IOP Conference Series: Earth and Environmental Science

PAPER • OPEN ACCESS

Optimization of material concentration and extraction time on yield and α-tocopherol content of sea lettuce (*Ulva lactuca* L.)

To cite this article: L P Wrasiati *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **230** 012054

IOP Conf. Series: Earth and Environmental Science 230 (2019) 012054 doi:10.1088/1755-1315/230/1/012054

Optimization of material concentration and extraction time on yield and α-tocopherol content of sea lettuce (*Ulva lactuca* L.)

L P Wrasiati[1], N L G D Yunita[1], L Suhendra[1] and I M A S Wijaya[2]

[1] Dept. of Agro-Industrial Technology, Faculty of Agricultural Technology, Udayana University, Kampus Unud Bukit Jibaran, Badung, Bali, Indonesia
[2] Dept. of Agricultural Engineering, Faculty of Agricultural Technology, Udayana University, Kampus Unud Bukit Jibaran, Badung, Bali, Indonesia

E-mail: wrasiati@unud.ac.id

Abstract. *Ulva lactuca* L. is a group of green algae (Chlorophyta). *Ulva lactuca* L. grows and spreads on rocky beaches in Indonesia and contains various bioactive compounds that are beneficial to health such as α-tocopherol. The purpose of this study was to determine the surface response methodology (RSM) equation, optimization, and overlaid point in obtaining the maximum yield and α-tocopherol extract of *Ulva lactuca* L. Treatment of the study was the concentration of the material (X_1) and the extraction time (X_2). Data analysis was performed using a surface response methodology (RSM) with a central composite design (CCD). The results of the research on yield and α-tocopherol show the maximum shape. The concentration of the ingredients and the extraction time in producing the yield (Y_1) of sea lettuce extract are following the equation Y_1 = -20.12 + 3.018 X_1 + 3.232 X_2 - 0.1228 X_{12} - 0.1728 X_{22} - 0.1459 X_1X_2 with a value of R^2 = 0.85. While the content of α-tocopherol (Y_2) follow the equation Y_2 = -205,242 + 43,849 X_1 + 6,031 X_2 – 2,530 X_{12} – 1,076 X_{22} + 661 X_1X_2 with a value of R^2 = 0.78. The optimization results showed that the yield value was 2.42% and the content of α-tocopherol compound of 18.16% was obtained from 9.27% material concentration and extraction time of 5.53 hours. The overlaid point produces maximum yield and α-tocopherol at two points. The first point is the material concentration of 9.3% and the extraction time of 5.7 hours, and the second point is the material concentration of 9.4% and the extraction time of 5.4 hours. The optimum yield and content of α-tocopherol of Lettuce (*Ulva lactuca* L.) can be determined by using Response Surface Methodology.

1. Introduction

Sea lettuce (*Ulva lactuca* L.) is a type of Chlorophyta or green algae and lives in shallow waters around the world, especially on rocky beaches. Sea lettuce habitats in Bali are Serangan Beach, Sanur, Nusa Penida, Sawangan Beach, and Nusa Dua [1, 2]. Previous research showed that sea lettuce contains 46-51% carbohydrates, 0.1-0.7% fat, 15-26% protein, 2-5% fiber, 16-23% ash, 20.9% water, and various vitamins B1, B2, B12, and C. Sea lettuce also contains antioxidant compounds such as 13.15% tocopherol and chlorophyll and Mg and Fe minerals [3]. Tocopherol is a class of vitamin E which is believed to be a source of antioxidants that can prevent lipid peroxidation from unsaturated fatty acids in cell membranes, help oxidize vitamin A, and maintain fertility [4]. The content of tocopherols found in sea lettuce has the potential to be developed and can be obtained by extraction.

In the extraction process of soursop leaves the best treatment was the ratio of the material with acetone 1:10 (w/v) and extraction time of 20 minutes [5]. Material and solvent ratios of 1:10 are equivalent to a material concentration of 9.09%. While based on research by Yulianthi et al. [6], ethanol: acetone (1: 9) v/v solvent with dielectric constant 21.06 was the best treatment to produce Sargassum polycystum extract with a yield of 2.61% and α-tocopherol levels 6,724 mg/100g. The method used in the extraction process also affects the results to be obtained. Extraction method that can be used to extract bioactive compound are maceration, percolation, soxhletation, reflux, and thin layer chromatography. In this study, soxhletation method is chosen because the process occurs continuously and the solvent can be used repeatedly [7]. Kadji et al. [8] stated that the soxhletation method produces higher yields compared to the maceration method. Soxhletation method with acetone solvent has the ability to extract antioxidant compounds in Spirulina platensis powder biomass. This is shown by the relatively high antioxidant activity produced at 64.712 ppm [9]. While the best time of extraction using the Soxhletation Method is 5 hours [10].

