The Influences of Extraction Conditions on the Content of β-glucan Extracted from *Schizophyllum Commune* Processed-Product Residue

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Abstract: In recent years, the interest in bio-based products, especially in the field of cosmetics, has risen extensively. Mushrooms have various derivative substances with excellent properties in terms of preventing and improving human skin structures. Especially, β-glucan which is water-soluble polysaccharides that can improve collagen generation and skin health. However, its relatively high production cost is concerned as the big problem for usage. Hence, utilizing the residue from food processing as a low-cost materials for β-glucan production would be economical. The objective of this study is to investigate the water extraction process for β-glucan from *Schizophyllum Commune* processed-product residue. The effects of solid-liquid ratio, extraction temperature and extraction time were evaluated. The experiments indicated that the suitable conditions for *Schizophyllum Commune* water extraction were the solid-liquid ratio of 1:10, the extraction time of 3 h and the extraction temperature of 70°C. The highest β-glucan content extracted from *Schizophyllum Commune* processed-product residue was 9.20%. Furthermore, microwave irradiation pretreatment before conventional extraction was determined to effectively increase beta glucan content to 11.77%.

Key words: Beta glucan, microwave-irradiation pretreatment, *Schizophyllum Commune*, water extraction.

1. Introduction

Beta glucan is a water-soluble polysaccharides of D-glucose monomers linked by β-glycosidic bonds. Their different linkage types depend on both source and isolation method. The properties of beta glucan are abundantly. In cosmeceuticals, beta glucan contains skin regenerative properties, which involve in regeneration of collagen-producing, promotion of anti-aging and anti-wrinkles. The interest in potential of beta glucan in cosmeceuticals application has risen extensively [1].

Mushroom have been reported to be an excellent source of beta glucan. Glucan is mostly found in the cell wall of several mushrooms with good moisturizing potential, which may contribute to skin aging treatment, and has potential for use in skin moisturizing and anti-aging formulations [2]. Schizophyllan is a non-ionic, water-soluble polysaccharides derived from *Schizophyllum Commune*, which consist of a linear chain of β-D-(1,3)-glycopynosyl groups and β-D-(1,6)-glycopynosyl groups [3].

The most appropriate extraction method depends on the sources and the structures of beta glucan. For

extracting water-soluble polysaccharides from fruiting bodies of fungi, extraction in hot water is the most common and convenient method. However, water extraction has drawbacks such as a long extraction time and high temperature. The applicable method such as microwave irradiation are useful in solving these problems along with the increased yields [4].

Microwave-irradiation technique have been reported to reduce extraction time and solvent volume as compared to conventional methods. However, using microwave radiation in extraction process is limited to small-molecule compounds due to thermal degradation [5]. Hence, short period of microwave radiation pretreatment is conducted to avoid the excessive temperature rise which could degrade the heat-sensitive bioactive substances [6].

The objective of this study is to investigate the influences of parameters on beta glucan contents from *Schizophyllum Commune* processed-product residue extraction. The solid-liquid ratio, the extraction time and the extraction temperature were evaluated to achieve the appropriate conditions. Microwave-irradiation pretreatment was investigated comparing to conventional extraction.

2. Materials and Methods

Fig. 1. *Schizophyllum Commune* processed-product residue dried powder.

2.1. Mushroom Powder Preparation

The processed-product residues of *Schizophyllum Commune* were obtained from a local mushroom farm (Chaiyo Farm, Surat Thani, Thailand). The mushroom residues were ground into fine powder by grinder. All samples were pretreated with 95% ethanol for 25 h and filtered by using No.1 Whatman filter paper. The samples were then oven-dried at 70°C for 24 h.

2.2. Water Extraction

Extraction of the dried residue powder was prepared by using distilled water as a solvent. The extraction experiments were performed with various conditions of the solid-liquid ratio (10–20 mL/g), the extraction temperature (50-70°C), and the extraction time (1-3 h). Each extraction was performed in triplicate. The crude extracts were separated by using vacuum filter with No.1 Whatman filter paper, then dried at 70 °C.

2.3. Microwave-Irridiation Pretreatment

Microwave irradiation was operated before the conventional extraction to improve beta glucan yields in the extract. Microwave-irradiation pretreatments were conducted by using Mars5 microwave machine with the different irradiation time and the microwave temperatures. After that, conventional extraction was operated at the extraction time of 3 h and the extraction temperature of 70°C. Beta glucan contents were determined by the method described below comparing to that of conventional extraction without

microwave irradiation.

