

EXTRACTION OF POLYSACCHARIDE FROM *SPIRULINA PLATENSIS* - ADVANTAGE OF FREEZE-THAW METHOD

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SUMMARY

Polysaccharide is one of the key natural products that profit human living conditions. This study aimed at investigating the polysaccharide extraction from *Spirulina platensis* (PSP) basing on previous research on optimization of experimental conditions. As a result, the efficiency of raw PSP for four extraction methods was 8.35%, 65%, 76.9% and 85.1% for hot water, lye, ultrasound-assisted and freeze-thaw extraction method, respectively, which was higher than previously reported results. Particularly, the efficiency of pure PSP extraction was highest with 14.89% of the dry matter of raw PSP for freezing-thawing method. To determine the major compounds constructing the pure PSP, the composition of PSP monosaccharides was determined by HPLC. The result showed that major PSP monosaccharides were glucose and galactose, of which glucose composition was up to 85%. Furthermore, pure PSP showed antioxidant activity with IC₅₀ values of 373, 403, 276, and 258 mg/mL for hot water, lye, ultrasound-assisted, and freeze-thaw extraction method, respectively, as compared to IC₅₀ of 101.7 mg/mL for ascorbic acid as control.

Keywords: polysaccharide, monosaccharide, antioxidation activity, *Spirulina platensis*, nutritional food

INTRODUCTION

Natural products have been constantly searched to combat aging to prolong youth and health safety. Indeed, the cost of skin rejuvenation is of particular interest to women, who are willing to invest a huge amount, about 20 - 30% of living-budget to do this. To meet this demand, many beauty establishments sprout up like mushrooms in response to the large number of female customers. However, manufacturers of beauty products are also under great pressure from counterfeit, unknown original products floating on the market, discrediting to women's confidence in the existing trademark of natural products, while almost of famous brands' beauty

products made from *Spirulina* are very expensive.

Spirulina is an edible and nutritious food, containing up to 60 - 70% protein, 6% - 12% polysaccharide, fatty acids and minerals, and many vitamins. In addition, *Spirulina* attracts attention because of biological activities in the human body and plays an unique role in various medicinal cases and health care (Yang *et al.*, 2012; 2009). *Spirulina* polysaccharide (PSP) has been reported to have anti-cancer, antioxidant, anti-aging, immunomodulatory and antiviral activity (Kurd, 2015; Lupatini *et al.*, 2017).

The polysaccharide properties could vary according to methods and forms of performing

extraction (Markou, 2012; Wang *et al.*, 2018; Wu, 2017). The extraction of PSP by ultrasound method resulted in a higher content than hot water, but the antioxidant activity of PSP decreased (Qu *et al.*, 2013). Method of PSP extracting by freezing and thawing defeated this disadvantage, but was more complicated in the implementation of experiments (Wang *et al.*, 2018).

Although PSP compounds have been studied in Vietnam on the antioxidant properties of *Spirulina*, assessing the biological activity of PSP (Le Thi Anh Dao *et al.*, 2018) and PSP extracted from shiitake mushrooms (Hà *et al.*, 2013) but, these works payed much attention to antioxidant activity of extract from *Spirulina* and other activities of PSP from many other plant sources. In other words, previous studies have just payed much attentions to characterizations of *Spirulina* (Võ Hoài Bắc *et al.*, 2018). Meanwhile, the extraction methods for obtaining PSP from *Spirulina* have been of little interest. Most studies focus on obtaining biological activities of PSP without mentioning the comparison of PSP extraction methods, which would affect PSP yield as well as the desirable biological characteristics.

In order to maximize the PSP content and to well illustrate its biological activities, this study compared the efficacy of extraction by four different methods such as hot water, ultrasonic-assisted, lye and freeze-thaw basing on polysaccharide yield extracted and purified from *Spirulina platensis* and their antioxidant activity. Additionally, PSP monosaccharide components were analyzed to determine the major compounds and its property in the free radical scavenging activity.

MATERIALS AND METHODS

Materials

S. platensis was harvested at the laboratory of University of Technology and Education, The University of Danang. Before extraction, *S. platensis* was dried and determined the dry

matter weight. The chemicals for analysis were from Merck, Vietnam and Sigma, America.