More in-depth research needs to be done to find the optimum point of production of sea lettuce extract using the concentration of ingredients and a combination of extraction time. Extraction will be carried out using Soxhlet tools and acetone solvents. In determining the optimum point of extraction to yield and α-tocopherol levels were analyzed using Response Surface Methodology (RSM). RSM is used to analyze problems several independent variables affect the response variable and the ultimate goal is to optimize the response [11]. Research using the RSM approach with a Central Composite Design (CCD) has been widely carried out, one of which is Suhendra's [12] study of changes in soluble protein during sesame seed germination. CCD is an experimental design that is used to construct a polynomial model of a mathematical function consisting of a factorial design with a central point. The aim of this study was to find the RSM equation and the shape of the surface response, optimization point, and overlaid of the yield and α-tocopherol extract of sea lettuce.

2. Materials and Methods

2.1. Materials

The materials used are sea lettuce (*Ulva lactuca* L.) obtained from Serangan Beach; South Denpasar The sea lettuce is washed with fresh water to remove dirt and some salt that is still attached. Then the sea lettuce is drained and dried air at room temperature for approximately 3 hours.

2.2. Experimental design

The experimental design used was the Central Composite Design (CCD) two factors. This study used material concentrations of 7.586%, 8%, 9%, 10%, 10.414% and extraction time of 3.586 hours, 4 hours, 5 hours, 6 hours, and 6.414 hours. The design results using CCDs were obtained as many as 13 units and carried out in duplicate.

2.3. Experimental set up

This research was conducted in two stages, namely the stage of making sea lettuce powder and making sea lettuce extract.

2.3.1. Making sea lettuce powder. Sea lettuce from Serangan Beach, South Denpasar was washed in running water, then cut into pieces 2 cm x 2 cm. Pieces of sea lettuce are dried by aerated then oven at $50^{o}C \pm 2^{o}C$ for 30 minutes until the ingredients are dry and easily destroyed. Dry sea lettuce is blended until smooth and sieved using a 60 mesh sieve.

2.3.2 Making sea lettuce extract. Making of sea lettuce extract is done by Soxhletation Method. Material dry powder sea lettuce is weighed according to treatment. Each material is then wrapped in filter paper and tied with thread. The solvent is weighed based on each treatment. The wrapped materials and solvent are then put into the Soxhlet for extraction. Extraction was carried out at the boiling point of acetone ($56^{o}C \pm 2^{o}C$) and extraction time according to treatment. Then the liquid extract was evaporated to obtain a crude extract. The crude extract was weighed to calculate the yield and analysed for α-tocopherol levels.

2.4 Analysis.
The parameters analysed in this research include extract yield and α-tocopherol content. The yield of sea lettuce extract is obtained following Sudarmadji et al. [13] calculations. The α-tocopherol content of sea lettuce extract was analysed using the modified method of Wong et al. [14].

3. Results and Discussion

3.1. Sea lettuce extract (Ulva lactuca L.*)*
The results (Table 1) showed that the average yield of sea lettuce extract was 2.19%. The highest yield was obtained from the treatment of 9% material concentration and 5 hours extraction time. The lowest yield was obtained at 8% material concentration and 4 hours extraction time.

Table 1. Yield (5) and α-tocopherol content (ppm) of sea lettuce extract.

