenedesmus Sp. Menggunakan Gelombang Mikro* Seminar fisika UNJ (Jakarta: UNJ)

[12] Montgomery DC (2001) *Design and Analysis of Experimental* (New York: John Wiley & Sons Inc,)

[13] Bas D and Boyaci I H 2007 Modelling and optimization I: usability of response surface methodology *J. Food Eng* **78** 836–845

[14] Raiss, S and Farzani R E 2009 Statistical process optimization through multi-response surface methodology. World Academy of Science *Engineering and Technology* pp. 267–271

[15] Radojkovic M, Zekovic Z, Jokic S and Vidovic S 2012 Determination of optimal extraction parameters of mulberry leaves using Response Surface Methodology (RSM) *Romanian Biotechnological Letters* **17(3)** 7295–7308

[16] Jia C, Han F, Miao X, Zhang Q, Yan A, Wu P 2019 Study on optimization of extraction process of anthocyanin from cherry wine lees *J. of Nutritional Health & Food Engineering* **9**

[17] Hu Y, Cui X, Zhang Z, et al. Optimisation of Ethanol-Reflux Extraction of Saponins from Steamed

[18] Iriawan N and Astuti SP 2006 Mengolah Data Statistik dengan Mudah Menggunakan Minitab 14. (Yogyakarta: Penerbit ANDI)

[19] 2018 *Panax notoginseng* by Response Surface Methodology and Evaluation of Hematopoiesis Effect *Molecules* **23(5)**1206

[20] Ariyanti D, Catarina SB and Cahyo A 2014 Modifikasi Tepung Umbi Talas Bogor (*Colocasia esculenta* L Schott) dengan Teknik Oksidasi sebagai Bahan Pangan Pengganti Tepung Terigu *Jurnal Reaktor* **15** 8

[21] Kumari K S, Babu I S Rao G H 2008 Process optimization for citric acid production from raw glycerol using response surface methodology *Indian Journal of Biotechnology* pp. 496–501.

[22] Keshani S, Chuah A L, Nourouzi M M, Russly A R and Jamilah B 2010 Optimization of concentration process on pomelo fruit juice using response surface methodology (RSM) *International Food Research J.* **17** 733-742

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