2.4. Beta Glucan Content

Beta glucan contents determination procedure was operated by using Megazyme test kit Cat. No. K-YBGL. This procedure contains two parts, which are the measurement of α glucan and the measurement of total glucan.

2.4.1. The measurement of α-glucan

100 mg of sample was added into a tube, and the tube was tapped to ensure that the entire sample fell to the bottom of the tube. 2.0 mL of ice-cold 2M KOH was added to that tube, and the tube contents were stirred using a magnetic stirrer in an ice-water bath for 20 min to dissolve the starch/glycogen. Eight milliliters of 1.2M sodium acetate buffer (pH 3.8) was added to the tube and then mixed on a vortex stirrer. A total of 0.2 mL of a mixture of amyloglucosidase (1630 U/mL) plus invertase (500 U/mL) (from Megazyme assay kit) was immediately added, and the tubes were incubated at 40°C for 30 min. 1.0 mL of the solution was centrifuged at 1500 g for 10 min in a centrifuge, and 0.1 mL of the supernatant solutions was analyzed for glucose with the glucose oxidase/peroxidase reagent. As shown in Fig. 2

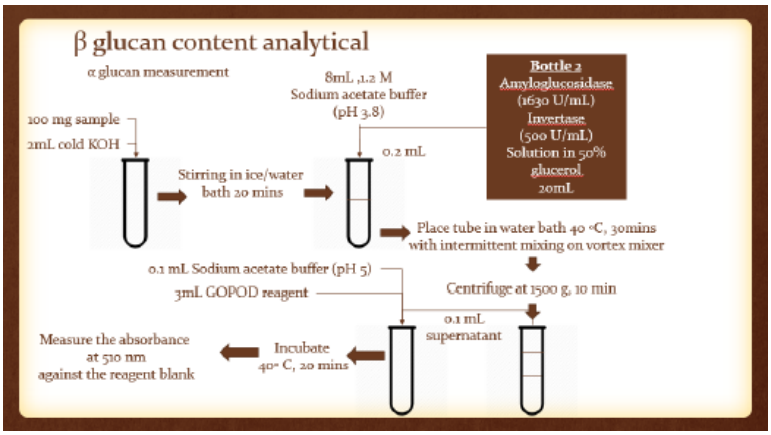
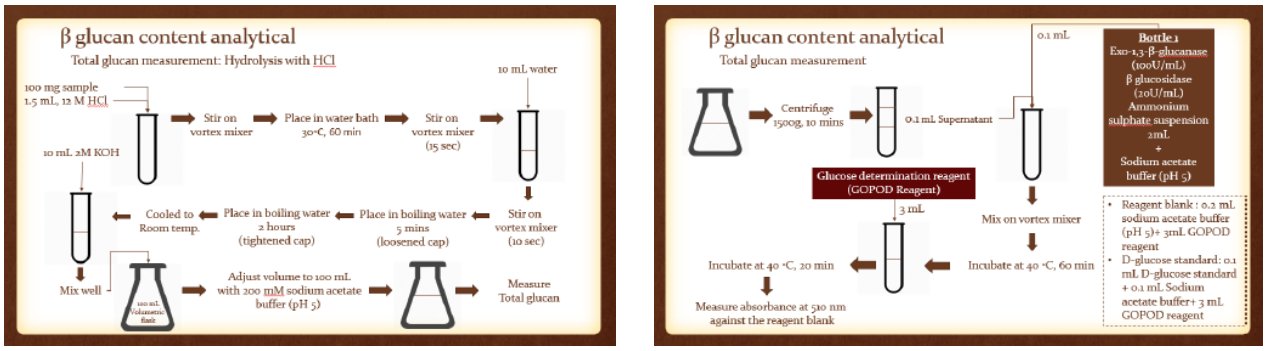


Fig. 2. α-glucan content measurement.

