Effect of strains and extraction methods on β-glucan production, antioxidant properties, and FTIR Spectra from Mushroom fruiting bodies of Schizophyllum commune Fr. in Thailand

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Abstract: Schizophyllum commune Fr, a native mushroom of Thailand, has a high nutrition value and it classified as a mushroom with medicinal properties, which can neutralize the growth of many cancer cells. Thus, the aim of this research was studied effect of S. commune strain and the extraction method on the quantity and properties of β-glucan. The five S. commune Fr strains consisted of Chanthaburi, 85-022, 85-023, 85-031, and 85-043, which used in this research. The β-glucan extraction method compared two different extraction: hot water (M1) and hot alkali extraction (M2), with control (native-MR). It found that Chanthaburi strain has the highest in β-glucan content 49.20 ± 0.35 % (w/w), and high potential antioxidant activity (79.14 ± 0.77 DPPH % and 50.92 ± 0.48 ABTS %) (p < 0.05). The extraction methods had no effect on the yiel of β-glucan, except antioxidant properties and chemical structure of extract substance. The extract substance from M2 has significant the highest potential antioxidant activity (80.22 ± 0.51 drink mushroom juice in can by using 1-day-old MR and adjust pH more than 7 can increase antioxidant properties of product.

Keywords: Schizophyllum commune Fr., Mushroom fruiting bodies, Schizophyllum commune Fr polysaccharide, β-glucan, β-glucan extraction method, FTIR Spectra

1. Introduction

Schizophyllum commune Fr., an edible mushroom, is native mushroom that grows on the logs in Thailand forest. It has common name “Krang”. Thai people, especially in the South, prefer to eat Krang mushroom, which found in sour soup or coconut milk curry, because of agood unique taste and high nutritional value [1]. S. commune Fr contains Schizophyllan (β-1, 3-glucan), anticancer substance [8], and antioxidant compounds that have anti-aging properties [15].

The chemical composition of substrate influenced β-glucan content, phenolic content, and antioxidant activity, which produced from S. commune [1]. S. commune, grown different region, has different chemical composition. It was reported that S. commune (MCCT 38) in Tripura, India consisted of 15.55 % crude protein, 42% total carbohydrates and 30.0 % crude fiber [2] which differ from S. commune (MCCT 38) in Nagaland, India consisted of 22.50 % crude protein, 32.43% total carbohydrates and 6.50% crude fiber [6]. Nevertheless, there is currently no information about the effect of S. commune Fr. Strains on the β-glucan production and effect of extraction method on antioxidant properties of β-glucan from S. commune Fr. Therefore, the aim of this research had two objectives. The first objective focus on the selection the strains of S. commune Fr for β-glucan production. The second objective is investigating the effect of extraction method on the amount of β-glucan production and its antioxidant properties. The results will be beneficial in many
industries, such as the food, pharmaceutical or cosmetics industries, especially local food production from natural mushroom. Because in this research, it is an adaptation of the extraction method of β-glucan by using filtering instead of centrifugation, which is a low-cost technology that can be produced by the community.

2. Materials and Methods

2.1 Materials

Five Pure mycelial culture strain of *S. commune* Fr were obtained from Chanthaburi mushroom farm (1 strain), and Department of Agriculture (4 strains), Thailand. The culture collection number from Chanthaburi mushroom farm was Chanthaburi, and Department of Agriculture was 85-022, 85-023, 85-031, and 85-043. The mycelium grown on sterile culture food bag at 30 °C for 7 days or until the mushroom fibers start to gather to grow into a mushroom. The sample used in the experiment was 1-day-old mushroom fruiting body, which came from the preliminary experiment; it found that the mushroom fruiting body had glucan content than in the mushroom mycelium and at the 1 day mushroom fruiting body the most. The mushroom samples were dried in hot air oven at 70 ± 5 °C until 2.50 ± 0.02 % moisture content. The dry samples were ground, sifted through an 80 mesh, and stored at room temperature in aluminum bag for further analysis.

