Utilization of spirulina as functional food: Phytosterol and amino acid profiles study

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Abstract. The potential of microalgae Spirulina sp. biomass as a raw material food is very large. Spirulina sp. is believed to promote health effects in human being. This study was aimed to assess the phytosterol content and amino acid profile of microalgae. Microalgae has been targeted because of several advantages, namely high area productivity, relative ease to cultivate, lack of need to compete with food products in terms of fertile areas, ability to adapt to environmental changes by generating secondary metabolites that are useful, and possibility to be used to refine and take nutritive from effluent. Spirulina sp. was collected from Yogyakarta, Indonesia and was tested for proximate content, β-sitosterol and stigmasterol content and amino acid profile. Spirulina sp. can be considered as a source of functional amino acid (FAA) in human nutrition as they are rich in glutamic acid (75,320.79 ppm), Leusin (58,568.49 ppm) and aspartic acid (43,830.78 ppm). Saponification fraction of Spirulina sp. contain 5.39±2.29 % β-sitosterol and 7.61±2.01 stigmasterol.

Keywords: amino acid, functional food, microalgae, phytosterol, spirulina

1. Introduction

The understanding of some people in modern times about food has developed. Food is not only an antidote to hunger, but food is expected to be able to maintain a healthy body. Awareness of the presence of good component components in foods that have certain positive physiological functions on body health raises new awareness of the importance of food selection as part of a healthy lifestyle. Foods that have positive physiological functions for health such as lowering cholesterol, lowering blood sugar and high blood pressure are known as functional foods [1]. According to the regulation head of drug and food control agency The Republic of Indonesia in 2011 functional food is processed food containing one or more food components in the amount that corresponds to the prescribed limits which are based on scientific studies have certain physiological functions outside their basic functions, Functional food is food that can be served, as well as consumed daily as a food or drink that has been proven to be safe and unproductive and has the same sensory characteristics as food in general, such as appearance, including color, texture, size, consistency, and taste acceptable to consumers [2].

The sea contains enormous natural richness where it contained a lot of bioactive compounds, one of which can be applied in various stages of manufacture of food [3]. Research has now been carried out to find natural resources as ingredients for functional food formulations. Some records prove that
microalgae have been used traditionally and anciently as a food for humans and a few dozen years ago microalgae like *Spirulina, Chlorella spp* and *Dunaliella* began cultivated for various purposes [4].

*Spirulina* sp. is a type of filamentous and multicellular cyanobacterium. *Spirulina* sp. lives in fresh water and has bioactive compounds that are beneficial to health such as protein, sterols, long chain fatty acids polyunsaturated, vitamins, pigments, and other compounds [5]. *Spirulina* sp. is rich in protein, so amino acids as part of protein are interesting to learn. The number of research on biochemistry, physiology and amino acid (AA) nutrition in growth, health and other human and animal diseases has increased in recent years. Nutritional studies show that administration of certain AA-rich diets (for example, arginine, glutamine, glutamate, leucine and proline) can increase the growth of small and skeletal intestinal muscles, modulate gene expression, or reduce excess body fat. Amino acids are the topics of interest to researcher because the amino acids have some physiological function to prevent and treat metabolic disease [6].

The potential of *Spirulina* sp. in lowering cholesterol can be studied from phytosterol content. Research on the role of phytosterols in various aspects of health has been widely acknowledged in new period. Several studies have indicate that phytosterol plays a role in lowering blood cholesterol and is used in the contraceptive industry, corticoids, diuretic male hormone progesterone, estrogen vitamin D [7], phytosterol has a large market demand. This encourages research that focuses on identifying natural resources for phytosterols. One of the promising alternative sources is microalgae [8]. This research focuses on *Spirulina* sp. as a functional food. Moreover, it focus on phytosterol properties and amino acid compound that are found in this *Spirulina* sp.

2. Materials and Methods

2.1. Materials

The raw materials used in this work were dried microalgae *Spirulina* sp. with moisture content of 12 ± 0.35%. The *spirulina* sp. was collected from Yogyakarta, Indonesia. The reagents used were mostly of analytical and technical grades. The equipment used were oven memmert, furnace furnace 6000, Soxhlet extractor Gerhardt, chiller, a tool of destruction kjedhal foss, hotplates, reflux, sonicator, centrifuge, evaporator, TLC scanner and laboratory glassware.

