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Comparative Study of Bioactive Substances Extracted from Fresh and Dried *Spirulina sp*.

Tri Winarni Agustini [a*], Meiny Suzery[b], Danny Sutrisnanto [c], Widodo Farid Ma'ruf[a], Hadiyanto[c]

[a] Department of Fisheries, Faculty of Fisheries and Marine Science, Diponegoro University, Jl. Prof Soedarto, SH, Tembalang, Semarang 50275. Indonesia

[b] Department of Chemistry, Faculty of Sceince and Mathematics, Diponegoro University, Jl. Prof Soedarto, SH, Tembalang, Semarang 50275. Indonesia

[c] Center of Biomass and Renewable Energy (C-Biore), Department of Chemical Engineering, Faculty of Engineering, Diponegoro University, Jl. Prof Soedarto, SH, Tembalang, Semarang 50275. Indonesia

Abstract

• The increase consumption of natural substances has brought about in increasing demand on biological source, including *Spirulina* sp. This *Spirulina sp.* has been recognized to provide some natural substances such as natural colorant, source of vitamins, protein and some minerals. Different material state of *Spirulina sp.* in the form of fresh and dried form may cause different quantity and quality of natural substances in microalgae which posses a wide range of change including nutritional and natural substances. The aims of the study was to investigate the bioactive compounds resulted from different form of Spirulian sp (fresh and dried) quantitatively. The materials used were fresh and dried Spirulina sp which was extracted by ethanol. Analysis were subjected for phytochemical screening, flavoniod, phenolic acid and antioxidant activity (DPPH scavenging activity). The results showed that total flavonoid of fresh and dried are: 25.6615±1.62 and 110.1356±12.5 quercetin/gr extract ; phenolic acid : 2.117±0.99 and 6.92±0.03 GAE/gr extract; IC50 : 33.0755, respectively. Fresh sample of *Spirulina platensis* shows better results in bioactive compounds quantitatively and qualitatively compared to that of dried sample, and drying process at 40-50^{o}C does not give any significant different on nutritional quality of dried sample of Spirulina platensis compared to fresh samples.

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Keywords: phenolic compound; flavonoid compound; fresh and dried sample

* Corresponding author: tagustini@yahoo.com; Telp.: +6224-7474698

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1. Introduction

Many researches have been conducted on bioactive compounds extracted from microalgae[1 – 4]. Some bioactive compounds can act as natural antioxidant that has been considered as safe and more accepted compared to that of synthetic antioxidant[5]. *Spirulina sp* include in the class of cyanobacterium that has been used as a source of protein, vitamin supplements and health drinks [6 – 8]. *Spirulina sp.* has a wealth of perfect nutrition, contains 25 kinds of vitamins and minerals for the health and longevity, body regulation, removal of toxins and strengthen the immune system[(5]

Spirulina platensis is one of cyanobacterium microalgae, multicelluler, and has spiral form that can grow well in either seawater and fresh water. Spirulina also contains pigment of chlorophyl and carotenoids as well as phenolic and flavonoid compounds which can act as natural antioxidant [5,8; 9]. Based on previous studies it is known that the cells of *Spirulina platensis* biomass will be much more soluble in polar solvents, such as water and buffer solution compared to the less polar solvents such as acetone or chloroform. The amount of solvent used in the bioactive extraction greatly affect to the outcome of the extract including the yield obtained, and the purity and stability of the extract. *Spirulina sp*. growing in Indonesia is expected to have more antioxidant compounds, because such microalgae has ability to survive from UV radiation and extreme environmental condition by producing metabolite seconder. It is able to protect human body from free radical damage, prevent from degenerative diseases and retard lipid peroxidation on food[5].