2.2 Methods

2.2.1 Glucan content determination

The Glucan contents was determined by using a β-Glucan Assay Kit (Megazyme International, Wicklow, Ireland). The principle of Mushroom and Yeast β-Glucan Assay Kit based on the determination of total glucan, which consists of α-Glucan and β-Glucan linkages. The bond of (1→3,1→6) -β - D-Glucan, (1→3) -β-Glucan and α-Glucan are dissolved and cut by concentrate hydrochloric acid at 100 °C for 2 h, and then the solution was incubated with exo-1, 3- β-glucanase and β-glucosidase in order to get complete D-glucose for analysis total glucan content. For α-Glucan, it was digested to be glucose with amyloglucosidase plus invertase, using GOPOD reagent to measure glucose content.

(1) Total glucan content

For total glucan content,10 mg of native-MR powder placed in Eppendorf tube then added 0.15 ml of 37% hydrochloric acid. The solution was mixed and incubated at 30 °C for 45 min (vortexed every 15 min). Then, 1 ml of distilled water was added, mixed and incubated at 100 °C for 2 h before added with 0.5 ml of 4 M KOH. The 200 µl solution was taken, adjusted volume to 1 ml with sodium acetate buffer pH 5 (800 µl) and mixed. After that, the mixtures were centrifuged at 13,000 x g for 5 min. Samples (20 µl) were taken to each well (in duplicates) before added with 10 µl of a mixture of exo-1, 3-β-glucanase plus β-glucosidase and then incubated at 37 °C for 90 min. Finally, 200 µl of glucose oxidase / peroxidase added and incubated at 37 °C for 30 min. The absorbance measured at 510 nm with spectrophotometer. The amount of total glucan content calculated from equation (1.1).

\[
\text{Total Glucan (}\%\text{w/w)} = \Delta E \times F/W \times 90
\]

When \(\Delta E\) is the absorbance

\(F\) is the factor to convert the absorbance to µg of D-glucose
W is the weight of sample (g)

(1) α-glucan and β-Glucan content

For α-glucan content, 100 mg of native-MR powder were placed in test tubes. Then 2 M KOH (2 ml) added and the pellets stirred with magnetic stirrer in ice bath for 20 min, after that 8 ml of 1.2 M sodium acetate buffer (pH 3.8) added to the mixture. Then, 1 ml of sample taken to an Eppendorf tube, added with 20 µl of Amyloglucosidase plus invertase and incubated at 40 °C for 30 min. Next, the mixture was centrifuged at 13,000 x g for 5 min. Supernatant (20 µl) were taken to microtiter plate. Glucose oxidase / peroxidase (200 µl) added to each well and incubated at 37 °C for 30 min. The absorbance measured at 510 nm with spectrophotometer. The amount of α-glucan content calculated from equation (1.2) or (1.3) depend on α-glucan content. For β-glucan content was calculated from total glucan minus α-glucan, showed in equation (1.4).

\[
\begin{align*}
\alpha-\text{glucan} > 10\% \ (w/w); & \quad \alpha-\text{Glucan} \ (%w/w) = \Delta E \times F/W \times 90 \\
\alpha-\text{glucan} < 10\% \ (w/w); & \quad \alpha-\text{Glucan} \ (%w/w) = \Delta E \times F/W \times 9.27 \\
\text{When} & \quad \Delta E \text{ is the absorbance} \\
F & \text{ is the factor to convert the absorbance to } \mu g \text{ of D-glucose} \\
W & \text{ is the weight of sample (g)}
\end{align*}
\]

(1.2) (1.3)

\[
\beta-\text{Glucan}(%w/w) = \text{Total Glucan} (%w/w) - [\alpha-\text{glucan}] \ (%w/w)
\]

(1.4)

2.2.2 Total phenolic compounds determination

The total phenolic content was determined, which modified method from Iqbal et al., 2005 [4]. Briefly, 3 g of native-MR powder mixed with 30 ml of 80% ethanol (w/v). The mixture shakes at 150 rpm for 24 h at room temperature. Then, the supernatant filtered through Whatman filter paper No. 1. The reaction mixture contained 50 µl of clear soluble, 200 µl of freshly prepared diluted Folin - Ciocalteu reagent from Merck and 0.5 ml of 7.5% sodium carbonate. The final mixture diluted to 7 ml with deionized water. The mixtures kept in dark at room temperature for 2 h to complete the reaction then the absorbance at 760 nm. The gallic acid used as a standard. The total phenolic content of sample calculated as gallic acid equivalents per g dry weight of extraction. The reaction conducted in triplicate and results averaged.