2.4.2. The measurement of total glucan by hydrolysis with hydrochloric acid

100 mg of samples was added to a tube, and the tube was tapped to ensure that the entire sample fell to the bottom of the tube. A total of 1.5 mL of 37% v/v (12M) hydrochloric acid was added to that tube, and the tube was capped and stirred on a vortex mixer. The tube was placed in a water bath at 30°C for 60 min, and the contents were stirred for 15 s on vortex mixer. Ten milliliters of water was added to the tube, the tube was capped, and the contents were vigorously stirred on a vortex mixer for 10 s. The cap was loosened, and the tube was placed in a boiling-water bath. After 5 min, the cap was tightened, and the incubation was continued for 2 h. The tube was cooled to room temperature, and the cap was carefully loosened. Then, ten milliliters of 2M KOH was added, and the contents were mixed well. The contents of the tube were transferred to 100 mL volumetric flasks, and the volume was adjusted to 100 mL with 0.2M sodium-acetate buffer (pH 5). The content of glucose in the solutions was analyzed by incubating an aliquot (0.1 mL) of the supernatant with 3.0 mL of GOPOD reagent at 40°C for 20 min. As shown in Fig. 3. (a) and (b).

Absorbances of α-glucan and total glucan were both measured at 510 nm with UV-spectroscopy. Since glucan content in mushrooms is a composite of α-glucan and β-glucan, β-glucan can be indirectly calculated by this method.



(a) (b)

Fig. 3. Total glucan content measurement.

3. Results and Discussion

3.1. The Effects of Solid-Liquid Ratio

The different solid-liquid ratios were set at 1:10,1:15 and 1:20 to investigate the influences of solid-liquid ratio when the other parameters were set as follow: the extraction time of 1 h and the extraction temperature of 30°C. Polysaccharides were precipitated from the crude extract with 95% ethanol for 24 h. Polysaccharides were separated and dried at 70°C. Beta glucan content was determined.

Table 1 indicates that beta glucan contents in the extraction with the solid-liquid ratios of 1:10 and 1:20 are equal. Therefore, solid-liquid ratio of 1:10 was considered to be the suitable condition for water extraction to maximize beta glucan content, meanwhile, an operation cost was reduced.

Table 1. The Effects of Solid-Liquid Ratio on Beta Glucan Content

Solid-liquid ratio (g/mL)	Polysaccharides (g)	Beta glucan content in polysaccharides (%)	Beta glucan content in extract (%)
1:10	0.67	21.61	0.29
1:15	0.42	20.24	0.17
1:20	0.44	32.94	0.29

3.2. The Effects of Extraction Time and Extraction Temperature

The effects of extraction time and extraction temperature of *Schizophyllum Commune* residue extraction were evaluated. The solid-liquid ratio was set at 1:10 for each experiment. The extraction time and temperature were varied as shown in Table 2 to determine the optimal condition.

Table 2. The Effects of Extraction Time and Temperature on Beta Glucan Content

Extraction temperature (°C)	Beta glucan content (%)		
	1 h	2 h	3 h
50	5.08	6.35	5.04
60	6.57	7.97	5.93
70	8.60	9.17	9.20

According to Table 2, beta glucan contents significantly increased with increasing extraction temperatures. The effects of extraction time and temperature on beta glucan content was also shown in the Fig. 4 below.

Fig. 4. indicates that among three extracion periods of time studied, the duration of 2 h showed the highest beta glucan content at almost every extraction temperature. However, at the extraction temperature of 70°C, 9.20% beta glucan which is the highest yeild was obtained at the extraction time of 3 h. The

diffusivity of solution is one of the condition that has an effect on extraction efficiency. It could be concluded that, the optimal conditions of *Schizophyllum Commune* processed-product residue extraction were the extraction time of 3 h and extraction temperature of 70°C.

