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# 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 nutritional value and is classified as a mushroom with medicinal properties that can neutralize the growth of many cancer cells. This research aimed to study the effect of *S. commune* strains and the extraction methods on the quantity and properties of β-glucan. The five *S. commune* Fr. strains used in this research consisted of Chanthaburi, 85-022, 85-023, 85-031, and 85-043. There were two different β-glucan extraction methods employed: hot water (M1) and hot alkali extraction (M2), which were compared with the control (native-MR). The results indicated that the Chanthaburi strain has the highest 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 did not affect the yield of β-glucan, except the antioxidant properties and chemical structure of the extract substance. The extract substance from M2 has significantly the highest potential antioxidant activity (80.22±0.51). A mushroom juice drink in cans was developed using 1-day-old MR and adjusted pH of more than 7, which can increase the antioxidant properties of the product.

## 1. Introduction

*Schizophyllum commune* Fr., an edible mushroom, is a native mushroom that grows on logs in Thailand's forests. Its common local name is "Krang", and Thai people, especially in the South, prefer to eat Krang mushrooms, which are found in sour soup or coconut milk curry, because of the good unique taste and high nutritional value (Basso *et al*., 2020). *Schizophyllum commune* Fr. contains Schizophyllan (β-1, 3-glucan), an anticancer substance (Lemieszek and Rzeski, 2012), and antioxidant compounds that have anti-ageing properties (Pirshahid *et al*., 2011). The chemical composition of substrate produced from *S. commune* influenced β-glucan content, phenolic content, and antioxidant activity (Klaus *et al*., 2011; Basso *et al*., 2020). *Schizophyllum commune* is grown in different regions and has various chemical compositions. It was reported that *S. commune* (MCCT 38) in Tripura, India consisted of 15.55% crude protein, 42% total carbohydrates and 30.0% crude fibre (Debnath *et al.*, 2017), which differs from the *S. commune* (MCCT 38) in Nagaland, India that consisted of 22.50% crude protein, 32.43% total carbohydrates and 6.50% crude fibre (Kumar *et al*., 2013). Nevertheless, there is currently no information about the effect of *S. commune* Fr. strains on the β-glucan production and the effect of the extraction method on the antioxidant properties of β-glucan from *S. commune* Fr. The aim of this research had two objectives, the first of which focuses on the selection of the strains of *S. commune* Fr for β-glucan production. The second objective is the investigation of the effect of extraction methods 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 mushrooms. This research 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 implemented by the community.

## 2. Materials and methods

### *2.1 Materials*

Five pure mycelial culture strains of *S. commune* Fr were obtained from a Chanthaburi mushroom farm (1

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

*2.2 Glucan content determination*

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

*2.2.1 Total glucan content*

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

$$\text{Total Glucan (\%w/w)} = \Delta E \times F/W \times 90 \quad (1)$$

Where ΔE is the absorbance, F is the factor to convert the absorbance to µg of D-glucose, and W is the weight of the sample (g).

*2.2.2 α-glucan and β-glucan content*

For the α-glucan content, 100 mg of native-MR powder was placed in test tubes. Then, 2 M KOH (2 mL) was added and the pellets were stirred with a magnetic stirrer in the ice bath for 20 mins, and after, 8 mL of 1.2 M sodium acetate buffer (pH 3.8) was added to the mixture. Then, 1 mL of the sample was placed into an Eppendorf tube, and 20 µL of Amyloglucosidase plus invertase was added, followed by incubation at 40°C for 30 mins. Next, the mixture was centrifuged at 13,000×*g* for 5 mins. Supernatants (20 µL) were placed into the microtiter plate. Glucose oxidase/peroxidase (200 µL) was added to each well and incubated at 37°C for 30 mins. The absorbance was measured at 510 nm with a spectrophotometer. The amount of α-glucan content calculated from Equations (2) or (3) depends on the α-glucan content. The β-glucan content was calculated from total glucan minus α -glucan, as shown in Equation (4).