Extraction of bioactive compound from microalgae can be done by several methods, including cold extraction (maceration, sonication) and hot extraction (soxhletation, hot water extraction etc). Extraction method can affect to the quality and quantity of nutritional and bioactive compounds extracted from microalgae. Soxhletation is considered more efficient method because increase surface contact between solvent and material, resulting in higher rendemen and more number of compounds extracted [10]. Therefore it is necessary to select the correct extraction method used in order to get the optimum condition of extraction. State of the raw material will also affect the number and quality of extracted compound inside the materials. This study were aimed to compare the nutritional and bioactive compound (qualitative and quantitatively) extracted from different state of raw material (fresh and dried Spirulina).

2. Materials and Methods

Materials used is *Spirulina platensis* in two different form, fresh Spirulina harvested directly from the cultivation area at Sukoharjo and dried Spirulina produced from fresh Spirulina and is dried directly by using oven at temperature 40^{o}C for 10 hours to get final water content below 10%. These two form of Spirulina were then extracted by using soxhletation (for dried Spirulina) and refluxion combined with sonication (for fresh Spirulina) and analysed for the nutritional and bioactive compound of proximate analysis, flavonoid, phenolic and antioxidant activity.

2.1. Extraction of Fresh Spirulina platensis

Fresh sample of *Spirulina platensis* was extracted by sonication at room temperature, 40 kHz for 1 hour to destruction the cell wall and followed by Reflux at temperature of 50-60^{o}C for 4 hours. Take 100 mL extract and is added ethanol with ratio of 2:1 and is used for further analysis.

2.2. Extraction of Dried Spirulina platensis

Dried Spirulina was extracted by *soxhletation*. Take 100 g of dried sample that has been covered by filter paper and cotton and put inside the soxhlete apparatus. Ethanol was used as extraction solvent.

2.3. Qualitatif test

The extract of Spirulina was subjected for phytochemical screening test to observe bioactive compounds contained in the sample qualitatively[11].

2.4. Quantitatif test

Quantitative test was conducted for total phenolic and flavonoid by using Folin-Ciocalteu method[12 – 14].

- ***Quantitative Analysis for Total Phenolic acid.***

Calibration curve of Gallic acid was made as equivalent to total phenolic compound containing in the sample. Concentration of Gallic acid standards were made by adjusting them according to the extract resulted. Take 0.05 mL from each concentration, added 0.4 mL aquadest and 2 mL reagen *Folin-Ciocalteu* and then homogenized. The homogenate was then left for 8 minutes before added with 1.6 mL Na_2CO_3 7.5%, and homogenized and left for another 30 minutes at room temperature until blue colour is performed. The absorbance of this homogenate was measured by spectrophotometer *UV-Vis* at 765 nm. The calibration curve was made by correlating gallic acid concentration (mg/L) with absorbance. Total phenolic was calculated as mg equivalent Gallic acid / gram dried sample.

- ***Quantitative Analysis for Total Flavonoid.***

Quantitative analysis of Total flavonoid is made by linear regression curve of Quercetin. Quercetin was diluted in ethanol and make some concentration of 20, 40, 60, 80 ppm. Take 0.5 mL from each concentration, homogenized them after adding 1.5 mL methanol and 0.1 mL $AlCl_3$ 10%. Left the homogenate for 6 minutes, and then add 0.1 mL Potasium acetate 1M and left for further 6 minutes. The homogenate is diluted up to 5 mL and left for 30 minutes before measuring the absorbance by using spectrophotometer *UV-Vis* at 415 nm. Total flavonoid was calculated as mg equivalen quercetin/gram dried sample.

2.5. Antioxidant activity test

Antioxidant activity test was conducted based on the method of Brand-Williams *et al.,* (1995); waterhouse A (1999); Orak HH (2006)

2.6. Analysis of proximate

Proximate analysis of *Spirulina platensis* was conducted on fresh and dried Spirulina by using AOAC (1995 and 1997) methods on water, ash, protein content, and fat content. Carbohydrate was calculated by difference.

3. Results and Discussion

3.1. Qualitative test

The results of phytochemical screening of *Spirulina platensis* with two different samples form (fresh and dried Spirulina) were presented in Table 1.

