2019-12

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Submission date: 24-May-2021 09:42PM (UTC+0800)
Submission ID: 1593151525
File name: 2019-12.pdf (398.67K)
Word count: 2990
Character count: 15839

DOI Number: 10.5958/0976-5506.2019.00869.6

Water Soluble Protein Optimized Extraction from Microalgae Spirulina Platensis

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Abstract

This research aimed to determine the factors influencing extraction of water soluble protein from *Spirulina platensis* which included the ratio of *Spirulina platensis* and aquadest, incubation time, and interaction of those two factors. This research contained two factors (ratio and incubation time) using factorial complete randomized experimental design with nine treatments and three replications. Ratios of *Spirulina platensis* and aquadest used were 1:40, 1:50, and 1:60, while the incubation times were 1, 2 and 3 hours. Parameter observed in this study was water soluble protein content of *Spirulina platensis*. Ratio of *Spirulina platensis* and aquadest significantly affected water soluble protein content ($p<0.05$). The highest content of water soluble protein was observed at the ratio of 1 : 50 (85.4742 µg/ml), while the highest water soluble protein content based on the incubation time was observed at 3 hours treatment ($p<0.05$). These results indicated that ratio and the incubation time influenced water soluble protein content on the extraction process of *Spirulina platensis* with the optimized extraction should be at the ratio of 1:50 for 3 hours.

Keywords: *extraction, optimization, Spirulina platensis, water soluble protein.*

Introduction

Spirulina platensis is one of the microalgae that has the potential as a food source. Complete nutrient content of *Spirulina platensis* and the presence of antioxidant compounds of ficocyanin pigments have been used as a dietary supplement[1]. The utilization of *Spirulina* is to have the value of high protein content mainly on dried *Spirulina* protein and blue pigment (ficocyanin), reaching 20% of dry weight[2]. The high protein content on *Spirulina platensis*, reaching 60 – 71%, could be used as a protein source[3]. Protein in *Spirulina* was composed of several amino acids, such as methionin, lysin, and systein, compared with the protein content in egg and milk[4]. *Spirulina*, which is a single cell organism, has other benefits as the source of carotenoids, chlorophyll, and other micronutrients[5].

Protein source in *Spirulina* was one of nutrient types that could potentially provide important functional properties, such as maintaining the food product characteristics for an emulsifier, thickener, natural dyes, gel-forming, and others[6]. *Spirulina* is produced in the form of drugs, supplements, juices, tablet, as well as functional food. *Spirulina* also served as a food source of food for better immune system and Super Oxyde Dismutase (SOD) content[5].

The utilization of *Spirulina* in the industry is not yet optimum. Optimization needs to be done to optimize the utilization of *Spirulina*. The existence of high-protein potential content in *Spirulina* could be developed quickly, as it had the ability to proliferate fast, season independence, not requiring an extensive land for culture[7].The optimization process is done to make an important protein extracts by determining the maximum variables and interaction on the various parameters that influenced the extraction outcome. Based on the research background, further research needed to be conducted for obtaining the optimum process conditions of extracting water soluble protein in *Spirulina platensis* which will be done in this study. Protein produced is expected to be widely exploited, one of which was the application of water-soluble protein in fruit juice.

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Methodology

This research used factorial complete randomized design (FCRD) experimental method with two factors, such as the ratio of *Spirulina* powder with aquadest and incubation time. These factors were divided into 9 treatments and 3 replications. The ratio treatments are 1: 40, 1: 50 and 1: 60, while incubation time treatments are 1, 2 and 3 hours. These treatments were based on Safi et al[8], who used 0.5 g of *Spirulina platensis* todissolve in 25 ml aquadest and incubated for 2 hours.

Sample preparations:

Water Soluble Protein Extract Production from *Spirulina platensis*

Protein extract production was done by measuring *Spirulina platensis* powder into the Erlenmeyer tube filled with aquadest and incubated based on the treatments given. Solutions made were homogenized using vortex and centrifuged at 6,000 rpm for 15 minutes.