No.	Treatment		Yield (%)	α-tocopherol content (mg/100g)
	Concentration (%)	Extraction time (hours)		
1	8	4	1.51	10,730
2	10	4	2.10	12,440
3	8	6	2.26	14,930
4	10	6	2.27	19,290
5	7.586	5	2.16	9,520
6	10.414	5	2.18	14,330
7	9	3.586	1.86	15,260
8	9	6.414	2.28	14,410
9	9	5	2.20	18,730
10	9	5	2.25	17,150
11	9	5	2.41	18,670
12	9	5	2.72	17,400
13	9	5	2.29	17,600

Based on the results of statistical analysis using the RSM method with a CCD model match obtained the equation $Y_1 = -20.12 + 3.018 X_1 + 3.232 X_2 - 0.1228 X_{12} - 0.1728 X_{22} - 0.1459 X_1X_2$. The strength of the relationship between variables (R) is equal to 0.85, which indicates that the two variables have a strong relationship. Based on the RSM equation shows the change in extraction time has the greatest effect on the yield of sea lettuce extract. The effect can be seen from the coefficient of extraction time has the greatest value compared to the concentration of material. This may occur because the determination of the range of material concentration code values is too narrow so the difference in results is not too different from the extraction time. The yield of sea lettuce extract in this study will increase with the length of the extraction process. The results of the research by Wahyuni and Widjanarko [15] showed that the extraction time gave a considerable amount of time for the solvent to penetrate the cell wall and the compounds contained in the material would be pulled out, resulting in high yield.

Extraction time will increase the penetration of the solvent into the raw material so that the compounds in the material will be extracted a lot until it reaches equilibrium. The increase in yield occurred up to 5 hours, but after 5 hours the yield began to be constant and slightly decreased. The image of increasing and decreasing the yield of sea lettuce extract can be seen in Figure 4. Based on the research of Yulianti et al. [6] extraction on stevia leaves with treatment of extraction time showed an increase in yield to a certain point, this happened because the penetration of the solvent into the material decreased. This is because the compounds contained in the ingredients are limited in number and the solvents used have limits on the ability to dissolve the existing material, so that even though the extraction time is extended, the compounds that are in the material are gone.

Statistical calculations show that the coefficient of determination R^2 = 72.84%, the value states that 72.84% of the yield value is influenced by treatment factors while 27.16% comes from the factors outside the observed treatment. Other factors that can affect the yield of sea lettuce extract such as the size of simplicia, type of solvent, solvent concentration, extraction temperature, the method used, comparisons of ingredients [16].

The significance of the probability value P < (0.01) for the first order and second order shows that the use of the RSM second order equation is very significant. The lack of fit model test results obtained P> (0.05) which means that the model mismatch is rejected. Thus, the second-order model equation is very valid and can predict responses, because an RSM equation is valid or not determined from the regression test and lack of fit data. Graphs of response surface and contour plots of yield can be seen in Figure 1.