$$\text{α-glucan} > 10\%\ \text{(w/w)};\ \text{α-Glucan (\%w/w)} = \Delta E \times F/W \times 90 \quad (2)$$

$$\text{α-glucan} < 10\%\ \text{(w/w)};\ \text{α-Glucan (\%w/w)} = \Delta E \times F/W \times 9.27 \quad (3)$$

Where ΔE is the absorbance, F is the factor to convert the absorbance to µg of D-glucose, and W is the weight of sample (g).

$$\text{β-Glucan (\%w/w)} = \text{Total Glucan (\%w/w)} - [\text{α-glucan}]\ \text{(\%w/w)} \quad (4)$$

*2.2 Total phenolic compounds determination*

The total phenolic content was determined with a modified method from Iqbal *et al.* (2005). Briefly, 3 g of native-MR powder was mixed with 30 mL of 80% ethanol (w/v). The mixture was shaken at 150 rpm for 24 hrs at room temperature. Then, the supernatant was 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 was diluted to 7 mL with deionized water. The mixtures were kept in dark at room temperature for 2 hrs to complete the reaction then the absorbance was analysed at 760 nm. Gallic acid was used as a standard. The total phenolic content of the sample was calculated as gallic acid equivalents per g dry weight of extraction.

 

The reaction was conducted in triplicate and the results were averaged.

*2.3 ABTH scavenging assay*

The ABTH radical scavenging activities were modified from the assay method of Iqbal *et al.* (2005). To prepare the ABTS radical cation, 2.45 mM of potassium persulfate (Merck, Germany) aqueous solution was added with 5 mM ABTS (Sigma-Aldrich, Germany) aqueous solution in equal quantities. The mixtures were kept in dark at room temperature for 24 hrs to complete the reaction. Then, 1 mL of the solution was diluted with 60 mL ethanol and used in the ABTS test. Next, 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 was measured at 734 nm. The scavenging effect of the ABTS free radicals was calculated as follow:

$$\text{Inhibition (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (5)$$

*2.5 DPPH scavenging assay*

The DPPH radical scavenging activities were the ability to reduce the free radical 2, 2 -diphenyl-1-picrylhydrazyl radical (DPPH, Sigma). The DPPH radical scavenging activity was modified from the assay method of Iqbal *et al.* (2005). Briefly, 2 mL of the extraction solution in No. 2.2.2 was 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 was measured at 517 nm. The scavenging effect of DPPH free radicals was calculated using Equation (5).

*2.6 Effect of extraction method*

The 1-day-old mushroom fruiting bodies of the *S. commune* Fr. strain, which has high glucan content, was selected for this study of the effect of the extraction methods. An accurate weight of 20 g of the native-MR dried powder was dissolved in 200 mL of deionized water, and then, the mixture solution was heated at 121° C for 15 mins. After that, the sample was allowed to cool down to 45°C and the clear part was extracted from the residue by filtering with Whatman paper No 1. The clear part solution was divided into two parts for comparison of the effect of the extraction method. For Method 1, part 1 was added with absolute ethanol into the ratio 3:1 ethanol/clear part and left overnight at -20°C, the clear part was extracted from the residue by filtering with Whatman paper No 1. The residue was washed with 80% ethanol three times and dried in a hot air oven at 80°C for 3 hrs and grounded to powder (M1 sample) for analysis. For Method 2, part 2 was added with 100 mL of 1 M NaOH, and the mixture solution was heated at 100° C for 24 hrs, the clear part was extracted from the residue by filtering with Whatman paper No 1. The residue was washed with 80% ethanol three times. The precipitate was dried in a hot air oven at 80°C for 3 hrs and grounded to powder (M2 sample) for analysis. A schematic diagram of the extraction process of polysaccharides by various extraction methods is shown in Figure 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 in order to compare them with the control (native-MR dry power).