2.2.3 ABTH scavenging assay

The ABTH radical scavenging activities was modified the assay method of Iqbal et al., 2005 [4]. To prepare ABTS radical cation, 2.45 mM of potassium persulphate (Merck, Germany) aqueous solution added with 5 mM ABTS (Sigma-Aldrich, Germany) aqueous solution in equal quantities. The mixtures kept in dark at room temperature for 24 h to complete the reaction. Then, 1 ml of the solution diluted with 60 ml ethanol and used in ABTS test. 0.1 ml of the extraction solution was added to 2 ml of ABTS+ solution and kept in the dark at room temperature for 10 min to complete the reaction. The absorbance measured at 734 nm. The scavenging effect of ABTS free radicals calculated as follows:

\[
\text{Inhibition} \ (%) = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100
\]

(1.5)

2.2.4 DPPH scavenging assay

The DPPH radical scavenging activities was the ability to reduce the free radical 2, 2'-diphenyl-1-picrylhydrazyl radical (DPPH, Sigma). The DPPH radical scavenging activity was modified the assay method from Iqbal et al., 2005 [4]. Briefly, 2 ml of the extraction solution in No. 2.2.2 mixed with 2 ml of 0.2 mM DPPH in ethanol and kept in
dark at room temperature for 30 min to complete the reaction. The absorbance measured at 517 nm. The scavenging effect of DPPH free radicals calculated as follows:

Inhibition (%) = \[(A_{control} - A_{sample}) / A_{control}\] x 100

2.2.5 Effect of extraction method

To study the effect of extraction method performed by modified from Suwanno et al., 2005 [19] and Mizuno and Nishitani (2013) [10]. Accurate weight 20 g of the native-MR dried powder dissolved in 200 ml of deionized water and then the mixture solution heated at 121 °C for 15 min. After that, allow the sample to cool down to 45 °C and extract the clear part from the residue by filtering with Whatman paper No 1. Then the clear part solution was divided into 2 parts for comparison the effect of extraction method; Method 1: the part 1 was added with absolute ethanol into the ratio 3:1 ethanol/clear part, and left overnight at (-20)°C, after that it was extract the clear part from the residue by filtering with Whatman paper No 1. The residue washed with 80% of ethanol for 3 times and dried in hot air oven at 80 °C for 3 h and ground to powder (M1 sample) for analysis. For methods 2: the part 2 was added with 100 ml of 1 M NaOH, and then the mixture solution heated at 100 °C for 24 h, after that it was extract the clear part from the residue by filtering with Whatman paper No 1. The residue washed with 80% of ethanol for 3 times. The precipitate was dried in hot air oven at 80 °C for 3 h and ground to powder (M2 sample) for analysis. A schematic diagram of the extraction process of polysaccharides by different extraction methods shown in Figure 1.1. The dried residues from method 1 and method 2 were analyzed for glucan content by using the method in No. 2.2.1, and antioxidant properties by using the methods in No. 2.2.2, No. 2.2.3, and No. 2.2.4 to compared with control (native-MR dry power).
2.2.6 Chemical structure of extraction substrate

The FT-IR absorption spectra of extraction substrate samples, from M1 and M2 method, were measured using Frontier Transform Infrared Spectrometer - FT-IR (PerkinElmer, model NIRA, Massachusetts, USA). The method used was an FT-IR in KBr solid. The 100-200 mg of KBr pulverized in an agate mortar and pestle, and mixed with 2 mg of the sample. The mixtures compressed with a pressure of 10 tons to tablet form for measuring with FT-IR. Each sample spectrum was collect by using reflectance mode, in a scanning range of 400-4000 cm⁻¹, and accumulation 60 scans. The FT-NIR spectra of each type were recorded with 3 replicates.

2.2.7 Statistical analysis

The data collected from triplicates. Analysis was perform by statistical package SPSS 17 for windows, p < 0.05 (two-tailed) was consider as statistically significant. All of data analyzed with Analysis of Variance (ANOVA) and multiple comparison F-test.