Protein Content Measurement using Lowry Method

This measurement analysis was begun with preparing 2000 ppm of BSA (*Bovine Serum Albumin*) as the main standard solution. This solution was made from 0.5 g solid BSA added with 250 ml aquadest. This solution was diluted using 40, 80, 120, 160, and 200 ppm concentration series. This solution was then added with 5 ml of modified reagent for 10 minutes and 0.5 ml of Follin-Ciocalteu reagent, while being shaken for 30 minutes. Solution was measured using 760 nm spectrophotometer[9].

Water Soluble Protein Content Analysis from *Spirulina platensis*

20 µl supernatant of *Spirulina platensis* was added with 1000 µlaquadest. This solution was added with 5 ml of modified reagent for 10 minutes and added with 0.5 ml of Follin-Ciocalteu reagent, while being shaken for 30 minutes. Solution was measured using 760 nm spectrophotometer[9].

Blueberry Juice Production

Fruit juice production was done using 5 concentration treatments of water soluble protein based on Yusmariniet al[10], containing 0%, 25%, 50%, 75%, and 100% modification. Juice was produced using blender by mixing all ingredients needed, such as blueberry, lemon, water, sugar, and honey. Blueberry juice was added with the water soluble protein from the optimized extraction of *Spirulina platensis*.

Hedonic test is done using 30 non-expert panellists from Faculty of Fisheries and Marine, UniversitasAirlangga, students. Indicators tested were appearance, colour, taste, aroma, and texture.Water soluble protein content result data was analysed using FCRD Analysis of Variance (ANOVA) statistical analysis with SPSS 16.0 software. Data containing significant difference was analysed continuously using Duncan's Multiple Range Test (DMRT). Hedonic test and juice protein content were analysed using descriptive method.

Results

Concentration Measurement Results of BSA (Bovine Serum Albumin)

Measurement of protein levels is carried out using the Lowry method. The method uses a solution of BSA as a comparison solution by measuring uptake on UV-VIS spectrophotometers at a maximum wavelength of 760 nm. The BSA concentration series used are 40, 80, 120, 160, 200 ppm and the results are tabulates in Table 1.Making standard solutions with various series aims to determine protein levels in a sample using straight line linear regression obtained from a standard solution graph[9]. Absorption values of UV-VIS Spectrophotometry from BSA obtained absorbance figures which became the y-axis and BSA concentration as x-axis in the form of a curve. The BSA curve and calibration equation can be seen in Figure 1. The measurement results of BSA solution absorption produced a curve with a positive slop approaching 1 (0.9945) with a linear regression equation $y = 0.0934x - 0.004$. The absorbance results of Spirulina platensis extract using UV-VIS Spectrophotometry were included in the equations and calibration curves of the BSA.

Table 1. UV-Vis spectrophotometer absorbance value of BSA series concentration

BSA Concentration (µg/ml)	Absorbance (λ= 760 nm)
40	0.091
80	0.169
120	0.291
160	0.373
200	0.456

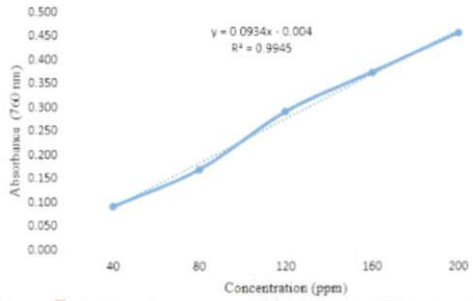

Figure 1. Calibration concentration against BSA absorbance solution

Water Soluble Protein Content Analysis based on Ratio of ***Spirulina platensis*** :Aquadest

Based on Table 2, the different ratio of *Spirulina platensis* and aquades affected the water soluble protein content is exposed. The highest water soluble protein was obtained from 1:50 ratio, while the lowest was observed at 1:40 ratio. Analysis result which had the closest level with 1:50 ratio was 1:60 ratio with 78.2769 µg/ml. However, 1:50 showed the highest water soluble protein content which used less solvent, making the extraction process using 1:50 ratio was more effective and efficient.