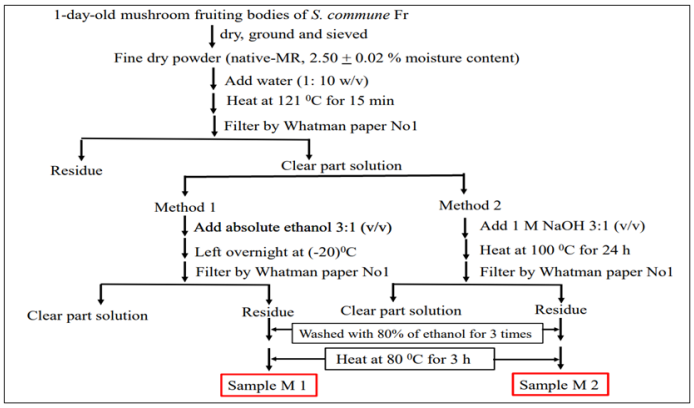


Figure 1. A schematic diagram of the extraction process of polysaccharides by different extraction methods from *Schizophyllum commune* Fr

*2.7 Chemical structure of extraction substrate*

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

*2.8 Statistical analysis*

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

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## 3. Results

### *3.1 Production of β-glucan*

β-glucan is a substance that has antitumor and antimicrobial properties (Manzi and Pizzoferrato, 2000), and a reduction of blood cholesterol and glucose levels (Nicolosi *et al*., 1999). Therefore, in this research, 1-day-old *S. commune* Fr. mushroom fruiting bodies (native-MR) dried powder from five strains was selected: Chanthaburi, 85-022, 85-023, 85-031, and 85-043, which are found in Thailand, for β-glucan production. The results in Table 1 show that the native-MR dried powder from the Chanthaburi strain was significantly the highest in total-glucan content at 49.76±0.35% (w/w) and β-glucan content at 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* (Mongkontanawat and Wongekalak, 2015) and *S. commune* Fr. mushrooms from local markets in Chanthaburi province, Thailand. (Mongkontanawat and Phuangborisut, 2015).

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$^{a}$ | 0.56±0.01$^{a}$ | 49.20±0.35$^{a}$ |
| 85-022 | 48.84±0.24$^{b}$ | 0.41±0.05$^{b}$ | 48.43±0.23$^{b}$ |
| 85-023 | 48.63±0.18$^{b}$ | 0.56±0.01$^{a}$ | 48.07±0.20$^{b}$ |
| 85-031 | 48.51±0.12$^{b}$ | 0.57±0.17$^{a}$ | 47.94±0.09$^{b}$ |
| 85-043 | 48.67±0.24$^{b}$ | 0.54±0.07$^{a}$ | 48.13±0.24$^{b}$ |

Values are presented as mean±SD of three replications. Values with different superscript within the same column are significantly different ($p < 0.05$).

The result of total phenolic compounds, DPPH, and ABTH are shown in Table 2. The Chanthaburi, 85-022, 85-023, 85-031, and 85-043 strains were analyzed for total phenolic compounds content by the Folin-Ciocalteu Colorimetric method, DPPH by the DPPH scavenging assay, and ABTH by the ABTH scavenging assay. The results showed that the strain of *S. commune* Fr. had no significant effect on total phenolic compounds content ($p > 0.05$). The *S. commune* Fr. from the Chanthaburi strain had significantly the highest DPPH (78.98 ± 0.35%) and ABTS (50.92 ± 0.48%) compared to that of the other strains ($p < 0.05$). From the results of this experiment, it was indicated that the strain of *S. commune* Fr. affected the β-glucan production and its chemical composition. The *S. commune* Fr. from the Chanthaburi strain was the most suitable for use as a raw material for β-glucan production. Therefore, Chanthaburi strain was selected for the study on the effect of extraction methods 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 |
|---|---|---|---|
| Chanthaburi | 78.98±0.35$^{a}$ | 50.92±0.48$^{a}$ | 2.62±0.18 |
| 85-022 | 78.06±0.36$^{b}$ | 49.92±0.58$^{c}$ | 2.60±0.09 |
| 85-023 | 78.35±0.71$^{b}$ | 49.79±0.47$^{c}$ | 2.60±0.17 |
| 85-031 | 78.29±0.49$^{b}$ | 50.43±0.53$^{b}$ | 2.64±0.16 |
| 85-043 | 78.12±0.45$^{b}$ | 49.62±0.43$^{c}$ | 2.54±0.21 |

$^{ns}$Not significant.