Extraction of PSP

Based on Wang *et al.*, 2013, *S. platensis* was extracted by four methods such as hot water, lye, ultrasound-assisted and freeze-thaw as follows: a) Hot water extraction. Add 40 g of *S. platensis* powder to 1.6 litre of water. The mixture was stirred vigorously in a water bath at 80°C for 8 hours, then centrifuged at 4300 rpm for 20 minutes. The solution obtained from the centrifuge was concentrated to 1/5 of the original volume. Afterwards, 95% ethanol was added to the concentrated solution with a ratio of 5/1 v/v. The mixture was placed in the refrigerator overnight, then centrifuged at 4300 rpm for 20 minutes. The precipitate was washed with acetone, filtered and then dried; b) Lye extraction. Add 40 g of *S. platensis* powder to 1.6 litre of water and adjust the pH to 10.0 by 1 M NaOH solution. The next extraction steps were similar to those in a); c) Ultrasound-assisted extraction. Add 40 g of *S. platensis* powder to 1.6 litre of water. The mixture was ultrasound for 50 minutes at 50°C and 320 W. The remaining extraction steps were similar to those in a); d) Freeze-thaw extraction. Add 40 g of *S. platensis* powder to 1.6 litre of water. The mixture was then frozen at -4°C for 1 hour and melted at 30°C for 1 hour. The remaining experimental steps were similar to those in a).

The efficiency of raw PSP extraction was calculated as follows:

$$E, \% = \frac{\text{raw PSP dry weight}}{\text{Biomass dry weight}} \times 100 \quad (1)$$

Purification and decoloration of PSP

Raw PSP obtained was purified and decolorized. The steps taken are as follows: raw PSP was dissolved in distilled water. The pH was adjusted to 7.0 with 1 M HCl or 1 M NaOH solution, then 3% of papain to the mixture was added and incubated at 50°C for 2.5 hours. Inactivate papain activity by adding 5% TCA and boiling. The mixture was stored at 4°C overnight in a refrigerator and then centrifugated to collect

the supernatant. 5% TCA solution was added to the supernatant which was then leaved overnight at 4°C in a refrigerator. Protein-extracted PSP solution was kept at 55°C and adjusted to 8.0 pH with a concentrated ammonia solution. 30% hydrogen peroxide solution was continued to add and leave the mixture stable for 2 hours. A precipitate, which was collected by adding 95% concentration of ethanol overnight at 4°C, was dissolved in distilled water and again precipitated with 95% of ethanol, repeat this step for 3 times. The final precipitate was washed with anhydrous ethanol, acetone and ether and then dried. The dried sample was then stored in the freezer at -20°C for further analysis.

Total sugar, total protein assay

For determining total carbohydrates of

$$DPPH \text{ scavenging activity } (\%) = \left(1 - \frac{A_t}{A_c}\right) \times 100 \quad (2)$$

where A_c is the absorbance of the control and A_t is the absorbance of test sample (Kedare, Singh, 2011; Rehakova *et al.*, 2008)

Analysis of monosaccharide by HPLC

For evaluating the valuable components of pure PSP in extract, the analysis of monosaccharide was necessary to analyse by HPLC-(Nguyen *et al.*, 2020).

RESULTS AND DISCUSSION

Extraction and purification of PSP

Results of four methods for extraction and purification of PSP were shown in Table 1. The difference between these methods definitely changed the efficiency of purification of raw PSP that depended on the extraction.

The efficiency of lye extraction was indicated to the highest value of pure PSP (10.8 %) in various researches (Wang *et al.*, 2018). As compared to publication of Markou *et al.*, 2012, with the composition of total sugar in PSP of 10%, the result in this study reached closer 15% with the highest pure PSP inherited to freeze-thaw extraction method, suggesting a

purified PSP, method of Dubois published in 1956 was used (Dubois *et al.*, 1956) using glucose as standard. Total protein in *Spirulina* was determined by Bradford method (Bradford, 1976) using albumin as standard.

DPPH test

3 mL of 0.1 mM DPPH solution in methanol was added to 1 mL of aqueous PSP to achieve PSP solutions with concentration in the range of 24 - 780 µg/mL, which were then incubated in room temperature for 30 mins. Free radical scavenging activity of each sample was determined by comparing its absorbance with that of a blank (ethanol), using ascorbic acid as a standard. The ability to scavenge the DPPH free radical was calculated using the following equation:

suitable extraction method for pure PSP according to a safe and economical production of PSP in large scale.