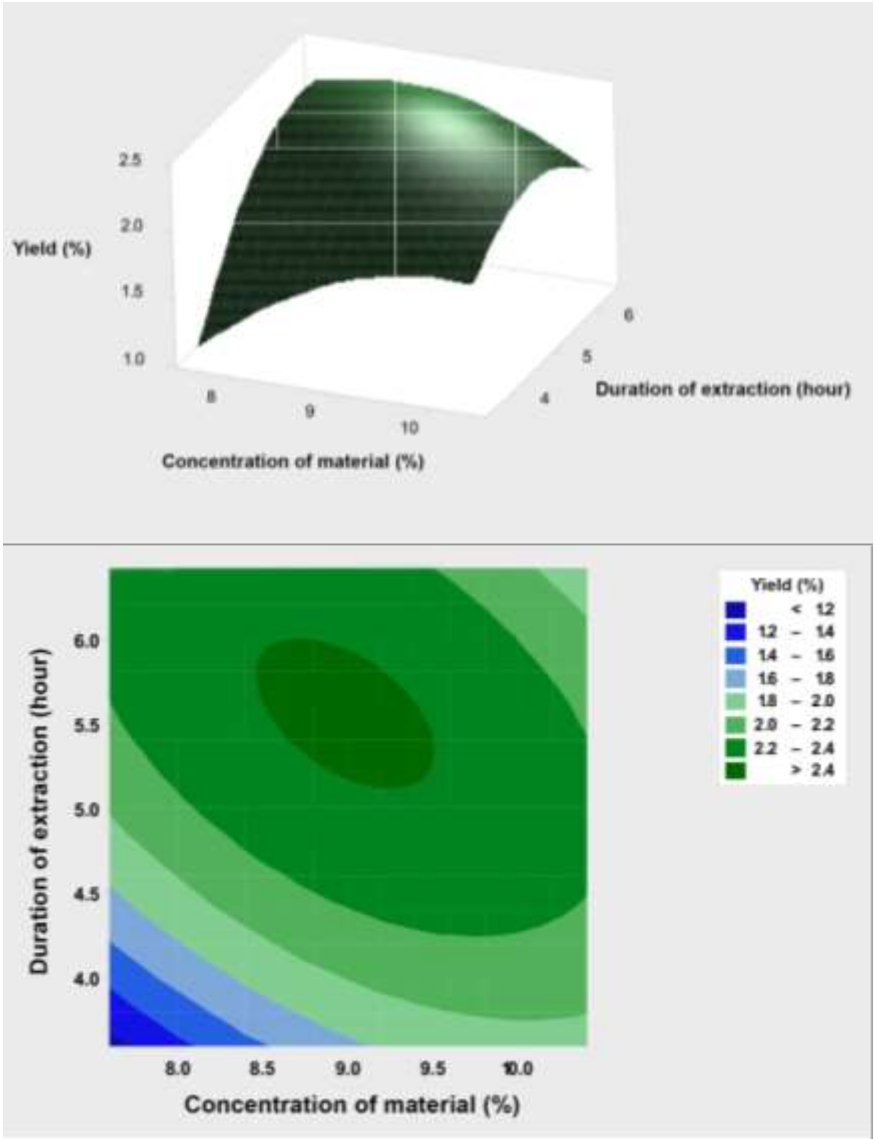

Figure 1. Surface and contour plot of yield.

Figure 1 shows that the curve that is formed resembles an inverted parabola which forms the maximum response. The increase in yield occurred up to 5 hours of extraction time, but after 5 hours the yield began to decline. Extraction time will increase the penetration of the solvent into the raw material so that the compounds in the material will be extracted a lot until it reaches equilibrium. The compounds contained in the ingredients are limited and the solvents used have limits on the ability to dissolve the ingredients, so that even though the extraction time is extended the compounds contained in the ingredients are gone. While the concentration of the material gives effect to the amount of circulation in the extraction process. At a concentration of 9% it has provided sufficient space for the solvent to come into contact with heat, so that the solvent will be easier to heat and the circulation will run optimally.

3.2. Levels of α-tocopherol Marine Lettuce Extract (Ulva lactuca L.)

Based on the results of the study showed an average level of α-tocopherol extract of sea lettuce was 15,250 mg / 100g equivalent to 15.25%. The highest average α-tocopherol level was obtained from the treatment of 9% material concentration and 5 hours extraction time. While the lowest α-tocopherol level was obtained in the treatment of 7.586% material concentration and 5 hours extraction time.

Statistical analysis using RSM with CCD model matching obtained equation Y_2 = -205,242 + 43,849 X_1 + 6,031 X_2 – 2,530 X_{12} – 1,076 X_{22} + 661 X_1X_2. The strength of the relationship between variables is R equal to 0.78 which indicates that the two variables have a strong relationship. If seen from the RSM equation shows that the change in concentration has the greatest influence on the levels of α-tocopherol extract of sea lettuce. This influence can be seen from the concentration coefficient which has the greatest value. The concentration of the material has a relationship with the ratio of the material to the solvent (b/v), which in the results of research by Handayani et al. [5] stated that the material ratio with solvent 1:10 (b/v) was the best treatment for the extraction of soursop leaves. The results of this study also showed the same thing that the decrease in the levels of α-tocopherol extract of sea lettuce is after 9% material concentration. This occurs because there is an activity of reducing the solvent's ability to dissolve the material. Levels of α-tocopherol increase with increasing concentration of material, but begin to decline after passing through the equilibrium point.

Statistical analysis showed that the coefficient of determination R^2 = 62.51%, which means that 62.51% of the levels of α-tocopherol were influenced by treatment factors and 37.49% came from factors observed outside treatment. Other factors that can affect the levels of α-tocopherol sea lettuce extract are temperature and solvent concentration [2].

The significance of the probability value $P < (0.01)$ for the first order and second order shows that the use of the RSM second order equation is very significant with the results of α-tocopherol levels of sea lettuce extract. The lack of fit model test results obtained $P > (0.01)$ which means that the mismatch is rejected. Thus, the second-order model equation is valid and can predict responses, because an RSM equation is valid or not determined by the value of the regression test and the lack of fit data. Graphs of Response Surface and Contour Plots can be seen in Figure 2.

Figure 2 shows a reversed parabolic shape so that it can be predicted that the response will be in the maximum shape. Levels of α-tocopherol increase with increasing concentration of material, but begin to decline after passing through the equilibrium point.