Table 1. Phytochemical screening Test of *Spirulina platensis* extract

Extract	Alkaloid	Phenolic compound	Triterpenoid and steroid	Flavonoid	Saponin
Dried	-	++	+++	++	++
Fresh	-	++	++	+	-

Note: - bioactive substance in the sample is not detected
+ bioactive substance is detected

Based on the results, there is a positive result for phenolic, triterpenoid and steroid, flavonoid on both samples, except for saponin only positive on dried sample. Different phytochemical compound will results in their bioactive activity[15]. Phytochemical compounds can be extracted by using suitable solvent. Polarity of solvent determine the

extracted phytochemical compound. This study used ethanol, which is considered as polar solvent. It has be proven that ethanol is safe for food, therefore it is recommended to use ethanol for microalgae extraction which will consequently safe for further processing.

Alkaloid compound is semi polar and show negative result on ethanol extraction. Alkaloid show negative (not detected) either on fresh and dried samples. The possibilities are due to alkaloid is considered as alkaline and tends to difficult to extract its compounds by chemical solvent and there may no alkaloid compound in the samples.

On the other hand, phenolic compound showed positive result. Phenolic compound is considered as big molecules and is composed from various structures with main characteristic of aromatic chain that has hydroxyl[14]. Phenolic is considered as acid with hydrogen can be easily remove. The most phenolic compound obtained in plants has low antioxidant activity but there is no side effect resulted as do for synthetic antioxidant[5, 16].

Triterpenoid and steroid showed positive results and high content of such compound were found in the samples. Triterpenoid and steroid are considered as non polar, therefor they can be extracted well by using non polar. However, the results showed positive, eventhougt ethanol used as extraction solvent is considered as polar solvent. This is because moment dipole of polar and semi polar compound will do induction to the non polar molecules which has no dipole, so that non polar compound can dissolve in semi polar and polar solvent. This statement is also supported [17] who stated that one factor causing non polar compound dissolved in semi polar and polar solvent due to there is moment dipol between polar and semi polar that will do induction non polar molecules which has no dipole so that there is electrostatic power resulted from polar and semi polar compounds.

Flavonoids is considered as polar compound and there is positive result for both samples. Ethanol as polar sovent can dissolve the flavonoids present in the samples.

Saponin showed positive result from dried samples only. Saponin is a polar substance, so that it can be easily dissolved in ethanol as polar solvent. But the presence of saponin in fresh sample is very small so that it can not be detected qualitatively. Saponin mainly in form of glicoside and is considered as polar substance[18]. Formation of foam in saponin test is a proven evident that saponin is present in the sample which can be converted into glucose and other compounds. In addition, saponin is glycoside complex with high molecular weight and present in plants, bacteria and low level animal[19].

3.2. Quantitative test

Total Phenolic and total flavonoid of *Spirulina platensis* extract form fresh and dried form were presente in Table 2 and Table 3.

Table 2 . Quantitative test of total phenolic of *Spirulina platensis* extract (fresh and dried form)

Sample	Quercetin/g extract							
	Dried sample				Fresh sample			
	1st	2nd	Wet basis	Dried basis	1st	2nd	Wet basis	Dried basis
E_{Et}	101,29	118,98	110,14 ± 12,50	119,43	26,81	24,52	25,66± 1,62	469,96
E_{EA}	213,70	232,16	222,93 ± 13,05	241,74	27,39	21,90	24,65± 3,88	451,47
E_{A}	182,02	225,87	203,94 ± 31	221,15	14,64	13,85	14,25± 0,58	260,99

Based on Table 2 and Table 3, it is obviously shown that fresh sample contained higher phenolic and flavonoid compounds compared to that of dried sample quantitatively. The big different of phenolic and flavonoid compounds resulted from fresh and dried sample were mainly due to the diferent in water content between this two samples. Fresh sample has water content almost 12 times (94.54%) than dried sample (7.78%). Flavonoid is polar compound, so that it can be easily dissolve in water[20]. In addition, flavonoid generally is attached to sugar group which consequently flavonoid can be easily dissolve in water or any other polar solvent[21].