2.2. Proximate analysis and mineral analysis

Proximate analysis were conducted according to the national standards of Indonesia. Moisture content analysis based on SNI 2354.2:2015 [9], protein content analysis based on SNI 2354.4:2006 [10], fat content analysis based on SNI 2354.3:2017 [11], ash content analysis based on SNI 2354.1:2010 [12]. Analysis of carbohydrate [13]. Mineral analysis were conducted according to AOAC 985.35/50.1.14.2005 [14].

2.3. Preparation extract

A total of 5 g of *Spirulina* was added to 50 mL of 7.5% KOH solution in ethanol and then in sonication for 1 hour. Furthermore, the mixture was put in reflux with heating treatment for 1 hour and was then left overnight. The separation of the filtrate from the precipitate was carried out by centrifugation (4,000 rpm, 10 min, 10°C). Partition of the extract was carried out by the addition of n-hexane (2: 1 v / v), followed by water addition until the n-hexane fraction reached pH 7. The obtained n-hexane fraction was then evaporated using an evaporator and dried using a concentrator [15].

2.4. Test levels of phytosterol

The test was done using TLC Scanner tool. The bottling of the 6-mm-wide strip test solution was conducted semi quantitatively using Linomat on a TLC plate that had been heated in an oven for 7 minutes at a temperature of 100°C. Bottuling is done with a distance of 1 cm from below, 1 cm from the right and 1 cm from the left of the KLT plate. The bottling rate was 50 nL/s. Vessel saturation was done for 30 minutes. Distance of development was 8 cm. The resulting development plate was scanned with a TLC scanner.
2.5. Analysis of amino acid

20 mg sample was hydrolysed with 1 ml 6N HCl, heated at 180°C for 60 min. The hydrolysate (100 µL) was then analysed for amino acid content using EZ:faast amino acid testing kit - Phenomenex against essential and non-essential amino acid standard (Phenomenex AG0-7184). Analysis of amino acid was conducted in duplicates [16].

3. Results and Discussion

3.1. Chemical content and phytosterol analysis

Chemical content and phytosterol analysis can be seen in Table 1.

| Chemical content and phytosterol content of Spirulina platensis. | Water content | Ash content | Lipid  | Protein  | Carbohydrate | B sitosterol | Stigmasterol |
|----------------------------------------------------------------|--------------|-------------|--------|----------|--------------|-------------|--------------|
| 7.98 ± 0.11                                                                    | 4.9 ± 0.073  | 4.26        | 61.96 ± 0.37  | 4.67 ± 2.83 | 5.39 ± 2.29  | 7.61 ± 2.01  |

Chemical content analysis result showed that Spirulina sp. is rich in protein. The percentage of protein was 61.96±0.37%. Based on the results of this study demonstrated that Spirulina sp. has a high protein content, almost doubled the soy beans which have a protein content 35%. Phytosterol is a steroid component found in plants that has a similar structure with cholesterol [17]. Phytosterol have various type two of them B-sitosterol and stigmasterol. The result using TLC method shows that non-saponification fraction extract of Spirulina sp. contains 5.39±2.29% B-sitosterol and 7.61±2.01% stigmasterol.

3.2. Amino acid

Spirulina sp. contains 10 essential amino acids and 8 non-essential amino acids. The highest amino acids in Spirulina sp. is glutamic acid followed by leucine and aspartic acid. Leucine is the highest essential amino acid and glutamic acid. Is the highest non-essential amino acid in Spirulina sp. Spirulina sp. contains high protein contents that is 60,96% dry weight. Beside high protein content Spirulina sp. are rich of amino acids. Spirulina sp. contains all essential and non essensial amino acids. The value of amino acids shows that the biological value of proteins in Spirulina sp. is very high. Amino acid of Spirulina platensis show in figure 1.

![Amino acid of Spirulina platensis.](attachment:image)

Spirulina sp. were known to contain stigmasterol and β-sitosterol. Stigmasterol and B- sitosterol are type of phytosterol. Phytosterol have function in lowering cholesterol because phytosterol are easier to hydrolize than cholesterol so that phytosterol is easier to binding to micelles that is important tool to binding cholesterol [18]. In this research the b-sitosterol and stigma sterol contents from non-saponification fraction extract were still low and it cannot be concluded that Spirulina sp. can be a
source of phytosterol. In the point of view of *Spirulina* sp. as source of functional food, it can be concluded that *Spirulina* sp. can be used as a source of protein that is rich in amino acid, and it also has other nutrients like carbohydrate, lipid and sterol.

4. Conclusion

The conclusion that can be drawn from this study is that *Spirulina* sp. contains phytosterol properties and amino acid compounds that are useful for functional food.

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