Table 2. Results data of water soluble protein levels based on Spirulina platensis ratio: aquades

Treatment	Protein Content ± SD (µg/ml)
1 : 40	50.8560 ± 21.97304
1 : 50	85.4742 ± 38.02541
1 : 60	78.2769 ± 20.39331

Note: Data was taken from the average of three replications ± Standard Deviation.

Water Soluble Protein Content Analysis based on Incubation Time

ANOVA results of the water soluble protein content analysis gave p value with 0.000 or smaller than 0.05 ($p<0.05$). This showed that there was a significant difference on the water soluble protein content produced. Based on Table 3 it can be seen that the incubation time in the extraction process affects the level of water soluble protein produced. Duncan's Multiple Advanced Test results showed that the treatment of incubation time for 3 hours had a value of 90.8870 µg / ml. The treatment of incubation time for 1 hour had the smallest value of 51.5103 µg / ml.

Table 3. Data on soluble protein levels based on incubation time

Treatment	Protein Content ± SD (µg/ml)
1 hour	51.5103 ± 17.87711
2 hours	72.2098 ± 38.18385
3 hours	90.8870 ± 21.09261

Note: Data was taken from the average of three replications ± Standard Deviation

Water Soluble Protein Analysis based on The Interaction of ***Spirulina platensis:*** Aquades Ratio and Incubation Time

Based on Table 4, it is shown that the difference in the ratio of Spirulina platensis and the different incubation times for each treatment affected the levels of water soluble proteins produced. Water soluble protein content from *Spirulina platensis* extraction was produced from two factors interaction, which was *Spirulina platensis:* Aquades ratio with incubation time. DMRT analysis showed that p value was 0.000 or smaller than 0.005 ($p< 0.05$), resulting a significant difference between treatment interactions. 1:50 ratio and 3 hours incubation gave the highest level of water soluble protein content with115.99 µg/ml.

Table 4. Results data on measuring water soluble protein in Spirulina platensis

Spirulina platensis : Aquadest	Incubation Time (hour)	Protein Content ± SD (µg/ml)
1 : 40	1	57.9937 ± 7.80056
	2	23.9110 ± 9.61106
	3	70.6633 ± 4.90641

Cont... **Table 4. Results data on measuring water soluble protein in Spirulina platensis**

1 : 50	1	41.7557 ± 29.88771
	2	98.6790 ± 17.13357
	3	115.9880 ± 8.46986
1 : 60	1	54.7817 ± 10.11966
	2	94.0393 ± 13.46156
	3	86.0097 ± 9.46111

Hedonic Test of Water Soluble Protein Application on Blueberry Juice Product

Figure 2 is the graphical representation of the hedonic test results. The concentration of water soluble protein added to blueberry juice includes treatment A (0%), treatment B (25%), treatment C (50%), treatment D (75%) and treatment E (100%). Appearance of blueberry juice with the addition of water soluble protein which has the highest value is in treatment A with a value of 7.53. This shows the value of 8 which means it is very much liked by the panellists. Treatment A with the addition of 0% water soluble protein has a good appearance.

In the colour parameters, the highest average value given by the panellists is in treatment A and D with a value of 7.03. This shows a value of 7 which means that treatments A and D have the colour preferred by the panellists. Treatment A with the addition of 0% water soluble protein has a purple colour produced from blueberries, whereas in treatment D it has blue-purplish colour due to the dominant addition of water soluble proteins produced from extraction Spirulina platensis contains dark blue.

The assessment of the aroma of blueberry juice with the addition of water soluble proteins is an assessment carried out based on the sense of smell. In the graph in Figure 2 shows the aroma parameter has the highest value of 6.87 in treatment D with the addition of 75% water soluble protein which means the aroma of blueberry fruit juice is favoured by panellists.

Assessment of taste parameters in blueberry juice is based on taste buds so the panellists are required to taste the product in order to provide an assessment. On the graph of the hedonic test the highest value is found in treatment D with a value of 6.70 which means that treatment D has a feeling that is favoured by panellists.