Decrease in α-tocopherol levels occurs because the solvent's ability to dissolve the material has reached the saturation point and the longer the extraction process will facilitate the process of α-tocopherol damage because the longer the material contact with heat.

3.3. Optimum condition of sea lettuce extract (Ulva lactuca L.)

The results of D-optimally analysis or optimization calculations are predicted to obtain 2.42% yield of sea lettuce extract and α-tocopherol levels of 18,163 mg/100g or 18.16% at 9.27% material concentration and extraction time of 5.53 hours. D-optimally results in this study indicate that the predicted concentration of material is at level 0. The optimization results can be seen in Figure 3.

3.4. Overlaid contour plots of yield and α-tocopherol

The relationship between response (yield and level of α-tocopherol) in Figure 4 shows that there are two points that bring the yield response and optimum levels of α-tocopherol extract of sea lettuce. The first point to obtain the optimum yield and levels of α-tocopherol can be done with the treatment of material concentration of 9.3% and extraction time of 5.7 hours. The second point can be done with the treatment of the material concentration of 9.4% and the extraction time of 5.4 hours. Each of these points can be selected according to needs and takes into account operational costs that are not too expensive, whether the extraction time treatment will be made for a long time or the concentration of the material made is higher.

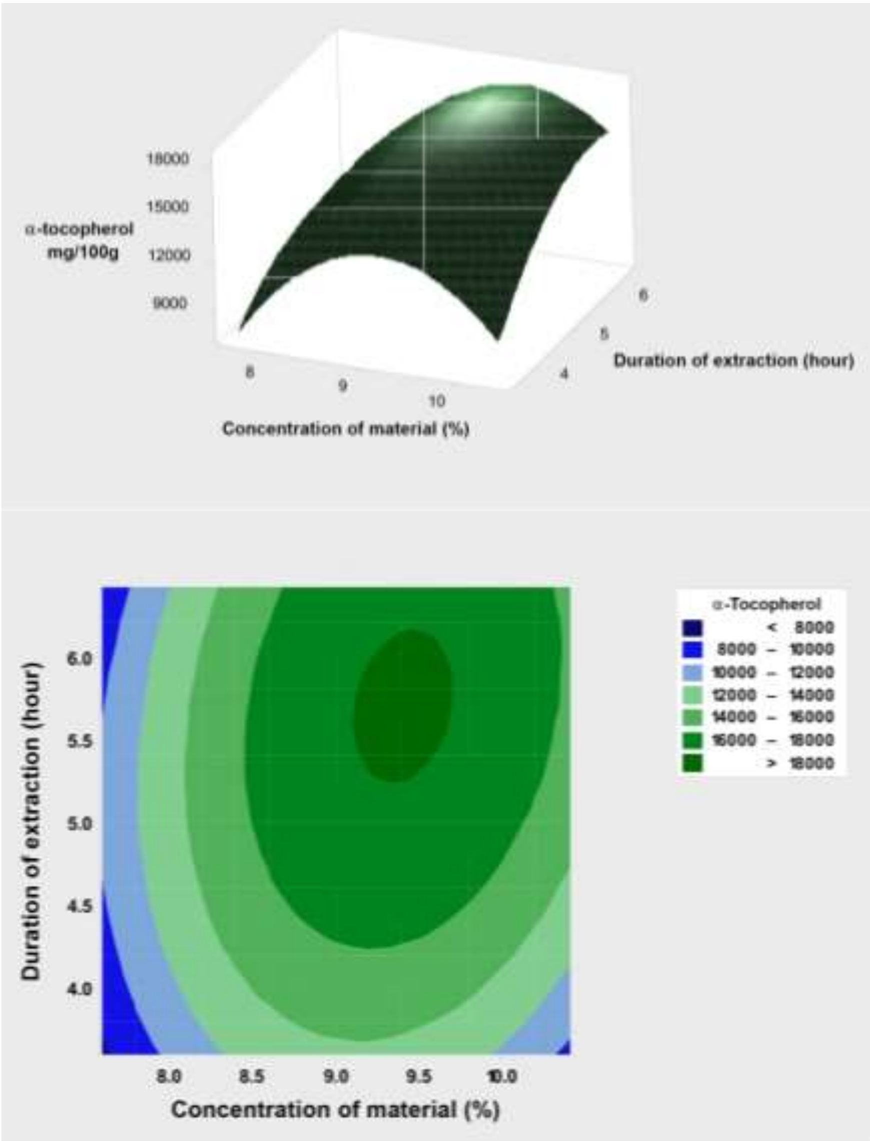

Figure 2. Surface and contour plots of α-tocopherol.

