

MICROWAVE-ASSISTED EXTRACTION OF PLEUROTUS SAJOR-CAJU POLYSACCHARIDES AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS

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Abstract

This research investigates the effect of microwave-assisted extraction (MAE) of *Pleurotus sajor-caju*, under variable MAE temperatures (80, 100, 120, and 140°C) and irradiation times (5, 10, and 15 min), on the precipitate yields and bioactive compound contents. For comparison purposes, autoclave extraction (without microwave) was carried out under variable extraction temperatures and times. Additionally, the physical characteristics of the MAE-precipitates were characterized. The results showed that the MAE-precipitate yields and extraction temperatures were positively correlated, whereas a prolonged irradiation time had no impact on the MAE-precipitate yield. The total phenolics and the antioxidant activities of the MAE-precipitates were significantly increased with the increase in the MAE temperatures, while the β -glucan content was insignificantly different beyond 100°C. The optimal MAE condition of *P. sajor-caju* was that of elevated extraction temperatures (120 or 140°C) and 5-min irradiation time. Moreover, the MAE-precipitates contained (1→3, 1→6)- β -glucan.

Keywords: Edible mushroom, *Pleurotus sajor-caju* polysaccharides, microwave-assisted extraction, bioactive compounds

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Introduction

Pleurotus sajor-caju (Fr.) Singer is an edible mushroom with high nutritional and medicinal benefits. Specifically, *P. sajor-caju* is an excellent source of proteins, fibers, minerals, and vitamins, with low calories (Carbonero *et al.*, 2012; Satitimanwiwat *et al.*, 2012; Jantaramanant *et al.*, 2014; Finimundy *et al.*, 2018). It is also rich in bioactive compounds, particularly polysaccharide β -glucan, which is recognized as a source of nutraceuticals with various health benefits (Carbonero *et al.*, 2012; Satitimanwiwat *et al.*, 2012; Finimundy *et al.*, 2018).

Mushroom polysaccharides are predominantly glucans of various structures, e.g., branched or linear and with an α or β -configuration. The most common mushroom glucan is (1 $\rightarrow$ 3, 1 $\rightarrow$ 6)- β -D-glucan, containing a single unit at the branching point (Carbonero *et al.*, 2012; Fricova and Kovalakova, 2013; Synytsya and Novak, 2014; Smiderle *et al.*, 2017). β -glucans from different sources exhibit different numbers of sugar moieties and linkages in the skeleton chain. Meanwhile, the activity of β -glucans depends on their structural characteristics, including molecular weight, polymer length, and degree of branching (Zhu *et al.*, 2015). Mushroom β -glucans are currently used for their antioxidant (Ferreira *et al.*, 2009), antidiabetic (Kanagasabapathy *et al.*, 2012), and anti-inflammatory properties (Elsayed *et al.*, 2014).

Conventional hot aqueous extraction methods using hot water or an alkali solution are commonly used for polysaccharide β -glucans extraction. However, those methods require long extraction times with low extraction yields (Santos-Neves *et al.*, 2008; Carbonero *et al.*, 2012; Smiderle *et al.*, 2017). Conversely, due to a shorter extraction time, lower solvent use, improved extraction yield, and environmental friendliness (Wang and Weller, 2006), microwave-assisted extraction (MAE) is thus an efficient alternative to conventional extraction in plant metabolite extraction. The enhanced extraction yield of MAE could be attributed to the effects of internal heating and dielectric polarization (Ballard *et al.*, 2010).

MAE efficiently and uniformly distributes energy to the solvent and solid plant matrix. The microwave energy is absorbed by water in the plant matrix, leading to internal superheating and cell disruption, which facilitates desorption of chemicals from the matrix and improves the recovery of nutraceuticals (Wang and Weller, 2006). Existing research utilized MAE under low and very high extraction temperatures (50-180°C) to extract polysaccharide β -glucans from edible mushrooms (Wang *et al.*, 2010; Zeng *et al.*, 2012; Smiderle *et al.*, 2017). Meanwhile, research on the effects of MAE, under moderate to high extraction temperatures with a relatively short extraction time, on polysaccharide β -glucan yields of *P. sajor-caju* and its physical characteristics is limited.

This research, thus, investigates the effect of MAE on the extraction yields of *P. sajor-caju* and bioactive compounds in the MAE-precipitates, including β -glucan, phenolics, and antioxidants. The MAE temperatures and irradiation times were varied between 80, 100, 120, and 140°C and 5, 10, and 15 min, respectively. The physical characteristics of native-MR (freeze-dried *P. sajor-caju* powder) and MAE-precipitates (MAE-P80, P100, P120, P140) were characterized. Additionally, conventional autoclave extraction (without microwave) was carried out under variable extraction temperatures (80, 100, and 120°C) and times (5, 10, and 15 min), and the results were compared with the MAE process.

Materials and Methods

Materials

Fresh fruiting bodies of *P. sajor-caju* were acquired from Lalompho Farm in Thailand's northeastern province of Nakhon Ratchasima. The fruiting bodies were first washed in distilled water to remove soil residues, and then sliced and stored at -60°C before lyophilization. The freeze-dried mushrooms were milled using a cross beater mill (Pulverisette 16, Fritsch GmbH, Idar-Oberstein, Germany) into powder

with a particle size less than 250 μm (Aguiló-Aguayo *et al.*, 2017). The freeze-dried *P. sajor-caju* powder (native-MR) was then vacuum-packed and retained at 4°C. In the study, all chemicals were of analytical grade, and the yeast beta glucan assay kit was from Megazyme International (Wicklow, Ireland).

