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OPTIMIZATION OF THE CONDITION OF PHYTOSTEROL EXTRACTION CONDITIONS FROM MICROALGAE *NANNOCHLOROPSIS* USING ETHANOL OF DIFFERENT PURITY LEVELS

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Abstract

Phytosterols are plant sterols, included in secondary metabolite compounds and have broad benefits in the health field. This study aims to observe the effect of ethanol solvency purity, time of extraction on phytosterol content. The extraction was carried out by saponification using a 7.5% KOH solution in ethanol. The variations in extraction time are 1, 2, 3, and 4 hours. Ethanol used varies purity levels, i.e technical 70%, technical 96%, and pure analysis. The variations in maceration time are 1, 2 and 3 days. The results showed that all ethanol solvents with different purity levels resulted in the highest crude phytosterol under long duration of 1 hour extraction time. The ethanol purity rate had a significant effect on the yield of phytosterol produced, i.e., 70% technical, 96% technical, and pure analysis consume 0.4%, 1.63% and 4.19% crude phytosterol, respectively.

Key words: Extraction, Phytosterol, Nannochloropsis

Introduction

Phytosterols are sterols that are naturally derived from plants. Chemically, phytosterols are similar to those obtained from animals. Sterols consist of three combinations of cyclohexane rings with various sterols (>40 phytosterols). Phytosterols consist of 28 to 30 atoms with steroids as structural framework with hydroxyl groups attached to C-3 of the A ring, and an aliphatic chain on C-17 atoms of the D ring (Pateh et al. 2009). More than 100 types of phytosterols have been reported in plants, but the most abundant are sitosterol, stigmasterol and campesterol (Berger et al. 2004, Kritchevsky and Chen 2005).

Recent years have done a lot of research on the role of phytosterol in various aspects of health. Phytosterols are thought to lower cholesterol in the blood, and are used in the industries of progesterone, corticoids, estrogens, contraceptives, diuretics, male hormones and vitamin D (Cabral and Fernandes 2007, Sukmaniah et al. 2008). With the widespread fitosterol market, many studies have focused on identifying new natural resources for phytosterols and several studies have shown that microalgae are an appropriate alternative source of these functional compounds (Francavilla et al. 2012).

Nannochloropsis sp. better known as the Sea Chlorela is a microalga belonging to the class of Eustigmatophyceae. These microalgae are about 1-2 µm in size and grow optimally in the waters with salinity 25-35 ppt, temperature 25-30°C, pH 8-9 and light intensity 1000-10000 lux (Isnansetyo and Kurniastuty 1995). *Nannochloropsis* commonly used in the field of aquaculture that is as a feed larvae, small fish and

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crustase. Excess *Nannochloropsis* include high nutrient content, easily cultured en masse, rapid growth, non-toxic, and contain antibiotic compounds (Fulk and Main 1991). Sterol is one of the important components found in the eukaryotic cell membrane (Volkman et al. 1998). Sterols are present in microalgae cell membranes that act as biomarkers to identify organic matter in the environment (Volkman et al. 1998).

Extraction of phytosterols can be performed by means of saponification method using ethanol solvent in KOH (Kim et al. 1990, Xiao et al. 2013). This study aims to see the effect of ethanol solvency purity and time of extraction on phytosterol content.

Materials and Methods

Chemical and equipment

Nannochloropsis sp., KOH, aquades, n-hexane and ethanol (pure analysis, technical 96%, and technical 70% were used for this study. As equipment glass container, reflux, centrifuge, evaporator, sonicator, and TLC scanner (CAMAG TLC Scanner 3 CAT No. 027.6485 SER. No. 160602) were used.

Methods

Analysis of proximate compositions

For analysis ash, water, fat, and protein content refers to SNI 01-2354 (1-4) - 2006 (BSN 2006 a,b,c&d). Analysis of carbohydrate and coarse fiber content refers to Apriyantono et al. (1989).

Preparation extract

A total of 5.0 g of *Nannochloropsis* microalgae was added with 50 ml of 7.5% KOH solution in ethanol and then in sonication for 1 hour. Furthermore, the mixture in reflux with heating treatment duration (1, 2, 3, and 4 hours) was then left to 1 night. The separation of the filtrate from the precipitate is carried out by centrifugation (4000 rpm, 10 min, 10°C). Partition of the extract was carried out by addition of n-hexane (2: 1 v/v), followed by water addition until the n-hexane fraction reached pH 7.0. The obtained n-hexane fraction was then evaporated using an evaporator and dried using a concentrator.

Phytochemical screening test

For phytochemical screening tests performed include alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids were used for this study.

Test levels of B-sitosterol and B-carotene

The test was done using TLC Sanner 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. Bottling 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 is done for 30 minutes. Distance of development is 8 cm. The resulting development plate is scanned with a TLC scanner.