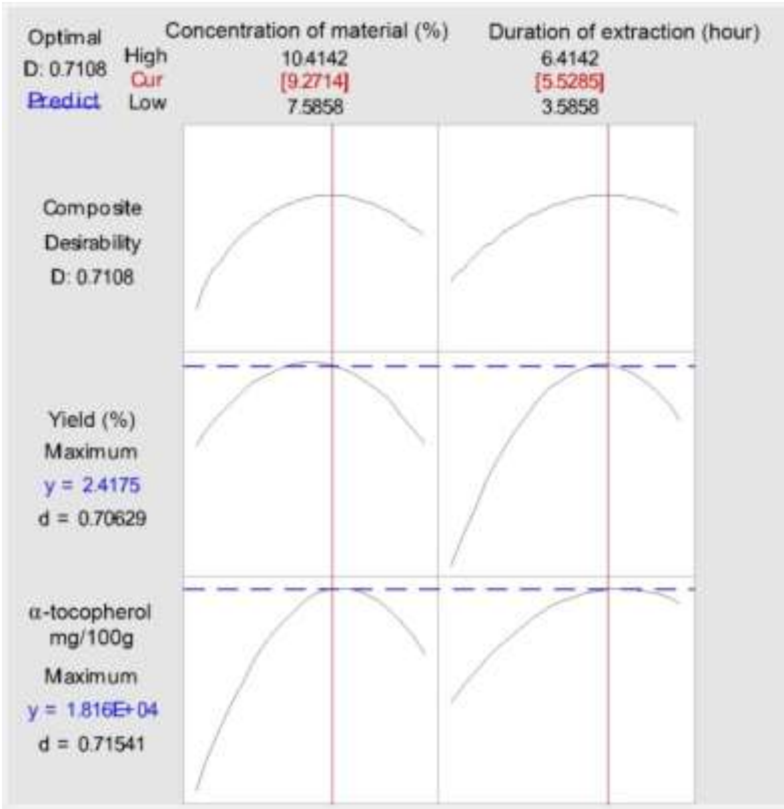

Figure 3. D-Optimally yield and α-tocopherol.

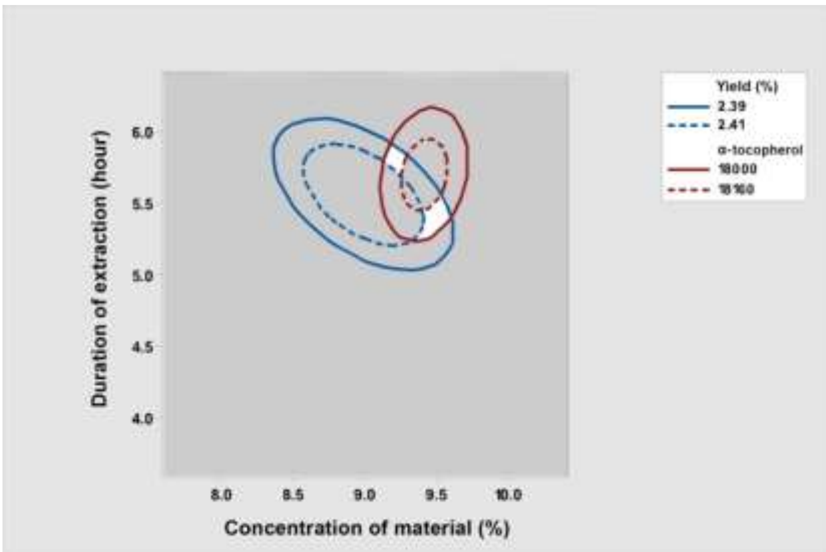

Figure 4. Overlaid contour plot yield and α-tocopherol.