Although this study aimed to investigating the PSP extraction, but composition of protein was also determined to compare with other researches cited above. The protein content from four extraction methods was indicated in Table 2. The percentage of protein in 100 g biomass of *S. platensis* was 38.42% with ultrasound-assisted extraction method (Lupatini *et al.*, 2017) while our study resulted in protein of 27.5% as recorded for mass of total soluble protein in 100 g of raw PSP.

Composition of monosaccharide

The analytical data of night monosaccharides indicated in Table 3 that xylose and ribose were not detected in four extraction methods. Moreover, the composition of glucose irrespective of extraction methods was highest than other monosaccharides. Compared to previous researches, glucose compound in this

study was also detected higher, for instance, Wang *et al.* (2018) found the composition of basic glucose at 21.3% against to other monosaccharides. Shekaram *et al.* (1987) also detected most of this compound at 75.1% (Shekaram, Venkataraman, Salimath, 1987). Meanwhile, glucose was found in Table 3 up to

more 80%, suggesting to high content of glucose in pure PSP. The second major composition was found as galactose, which was detected to more 7.5% for hot water extraction method. This result exhibits an important aspect in human healthy, the composition of glucose would contribute on treating rheumatic pains.

Table 1. Compositions of PSP extracted and PSP purified.

	Hot water extraction	Lye extraction	Ultrasonic-assisted extraction	Freeze-thaw extraction
Raw PSP				
Dry matter, g	17.087 ± 0.78	16.25 ± 1.2	19.225 ± 1.2	21.263 ± 0.52
Efficiency, %	68.35	65	76.9	85.1
Pure PSP				
Dry matter, g	1.98 ± 0.06	1.917 ± 0.05	2.721 ± 0.05	3.167 ± 0.07
Composition of pure PSP, %	11.59	11.79	14.15	14.89

Table 2. Composition of protein (%) in fractions of *S. platensis* with its concentration in biomass of 57.02 ± 2.2%.

Extraction method	Hot water	Lye	Ultrasonic-assisted	Freeze-thaw
Raw PSP, mg/100g	27	25	22.5	24.25
Pure PSP, %	3.4	2.3	3.1	2.65

Table 3. Monosaccharide content (%) in pure PSP derived from different extraction methods.

Monosaccharide	Hot water hydrolysate*	Lye hydrolysate*	Ultrasonic-assisted	Freeze-thaw
Fucose	0.97	0.90	1.10	1.12
Rhamnose	3.57	2.56	1.73	2.10
Arabinose	2.30	2.10	3.30	4.52
Galactose	7.67	7.12	4.50	4.05
Glucose	82.53	83.25	84.97	83.80
Mannose	3.00	4.07	4.17	4.20
Xylose	ND	ND	ND	ND
Fructose	0.00	0.00	0.23	0.21
Ribose	ND	ND	ND	N.D.

ND, not detected

*, hydrolysate was analyzed with three repeats

Antioxidant activity

Antioxidant activity of pure extracted PSPs from four extraction methods were shown in Figure 1. With fluctuations in the extraction method, PSPs exhibited slight changes through the DDPH scavenging effects. As shown in Figure 1, pure PSPs exhibited strong antioxidant activities through ICs at 780 µg/mL over 60%. Compared to four extractions, the freeze-thaw method was found to have highest efficiency of extraction as well as scavenging oxygen. With the highest value in the

concentration of pure PSP in the freeze-thaw extraction method, the inhibition of oxidation was tested by DPPH and valued to IC₅₀ of 258.8 µg/mL against to IC₅₀ of ascorbic acid of 101.7 µg/mL (Table 4). This result exhibited a strong antioxidation of pure PSP extracted by the freeze-thaw method in comparison to ascorbic acid. Meanwhile, various natural products have the capacity of antioxidation such as tronchuda cabbage seeds with IC₅₀ of 356 µg/mL (Pereira, Ferreres, Valentão, Andrade, 2011), green tea with 0.48-1.16 mg DW/mg DPPH (Balci, Özdemir, 2016).