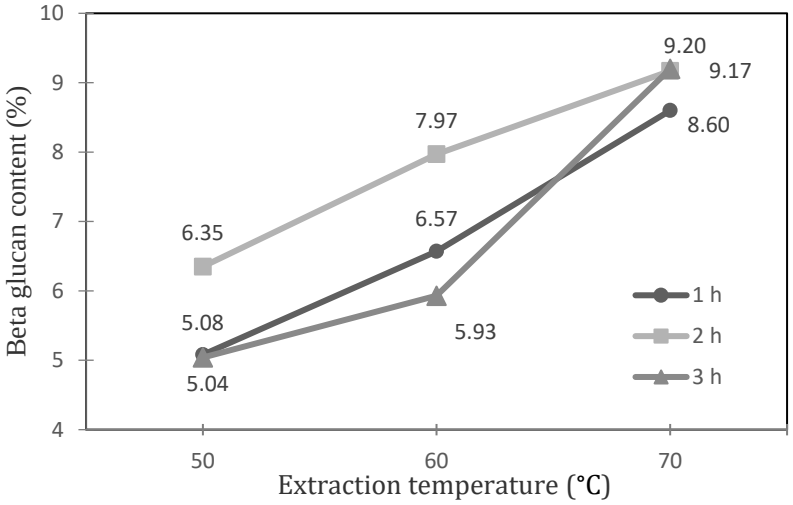


Fig. 4. The effects of extraction time and temperature on beta glucan content.

3.3. The Effects of Microwave-Irradiation Pretreatment

To determine the effects of microwave-irradiation pretreatment on increasing beta glucan content from *Schizophyllum Commune* extracts, microwave pretreatment was operated using MARS5, the power was set at 600 Watts, and 4 XP-1500 vessels were used. After microwave pretreatment, the samples were extracted with the conventional extraction at the extraction time of 3 h and the extraction temperature of 70°C.

Table 3 indicates that beta glucan contents increased when microwave pretreatment involved with the conventional extraction. At the same microwave-irradiation time, beta glucan content resulted from higher microwave temperature is significantly higher than that of low microwave-irradiation temperature.

Table 3. The Effects of Microwave-İrradiation Pretreatment on Beta Glucan Content

	MW irradiation time (min)	Microwave temperature (°C)	Beta glucan content (%)
Without microwave-irradiation pretreatment	-	-	9.20
With microwave-irradiation pretreatment	3	65	9.62
	3	85	11.77

Extraction with microwave-irradiation pretreatment at the temperature of 65°C and 85°C, beta glucan contents increased by 4.5% and 27.93%, respectively. It is because during microwave-irradiation pretreatment, the energy was efficiently absorbed. Therefore, the thermal and mechanical stress severely changed the physical properties of the cell walls which contains beta glucan. The cell wall structure was collapsed and improved the capillary-porous structure of the tissues, facilitating faster diffusion out of the substances [7].

4. Conclusion

The most common and convenient method to extract beta glucan from *Schizophyllum Commune* processed-product residue was water extraction. Beta glucan content increased with increasing the extraction time and the extraction temperature. The results showed that the influential parameters were

the solid-liquid ratio of 1:10, the extraction time of 3 h and the extraction temperature of 70°C. Microwave-irradiation pretreatment was a useful method to improve *Schizophyllum Commune* processed-product residue extraction due to the fact that beta glucan content in the extract increased by 27.93% compared to that of conventional extraction.

Conflict of Interest

The authors declare no conflict of interest.

Author Contribution

SN conducted the research, analyzed the data and carried out the drafted paper. CS and DC conceived the paper, participated in its design coordination and co-wrote the paper. All authors read and approved the final version.

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References

[1] Du, B., Bian, Z. X., & Xu, B. J. (2014). Skin health promotion effects of natural beta-glucan derived from cereals and microorganisms: A review. *Phytother. Res., 28*, 159-166.

[2] Wu, Y. Z., Choi, M. H., Li, J. S., Yang, H. T., & Shin, H. J. (2016). Mushroom cosmetics: The present and future. *Cosmetics, 3(22)*, 1-13.

[3] Kumari, M., Survase, S. A., & Singhal, R. S. (2008). Production of schizophyllan using schizophyllum commune NRCM. *International Journal of Biological Macromolecules, 2016(96)*, 100-105.

[4] Zhu, F. M., Du, B., & Xu, B. J. (2016). A critical review on production and industrial applications of beta glucans. *Food Hydrocolloids, 52*, 275-288.

[5] Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med. Aromat. Plants, 4(196)*, 1-6.

[6] Song, C. F., Wang, S. G., Yang, J., Cui, Z. W., & Gu, Y. H. (2015). Optimization of vacuum-microwave radiation pretreatment on extraction of ganoderma polysaccharides. *Mathematical Problems in Engineering, 2015*, 792832.

[7] Flórez, N., Conde, E., & Domínguez, H. (2015). Microwave assisted water extraction of plant compounds. *J. Chem. Technol. Biotechnol., 2015(90)*, 590-607.

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