Table 3. Quantitative test of total flavonoid of Spirulina platensis extract (fresh and dried form)

Type of Solvent	GAE/g extract							
	Dried sample				Wet sample			
	1st	2nd	Wet basis	Dried basis	1st	2nd	Wet basis	Dried basis
E_{et}	6,89	6,94	6,92± 0,03	7,50	2,82	1,41	2,11±0,99	38,64
E_{EA}	15,43	5,49	10,46± 7,02	11,34	2,83	1,26	2,04±1,10	37,36
E_{A}	19,69	8,10	13,89± 8,19	15,06	4,66	2,28	3,47±1,68	63,55

3.3. Antioxidant Activity

Antioxidant activity of the *Spirulina platensis* samples were analysed by using method of [12] and the results is presented in Fig.1 and Fig.2. Antioxidant activity of the samples were determined by using DPPH (Diphenil pycril hydrazil) method. Etil acetat is considered as semi polar solvent and suitable to extract the bioactive compound in Spirulina [9]. However, the antioxidant activity resulted is very low. It means that the type of solvent not only important for getting high yield but also it is very important to consider the activity of bioactive extracted. Utilisation of other solvent is necessary to carried out, for example by using ethanol to extract bioactive compound from *Spirulina platensis* may give better result for their activity.

Based on Fig.1 and 2, it is obvious that the higher concentration of extract, the higher precentage of inhibition. This is in line with the result that the higher concentration of extract, the higher percentage of inhibition[22]. This is because on high cencentration of extract, the higher antioxidant present in the sample so that consequently the level of free radical inhibition by antioxidant contained in the sample is also higher. Antioxidant activity can be measured by the number of reducing intencity purple color of DPPH which is linier with reduction of DPPH concentration. [23]. Such reduction was due to reaction between DPPH and hydrogen atom released and characterised by changing from purple to yellow color of DPPH.

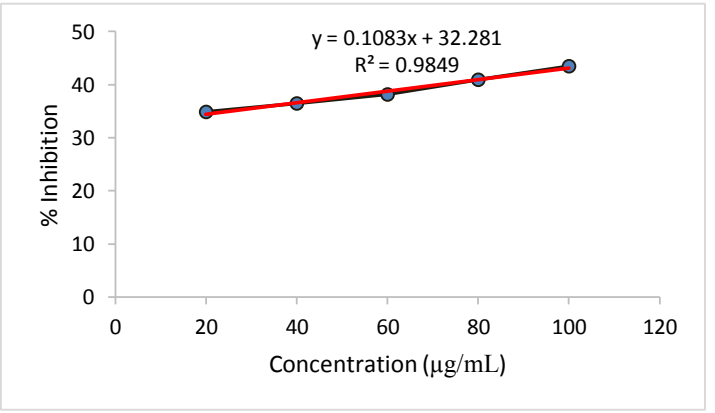

Fig.1. Antioxidant activity of *Spirulina* sp (dried form)

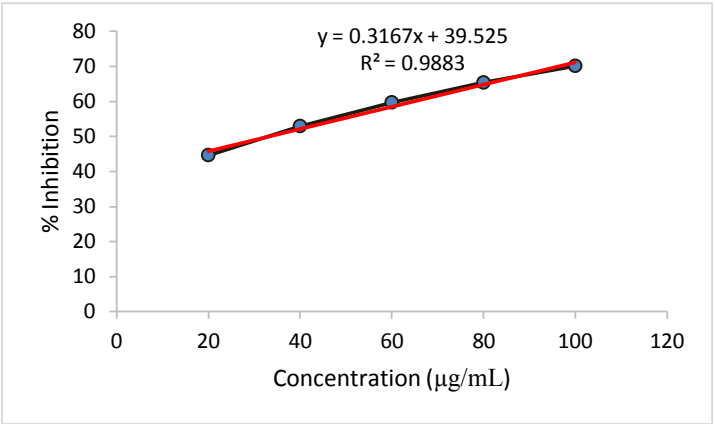

Fig.2. Antioxidant activity of *Spirulina* sp (fresh form)