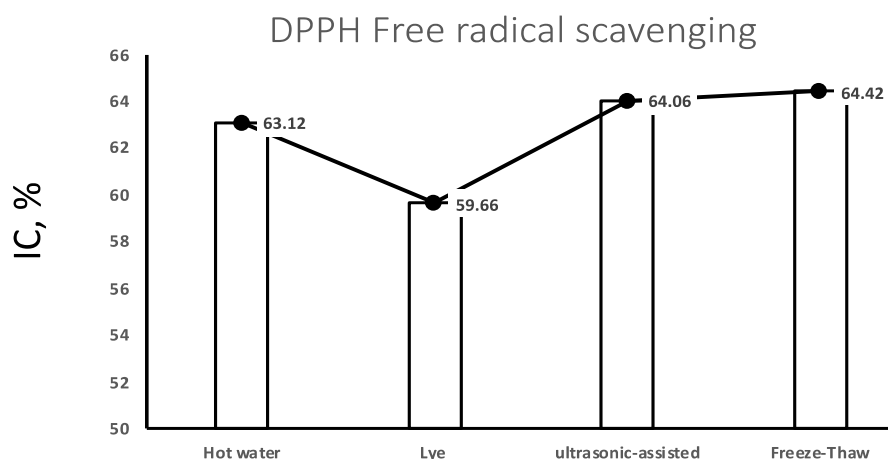


Figure 1. Antioxidant activity of pure PSP in content of 780 µg/mL by four extraction methods; DPPH is a free radical that reacts with polysaccharide able to free a hydrogen.

Table 4. IC₅₀ of pure PSP from four extraction methods compared to ascorbic acid.

Extraction method	Hot water	Lye	Ultrasonic-assisted	Freeze-Thaw	Acorbic acid
Concentration of PSP aqueous, µg/mL	373.13	403.86	276.34	258.87	101.71

CONCLUSION

This study carried out another aspect of polysaccharide extraction from *Spirulina platensis* basing on previous researches. Our results indicated that the extraction efficiency of raw and pure PSP by freeze-thaw method was higher than other three methods. This is able to

profit global production of polysaccharide from *S. platensis* such as applying a simple extraction method, economical in operating a process. Moreover, the pure PSP consists of special characteristic such as the antioxidation activity that evaluated through value of IC₅₀ compared to ascorbic acid.

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CHIẾT XUẤT POLYSACCHARIDE TỪ TẢO XOẮN *SPIRULINA PLATENSIS* - LỢI THẾ CỦA PHƯƠNG PHÁP ĐÓNG BĂNG-TAN BĂNG

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TÓM TẮT

Polysaccharide là một trong các sản phẩm tự nhiên mang lại lợi ích cho con người. Bài báo này đặt mục tiêu nghiên cứu chiết xuất polysaccharide từ tảo xoắn, dựa trên các nghiên cứu trước đây về tối ưu hoá các điều kiện chiết tách polysaccharide bằng bốn phương pháp chủ yếu như nước nóng, kiềm, siêu âm và đóng băng-tan băng. Kết quả cho thấy rằng hiệu suất chiết xuất polysaccharide thô cao hơn các nghiên cứu đã được nêu, và đạt các giá trị tương ứng cho phương pháp chiết nước nóng, kiềm, siêu âm, đóng băng-tan băng là 68,35%, 65%, 76,9% and 85,1%. Đặc biệt là hiệu suất thu PSP tinh sạch đạt cao nhất, tới 14,89% khi sử dụng phương pháp đóng băng-tan băng. Để xác định thành phần đường đơn cấu thành PSP, thành phần monosaccharide của PSP sau tinh chế được phân tích bằng HPLC. Kết quả cho thấy glucose và galactose là thành phần chính với glucose lên đến 85%. Một tính chất quan trọng khác đó là khả năng chống oxi hoá của PSP sau tinh chế thể hiện ở IC₅₀ được thực hiện đối với các nồng độ 373, 403, 276, and 258 mg/mL tương ứng phương pháp chiết nước nóng, kiềm, siêu âm, đóng băng- tan băng so với 101,7 mg/mL của đối chứng là axit ascorbic.

Từ khóa: *polysaccharide, monosaccharide, antioxidation activity, Spirulina platensis, nutritional food*