Values are presented as mean±SD of three replications. Values with different superscript within the same column are significantly different ($p < 0.05$).

### *3.2 Effect of β-glucan extraction method*

In this research, the objective was to study the effect of the extraction methods on the obtained glucan content and antioxidant properties, which will lead to the development of community canned mushroom juice products from *S. commune* Fr. in Thailand. The filter paper method was chosen instead of sedimentation by centrifugation to extract the glucan. β-glucan content (%, db) of the M1 extraction substrate, the M2 extraction substrate, and the dried native-MR (control) was analyzed using the Beta Glucan (Yeast and Mushroom) Assay Kit from Megazyme International, as shown in Table 3. The β-glucan content in the M1 extraction substrate, M2 extraction substrate, and dried native-MR (control) were 48.90%, 49.23%, and 49.20%, respectively, which are high compared with the β-glucan content in the edible mushrooms (4.71 to 46.20% db) as found by Lee and Kim (2005). Most of the 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 is consistent with the research of Klaus et al. (2011), who studied antioxidative activities and chemical characterization of polysaccharides extracted from the basidiomycete *S. commune*, which grows on Mountain Avala, Republic of Serbia. The extraction methods had no significant effect on the amount of total-glucan, α-glucan, and β-glucan of the extraction substrates ($p > 0.05$), but had an effect on the antioxidant properties and chemical structure of the extraction substrates, as shown in Table 4 and Figure 2. Table 4 shows that the extraction method affected the antioxidant properties of extracts. The extract from the M2 method had significantly the highest DPPH (80.22±0.51%) and ABTS (58.16±0.53%) ($p < 0.05$).

FT-IR spectroscopy is a technique that is used in the structural analysis of polysaccharides. The FT-IR spectra shows the molecular vibrations of covalent bonds at the infrared region range (4000 - 400 $cm^{-1}$). Figure 2 shows

 

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 Content[ns] (% w/w) | α-glucan Content[ns] (% w/w) | β-glucan Content[ns] (% 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 |

[ns]Not significant.
Values are presented as mean±SD of three replications.

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 |
|---|---|---|---|
| Control (native-MR) | 78.98±0.35[b] | 50.92±0.48[c] | 2.62±0.18 |
| M1 | 80.15±0.51[a] | 57.51±0.48[b] | 2.90±0.15 |
| M2 | 80.22±0.51[a] | 58.16±0.53[a] | 2.87±0.06 |

[ns]Not significant.
Values are presented as mean±SD of three replications. Values with different superscript within the same column are significantly different ($p < 0.05$).

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 indicate that both of the extraction methods affected the chemical structure of the extracted substrate when compared with the control sample, especially at the frequency ranges of 1567 - 1570 $cm^{-1}$, 1405 - 1409 $cm^{-1}$, and 878 - 876 $cm^{-1}$.

The results showed that heat and alkalinity had an effect on the structure and antioxidant properties of *S. commune* Fr. β-glucan, which is consistent with the research of Polacios *et al*. (2012) and Gieroba *et al*. (2020). Polacios *et al*. (2012) found that the extraction method influenced the structure of the polysaccharide extracted from *Pleuratus ostreatus* fruiting bodies. The extracted polysaccharides structure of *Pleuratus ostreatus* fruiting bodies with cold water was formed by α (1→3,1→6) linked galactopyranosyl residues, whereas the extracted polysaccharides structure with hot water and with hot aqueous NaOH consisted of glucose-linked units, and the molecular arrangement by complexation with Congo red of β-linked polysaccharide from hot aqueous NaOH displayed a triple helix conformation. The (1→3)- β- D- glucan polymer gelled at 80°C has a distinctly different structure than the matrix gelled at 90° C (Gieroba *et al*., 2020). The important FT-IR absorption spectra region of the extracted polysaccharides from *S. commune* Fr. consisted of the sugar region (1200 - 950 $cm^{-1}$) and anomeric region (950 - 750 $cm^{-1}$), and the heat used for extraction or the pH affected the structure of the extracted β-glucan. The differences in the chemical structure of the extracted β-glucan led to different antioxidant properties.