4. Conclusion

Material concentration (X_1) and extraction time (X_2) in producing the yield of sea lettuce extract with the equation $Y_1 = -20.12 + 3.018\ X_1 + 3.232\ X_2 - 0.1228\ X_{12} - 0.1728\ X_{22} - 0.1459\ X_1X_2$ with values $R^2 = 0.85$ and levels of α-tocopherol $Y_2 = -205{,}242 + 43{,}849\ X_1 + 6{,}031\ X_2 – 2{,}530\ X_{12} – 1{,}076\ X_{22} +$

661 X_1X_2 with a value of $R^2 = 0.78$. The shape of the surface yield response and α-tocopherol show the maximum shape resembling a reversed parabola. The optimal concentration of material and extraction time for extraction of sea lettuce is at 9.27% and 5.53 hours respectively. Two points of overlaid that bring maximum yield and α-tocopherol. The first point is the material concentration of 9.3% and the extraction time of 5.7 hours. The second point is the material concentration of 9.4% and the extraction time of 5.4 hours.

References

[1] Ortiz J, Romero N, Robert P, Araya J, Lop ez-Hernandez J, Bonzzo C, Na-varrete E, Osorio A, Rios A 2006 Dietary fiber, amino acid, fatty acids and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvilaea antica Food Chem.* **99** 1 98-104.

[2] Dewi N N D T, Wrasiati L P, Son G P G 2016 Effect of ethanol solvent concentration and maceration temperature on yield and chlorophyll content of sea lettuce extract encapsulation products (*Ulva lactuca* L) *Jurnal Rekayasa dan Manajemen Agroindustri* **4** 3 59-70. [In Indonesian]

[3] Xiao-Ling L, Rong C, Zai-Yong Y 2003 Elementry study on nutritional composition of the green algae, *Ulva lactuca* in the South China Sea *J. Nat. Sci.* **6** 2 79-83.

[4] Rohmatussolihat 2009 Antioxidants rescue human body cells *Bio Trends* **4** 1 5-9.

[5] Handayani H, Sriherfyna F H, Yunianta 2016 Extraction of soursop leaf antioxidants ultrasonic bath method (study of material ratio: solvent and extraction time) *J. Food Agro-ind.* **4** 1 262-272.

[6] Yulianthi N N S, Suhendra L, Wrasiati L P 2017 Effect of solvent type comparisons on the content of phenol compounds, a-tocopherol, and carotenoids of Sargassum Polycystum extract *Jurnal Rekayasa dan Manajemen Agroindustri* **5** 4 1-10. [In Indonesian]

[7] Sudjadi 1986 Separation Methods UGM Press Yogyakarta Indonesian [In Indonesian]

[8] Kadji M H, Runtuwene M R J, Citraningtyas G 2013 Phytochemical analysis and antioxidant activity of ethanol extract of soyogik leaf (*Saurauia bracteosa* DC) FMIPA UNSRAT Manado Indonesia [In Indonesian]

[9] Anam C, Agustini T W, Romadhon 2014 Effect of different solvents on extraction of platinum Spirulina powder as an antioxidant with the Soxhletation method *J. Fish. Prod. Biotechnol.* **3** 4 106-112.

[10] Adiyasa I K G P, Wrasiati L P, Wartini N M 2015 Effectiveness of the type of solvent and extraction time on the concrete characteristics of mandarin orange peel essential oil (*Citrus reticulata*) *Jurnal Rekayasa dan Manajemen Agroindustri* **3** 4 21-29. [In Indonesian]

[11] Montgomery D C 2001 Design and Analysis of Experiments 5th Edition John Wiley & Sons, Inc, New York USA

[12] Suhendra L 2010. The study of protein changes during dissolution of sesame seeds (*Sesamun indicum* L.) using the response surface methodology approach *Agrotekno*. **11** 2 15-21. [In Indonesian]

[13] Sudarmadji S, Haryono B, Suhardi. 1989 Analysis of Food and Agricultural Materials Liberty Yogyakarta Indonesia. [In Indonesian]

[14] Wong M L, Timms R E, Goh E M 1988 Colorimetric determination of total tocopherols in palm oil, olein and stearin *J. Am. Oil Chem. Soc.* **65** 2 258-261

[15] Wahyuni D T, Widjanarko S B 2015 Effect of solvent type and extraction time on pumpkin carotenoid extract with ultrasonic wave method *Jurnal Pangan dan Agroindustri* **3** 3 390-401. [In Indonesian]

[16] Arbi B, Farid M, Romadhon 2016 Activities of marine lettuce bioactive compounds (*Ulva lactuca*) as antioxidants in fish oil Indonesian *J. Fish. Sci. Technol.* **12** 1 12-18.