3. Results

3.1 Production of β-glucan

β-glucan is a substance that had anti-tumor, antimicrobial [9] and reduction of blood cholesterol and glucose levels [13]. Therefore, in this research was to select 1-day-old S. commune Fr mushroom fruiting body (native-MR) dried powder from 5 strains; Chanthaburi, 85-022, 85-023, 85-031, and 85-043, which found in Thailand, for β-glucan production. The result shown in Table 1, the native-MR dried powder from Chanthaburi strain had significantly the highest in total-glucan content 49.76 ± 0.35% (w/w) and β-glucan content 49.20 ± 0.35 % (w/w) (p < 0.05). The strain of S. commune Fr affects the amount of total-glucan and β-glucan similar to that of Hypsizygus marmoreus [11] and S. commune Fr mushroom from local market in Chanthaburi province, Thailand [12].

Table 1. Mean of glucan content of native-MR dried powder from S. commune Fr, which different strains.

| Samples Strain | Total-glucan Content (% w/w) | α-glucan Content (% w/w) | β-glucan Content (% w/w) |
|----------------|-------------------------------|---------------------------|--------------------------|
| Chanthaburi    | 49.76±0.35 ±                  | 0.56±0.01                 | 49.20±0.35               |
| 85-022         | 48.84±0.24                   | 0.41±0.05                 | 48.43±0.23               |
| 85-023         | 48.63±0.18                   | 0.56±0.01                 | 48.07±0.20               |
| 85-031         | 48.51±0.12                   | 0.57±0.17                 | 47.94±0.09               |
| 85-043         | 48.67±0.24                   | 0.54±0.07                 | 48.13±0.24               |

The values are the mean of three replications ± standard deviation.

Different letters in each column represent significant differences between treatments (p < 0.05). The result of total phenolic compounds, DPPH, and ABTH shown in Table2. The Chanthaburi, 85-022, 85-023, 85-031, and 85-043 were analyzed total phenolic compounds content by Folin-Ciocalteu Colorimetric method, DPPH by DPPH scavenging assay, and ABTH by ABTH scavenging assay. The result shown that the strain of S. commune Fr had no significantly affect total phenolic compounds content (p ≥ 0.05). The S. commune Fr from Chanthaburi strains had significantly the highest DPPH (78.98 ± 0.35%) and ABTS (50.92 ± 0.48 %) than that of the other
strains (p < 0.05). From the result of this experiment, it indicated that strain of *S. commune* Fr affected the β-glucan production and its chemical composition. The *S. commune* Fr. from Chanthaburi strains was the most suitable for use as a raw material for the β-glucan production. Therefore, Chanthaburi was select to study the effect of extraction method on the amount and quality of β-glucan.

Table 2. Total phenolic content and antioxidant properties of *S. commune* Fr from 1-day-old MFB dried powder, which different strains.

| Samples Strain | DPPH (%)  | ABTS (%)  | Total Phenolic content (mg gallic acid/ml sample)ns |
|----------------|-----------|-----------|--------------------------------------------------|
| Chanthaburi    | 78.98±0.35a | 50.92±0.48a | 2.62±0.18                                             |
| 85-022         | 78.06±0.36b | 49.92±0.58c | 2.60±0.09                                             |
| 85-023         | 78.35±0.71b | 49.79±0.47c | 2.60±0.17                                             |
| 85-031         | 78.29±0.49b | 50.43±0.53b | 2.64±0.16                                             |
| 85-043         | 78.12±0.45b | 49.62±0.43c | 2.54±0.21                                             |

The values are the mean of three replications ± standard deviation.
Different letters in each column represent significant differences between treatments (p < 0.05).

ns mean no significant difference (p > 0.05).

3.2 Effect of β-glucan extraction method.

In this research, the researcher would like to study the effect of the extraction method on the obtained glucan content and antioxidant properties, that will lead to the development of community canned mushroom juice products from *S. commune* Fr, Thailand. The researcher chose to use the filter paper method instead of sedimentation by centrifugation to extract the glucan. β-glucan content (% db) of M1 extraction substrate, M2 extraction substrate, and dried native-MR (control) was analyzed using the Mushroom and yeast beta glucan assay kit from Megazyme International, shown in table 3. The β-glucan content in M1 extraction substrate, M2 extraction substrate, and dried native-MR (control) were 48.90%, 49.23%, and 49.20 %, respectively, which were high compared with the β-glucan content in the edible mushroom (4.71 to 46.20% db) found by Lee and Kim (2005). [7]