## 4. Conclusion

The research found that the strains of *S. commune* Fr. in Thailand have an effect on the amount of extracted β-glucan. The 1-day-old MR *S. commune* Fr. from Chanthaburi was proved to be a good source for β-glucan production. For the effect of the extraction method, it was found that temperature and pH in the extraction have an effect on the structure and antioxidant properties of β-glucan. The extraction from 1-day-old MR *S. commune* Fr. from Chanthaburi with 1 M of NaOH at 100°C for 24 h yielded β-glucan with more antioxidant properties than β-glucan from hot water extraction at 121°C for 15 mins.

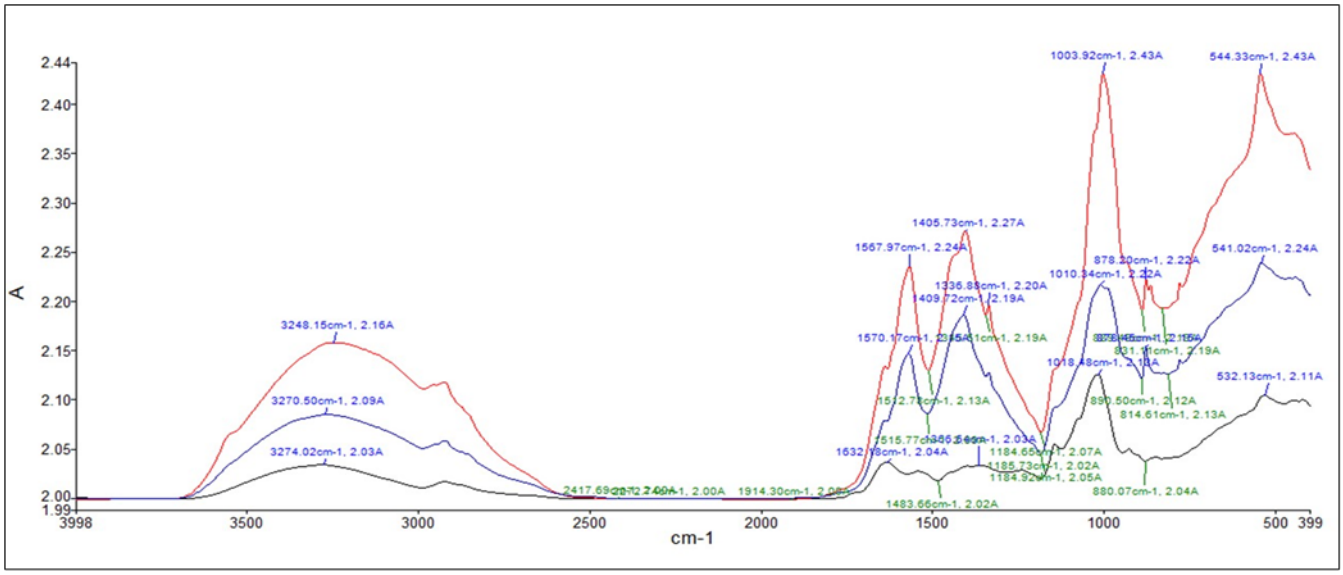


Figure 2. Infrared spectra of *S. commune* Fr from 1-day-old MFB dried powder, which different extraction method. Red line: M1 method (Hot water extraction method), Blue line: M2 method (Hot water- Hot alkali extraction method), Black line: Control (native-MR dried powder)

 

The results of this research led to the development of a ready-to-drink mushroom juice in cans by using 1-day-old MR *S. commune* Fr. and adjusting the pH to more than 7, which can increase the antioxidant properties of the product.

## Conflicts of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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