The hedonic test results on the texture parameters of blueberry juice which have been added to water soluble protein showed the highest value in treatment B with the addition of water soluble protein by 25%. Treatment B shows a value of 6.77, which means the texture of the preferred blueberry juice is treatment B. The average value of all parameters in the hedonic test shows numbers 6 and 7, which means that fruit juice which has been added to water soluble protein from the resulting Spirulina platensis and well received by panellists.

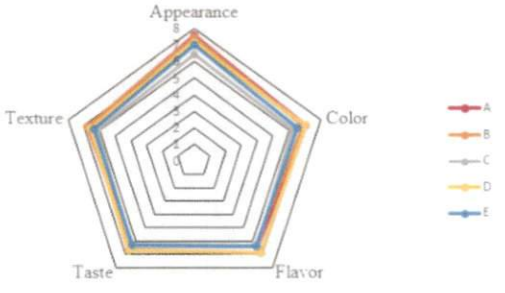

Figure 2. Hedonic test results

Protein Content Analysis on Blueberry Juice

Based on Table 5, it can be seen that the addition of different water soluble proteins to each treatment affects the protein content of the blueberry juice products produced. The highest protein content was obtained in treatment E, while the lowest was in treatment A. This shows that the higher the concentration of water soluble proteins added, the higher the protein content produced.

Table 5. Protein level test results of blueberry fruit juice with addition of water soluble protein from Spirulina platensis

Treatment group	Protein (%)
A	3.45
B	4.82
C	5.24
D	6.71
E	7.68

Discussion

Different Spirulina platensis: Aquades Ratio against Water Soluble Protein

Data result analysis showed that there was a significant difference at the ratio of Spirulina with incubation time ratio, making them as generated water soluble protein content. Table 2 showed more aquades solvent used would make an increased product of water soluble protein. This was because Spirulina platensis content would be solved only at the polar soluble solvent for Spirulina platensis[2].

Solvent selection was very important, as it determines the bioactive compound which was taken in the extraction process. Extraction process included the maceration process, the larger ratio of simplicial against the extracting, the higher content result obtained[11].

Different Incubation Time against Water Soluble Protein Extraction Result

Data analysis of protein from Spirulina platensis water-soluble based on 1, 2, and 3 hours showed longer of incubation time would produce more water soluble protein. The treatment time of incubation for 3 hours has a value of 90.8870 µ g/ml. According to Wang et al[12], longer extraction time would produce more time between solvent and main materials, making them into mass diffused precipitation until reaching a balance of excluding material extraction, thus incubation time influenced the extract production process obtained.

Interaction of Ratio and Incubation Time against Water Soluble Protein Content

The interaction of these two factors, namely the ratio of Spirulina platensis: aquades and incubation time showed significant difference on all treatments that produced water soluble protein. Table 4 shows that the treatment ratio of 1:50 with 3 hours incubation time had the highest water soluble protein content with 115.99 µg/ml. This research used aquades as a maceration solvent. Long and non-movement maceration process allowed many protein compounds got extracted[13].

Soaking process would crash the cell walls and membranes due to the difference pressure on the inside and outside of the cell[14]. According to Safi et al[8], cell wall of Spirulina platensis had a characteristic that was fragile, as it was composed by the absence of peptidoglycan cellulose.

Hedonic Test

Hedonic test resulted blueberry fruit juices with addition of water soluble proteins a preferred mark from all panellists. All treatment showed blueberry juice with the addition of water soluble protein was acceptable by the panelists. Protein content on juice increased along with the increased protein concentration. According to Febriantiet al[15], protein content in drinks with protein added would be more appropriate for people who had food restrictions. People suffered kidney failure was more advisable to get the diet food by way of reducing the protein intake[16].

Conclusions

Different ratio, incubation time, and indicators between those two factors affected the water soluble protein content produced. Optimum ratio was observed at 1:50, while incubation time was observed at 3 hours incubation. The best protein content was also observed at the interaction of 1:50 ratio for 3 hours incubation.

Ethical Clearance: Taken from the committee

Source of Funding: Nil

Conflict of Interest: Nil

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