The result of antioxidant activity of the samples by using DPPH scavenging activity method is represented by IC_{50}, meaning that concentration of extract required to inhibit 50% free radical DPPH. Antioxidant activity is performed by IC_{50} which define as concentration of sample required to inhibit 50% free radical of DPPH[5, 24]. IC_{50} of *Spirulina platensis* extracted with ethanol extraction for fresh and dried sample and quercetin as control is presented in Table 4.

Table 4. IC_{50} of extracted *Spirulina platensis* in fresh and dried samples compared to quercetine.

Sample	IC_{50} (ppm)
Quercetine	**21.616**
Dried sample	**33.075**
Fresh sample	**163.61**

Based on Table 4, it is shown that IC_{50} of fresh and dried samples are different compared to quercetine. Dried sample of *Spirulina platensis* perform high IC_{50} of 33.075 ppm, which is considered as very strong antioxidant. For the fresh sample, IC_{50} is 163.61 ppm and it is considered as weak antioxidant. Both two samples have lower IC_{50} compared to Quercetine (21.616 ppm). Study has been conducted on DPPH scavenging activity of Spirulina extract compared to Vitamin C and E which perform that IC_{50} was around 40 ppm that is comparable to our result[5]. According to research result, a compound can be characterized as very strong antioxidant (IC_{50} < 50 ppm), strong antioxidant (IC_{50}: 50 – 100 ppm), middle (IC_{50}: 100 – 150 ppm), weak (IC_{50}: 150 – 200 ppm), and very weak (IC_{50}: > 200 ppm). Moreover, Andyani et al.(2008) reported that a compound is considered to have antioxidant activity if it has IC_{50} less than 200 ppm[22].

From this results, there is a correlation between antioxidant activity and phytochemical screening test in which dried sample of Spirulina gave more higher extracted bioactive compound compared to fresh sample and consequently perform high antioxidant activity.

3.4. Proximate analysis

Fresh sample and dried sample of Spirulina have different proximate data as shown on Table 5. Based on the results it was obviously found that fresh sample perform comparatively the same nutritional value compared to that of dried sample of Spirulina. Protein and ash content of dried sample is higher than fresh sample. In this case the effect of heating process applied during processing of dried Spirulina does not give significant effect to nutritional decrease of dried sample. The dried sample was subjected to heating process at 40-50^{o}C for 10 hours, which is considered to have very less effect to the nutritional quality of protein. Protein would subject to denaturation at temperature of 70^{o}C which consequently results in nutritional damage of the sample. In this study, heating process took place at fairly low temperature and thus processing can maintain the nutritional quality of dried sample.

Table 5. Proximate analysis of Spirulina platensis (fresh and dried samples)

Concentration of compound (%)	Dried sample		Fresh sample	
	Wet basis	Dried basis	Wet basis	Dried basis
Water	7.78	-	94.54	-
Protein	67.18	72.85	3.28	60.07
Lipid	2.64	2.86	0.27	4.94
Carbohydrate	11.74	12.73	1.47	26.92
Ash	10.66	11.56	0.44	8.06

However, this drying process gave effect to the number of bioactive compounds present in dried sample as shown on Table 2 and Table 3. Dried sample perform to have less number of bioactive compounds compared to that of fresh sample. This phenomenon can be described that some of bioactive compound are mainly heat-sensitive compound, so that they will perform decreasing number due to heating process even at low temperature.

4. Conclusion

- Fresh sample of *Spirulina platensis* shows better results in bioactive compounds quantitatively and qualitatively compared to that of dried sample, and
- Drying process at 40-50^{o}C does not give any significant different on nutritional quality of dried sample of *Spirulina platensis* compared to fresh samples.

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