Most of glucan content in both dried extraction substrates and native-MR dried powder were β-glucan (48.90 – 49.23 g/100 g of native-MR dried powder), which consistent with the research of Klaus et al., 2011, [5] who studied antioxidative activities and chemical characterization of polysaccharides extracted from the basidiomycete *S. commune*, which grow in the mountain Avala, Republic of Serbia. The extraction methods had no significantly on the amount of total-glucan, α-glucan, and β-glucan of extraction substrates (p >0.05), but had effect on the antioxidant properties and chemical structure of extraction substrate, showed in Table 4, which found that antioxidants efficiency is higher with heat or alkaline extraction. The M2 extraction substrate had significantly the highest DPPH (80.22±0.51 %) and ABTS (58.16 ± 0.53%) (p < 0.05).

FT-IR spectroscopy was a technique that was use in the structural analysis of polysaccharides. The FT-IR spectra shown the molecular vibrations of covalent bonds at the infrared region range (4000-400 cm⁻¹). Fig 2 show the FT-IR absorption spectra of M1 and M2 dried extraction substrate compared with the dried native-MR (control). The infrared absorption characteristics of polysaccharides show that the both extraction methods affected the chemical structure of the extracted substate when compared with the control sample especially at frequency range 1567-1570.
The result of FT-IR spectroscopy showed that heat and alkalinity had an effect on the structure of *S. commune* Fr β-glucan, which was consistent with the research of Gieroba *et al.* [3] that found that the 1,3-β-D-glucan polymer gelled at 80 °C has a distinctly different structure than the matrix gelled at 90 °C. The important FT-IR absorption spectra region of polysaccharides consisted of the sugar region (1200-950 cm⁻¹) and anomeric region (950-750 cm⁻¹) [16], which heat used for extraction or the pH has an effect on the structure of the extracted β-glucan, thus effect on its antioxidant properties.

**Table 3.** Mean of glucan content of *S. commune* Fr from 1-day-old MFB dried powder, which different extraction methods.

| Extraction method | Total-glucan Contentns (%) w/w | α-glucan Contentns (%) w/w | β-glucan Contentns (%) w/w |
|-------------------|--------------------------------|-----------------------------|-----------------------------|
| Control (native-MR) | 49.76±0.35                   | 0.56±0.01                   | 49.20±0.35                   |
| M1                | 49.47±0.18                    | 0.57±0.04                   | 48.90±0.21                   |
| M2                | 49.76±0.12                    | 0.53±0.01                   | 49.23±0.11                   |

The values are the mean of three replications ± standard deviation.

**ns** mean no significant difference (p > 0.05).

**Table 4.** Total phenolic content and antioxidant properties of *S. commune* Fr from 1-day-old MFB dried powder, which different extraction methods.

| Extraction method | DPPH (%) | ABTS (%) | Total Phenolic content (mg gallic acid/ml sample)ns |
|-------------------|----------|----------|-----------------------------------------------|
| Control           | 78.98±0.35 b | 50.92±0.48 c | 2.62±0.18                                      |
| M1 method         | 80.15±0.51 a | 57.90±0.48 b | 2.90±0.15                                      |
| M2 method         | 80.22±0.51 a | 58.16±0.53 a | 2.87±0.06                                      |

The values are the mean of three replications ± standard deviation.

Different letters in each column represent significant differences between treatments (p < 0.05).

mean no significant difference (p > 0.05).
In this research, it found that the strains of *S. commune* Fr in Thailand had effect on the amount of extracted β-glucan. The 1-day-old MR *S. commune* Fr from Chanthaburi was the good source for β-glucan production. For the effect of extraction method, it found that temperature and pH in extraction have effect on the structure and antioxidant properties of β-glucan. The extraction 1-day-old MR *S. commune* Fr from Chanthaburi with 1 M of NaOH at 100 0°C for 24 h yielded of β-glucan with more antioxidant properties than β-glucan from hot water extraction at 121 °C for 15 min. The results of this research led to the development of ready-to-drink mushroom juice in can by using 1-day-old MR *S. commune* Fr and adjust to pH more than 7 can increase antioxidant properties of product.

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