Results and Discussion

Proksimat composition of Nannochloropsis oculata

Table 1. Proportional composition of *Nannochloropsis* oculata microalgae

Proximate compositions	Yield (%)
Moisture	14.82 ± 0.12
Ash	39.36 ± 0.07
Carbohydrate	4.36 ± 0.04
Proteins	29.06 ± 0.46
Lipids	4.29 ± 0.41

The proximate composition of the *N. oculata* microalgae was or are shown in Table 1. *N. oculata* had high ash and protein content, 39.36% and 29.06%. While carbohydrate and fat content had a low value of 4.365 and 4.29%. This *N. oculata* proximate composition is lower than that of an earlier study by Sorgeloos (1996) who reported that the protein content of *Nannochloropsis* sp. by 37%, carbohydrates 18% and fat 7.8%. Similarly, the study of Sanjeewa et al. (2016) who reported that *N. oculata* had a protein content of 30.52%, 30.64% ash, and 7.99% fat. However, the differences of nutrient values in microalgae might be influenced by the growing environment such as pH, salinity, temperature, and light intensity.

Rendement of crude phytosterol

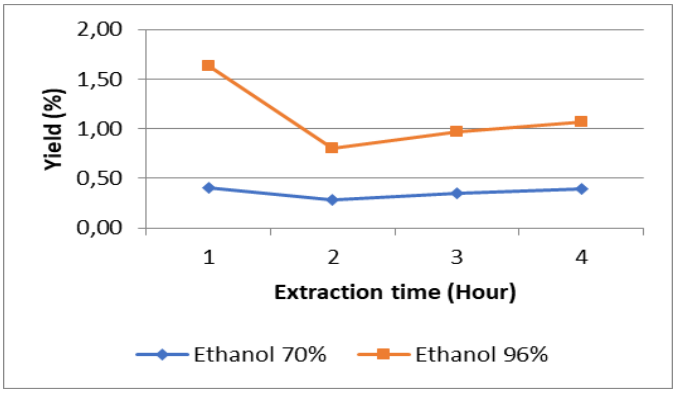

Fig. 1. Rendement of crude fitosterol from *N. oculata.*

The value of crude phytosterol rendement from *N. oculata* is shown in Fig. 1. The use of ethanol with higher purity level results in higher tenement value. Phytosterols are known to dissolve in organic solvents and most solvents have one alcohol functional group (Kanimozhi and Bai 2012). The lower the purity level of the alcohol used, the more water contained in the solvent. The higher water content will cause the low solubility of non-polar compounds such as phytosterols. Thus, the level of phytosterol produced will also be lower.

Meanwhile, in the long treatment time of extraction showed that the highest rendement value was obtained at 1 hour of extraction, except with the optimum ethanol 96% solvent utilization at 2 hours of extraction.

Phytochemical screening analysis of crude phytosterol

Components contained in crude fitosterol are analyzed by their compounds in a qualitative manner using color test tests with several reactions including alkaloid compounds, saponins, tannins, phenolic, flavonoids, triterpenoids, steroids and glycosides. The results of phytochemical screening of crude fitosterol from *N. oculata* are presented in Table 2.

Table 2. Phytochemical screening test results of crude phytosterol

Phytochemical	Crude phytosterol	
	Ethanol 70%	Ethanol 96%
Alkaloid	+	+
Saponin	-	+
Tannin	-	-
Phenolik	+	+
Flavonoid	+	-
Triterphenoid	-	+
Steroid	+	+
Glykoside	+	+

The results of phytochemical screening indicate a treatment that results in negative triterpenoids and steroids. Though phytosterol compound itself is shown with positive results on triterpenoid and steroid tests. In addition, screening did not show a uniform result. This may be due to the very small number of samples so that detection is extremely difficult. According to Pascal and Segal (2006), phytosterols consist of five forms, namely as free alcohol, fatty acid esters, sterile glycosides, sterilized glycosides terasilasi, and acid esters. Thus, phytosterols will positively contain triterpenoids, phenolic, steroids, and glycosides.

Analysis of B-carotene, B-sitosterol, and stigmasterol levels

Beta sitosterol has many health benefits. It is known that beta sitosterol is found naturally in vegetables and fruits, but the ingredients are too low to provide a medicinal effect.

Table 3. Levels of B-carotene, B-sitosterol, and stigmasterol per weight of crude fitosterol

Parameter	Yield per weight of crude phytosterol extract (%)		Yield per weight of microalgae (g/kg microalgae) (%)	
	Ethanol 70%	Ethanol 96%	Ethanol 70%	Ethanol 96%
B-carroten	6.14	2.74	0.25	0.45
B-sitosterol	10.99	6.44	0.44	1.05
Stigmasterol	21.58	17.53	0.87	2.86

Conclusion

Microalgae *Nannochloropsis oculata* might be used as an alternative source of phytosterol raw materials. The use of 96% technical ethanol resulted in higher rendement of crude phytosterols compared to the use of 70% technical ethanol. While the long treatment time of extraction yielded optimum yield at 1 hour reaction with higher levels of B-carotene, B-sitosterol, and stigmasterol in 96% ethanol use compared to 79% ethanol.

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