Experimental Methods

Microwave-assisted Extraction (MAE) of *P. sajor-caju*

Laboratory-scale MAE of *P. sajor-caju* was carried out following Smiderle *et al.* (2017) with modifications. A 0.25 g mushroom powder was weighed in a 100 mL extraction tube containing a small magnetic stirrer, and 15 mL deionized water (1:60, w/v) was added. MAE was conducted under different extraction temperatures (80, 100, 120, and 140°C) and irradiation times (5, 10, and 15 min). The ramping time (time required to reach the target extraction temperature) was 5 min, using a Multiwave 3,000 microwave reaction system (Anton Paar GmbH, Graz, Austria) with an infrared temperature sensor. The extraction was carried out at a maximum power of 1,200 W and pressure of 1.5 MPa with 2,455 MHz frequency and 200 rpm magnetic agitation. After extraction, the tube was cooled down to 45°C within 10 min. The extract was then separated by centrifugation from the residue (10,000 rpm, 4°C, 10 min), and ethanol (95%) was added into the extract (3:1, v/v) and retained at 4°C for 24 h to allow for complete precipitation of polysaccharides. The sample was further centrifuged (10,000 rpm, 4°C, 10 min) and the precipitate of each extract was lyophilized, and the precipitate yield determined. Figure 1 illustrates the schematic of the MAE extraction of polysaccharides from the fruiting bodies of *P. sajor-caju*. The MAE-residue and MAE-precipitate (mainly polysaccharides) yields were calculated by:

$$\text{Yield of residue (\%)} = \frac{\text{weight of residue after MAE (g)}}{\text{weight of the mushroom powder (g)}} \times 100 \quad (1)$$

$$\text{Yield of precipitate (\%)} = \frac{\text{weight of precipitate after recovered from the MAE extract (g)}}{\text{weight of the mushroom powder (g)}} \times 100 \quad (2)$$

Autoclave Extraction of *P. sajor-caju*

For comparison, autoclave extraction (AE) was performed using an autoclave (SX-700, TOMY, Tokyo, Japan). A 0.25 g native-MR was weighed in a 50 mL conical tube, and 15 mL deionized water (1:60, w/v) was added. The AE was carried out at 80, 100, and 120°C for 5, 10, and 15 min (without microwave). After cooling, the extract was separated from the residue by centrifugation (10,000 rpm, 4°C, 10 min), and ethanol (95%) was added into the extract (3:1, v/v) and retained at 4°C for 24 h to recover the polysaccharides. The sample was further centrifuged (10,000 rpm, 4°C, 10 min) and the precipitate of each extract was lyophilized and the precipitate yield determined. The AE-residue and AE-precipitate yields were also calculated by Equations 1 and 2.

Chemical Composition Analysis

The chemical composition, including protein, fat, ash, and carbohydrates, of the native-MR and MAE-precipitates (under 5-min irradiation time given that the extended irradiation time (i.e., 10 and 15 min) had no effect on the MAE-precipitate yields) were analyzed in accordance with the Association of Official Analytical Chemists methods (AOAC International, 2000). The samples were oven-dried at 105°C to constant weight and the

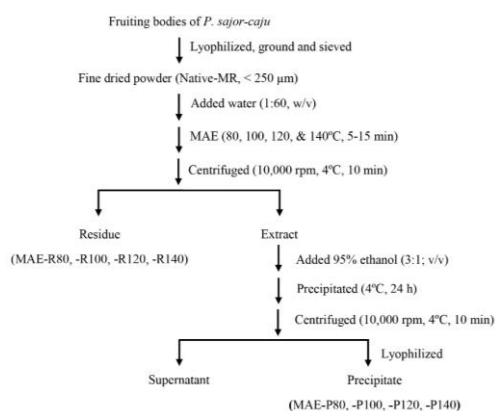


Figure 1. Schematic of microwave-assisted extraction (MAE) of polysaccharides from the fruiting bodies of *P. sajor-caju*

moisture content determined. The crude protein content ($N \times 4.38$) was analyzed by the Kjeldahl method, crude fat by petroleum ether using a Soxhlet apparatus, and ash content by a muffle furnace at 550°C until free of black coloration. Total carbohydrates and energy were calculated by Equations 3 and 4, respectively (Heleno *et al.*, 2015; Finimundy *et al.*, 2018).

$$\text{Total carbohydrates (\%)} = 100 - \text{protein content (\%)} - \text{fat content (\%)} - \text{ash (\%)} \quad (3)$$

$$\text{Energy (kcal)} = (4 \times \text{g protein}) + (4 \times \text{g carbohydrates}) + (9 \times \text{g fat}) \quad (4)$$

β-glucan Content Analysis

The β-glucan contents of the native-MR and MAE-precipitates were determined using an enzymatic yeast β-glucan assay kit (Megazyme International, Wicklow, Ireland). The total glucans, including α-glucan and β-glucan linkages, were solubilized in sulfuric acid (H₂SO₄) at ice-cold temperature. A potassium hydroxide (KOH) solution was used to neutralize and the mixture was filtered with Whatman filter paper no. 1. A mixture of exo-1,3-β-glucanase and β-glucosidase was added into the mixture and hydrolyzed for glucose. The free glucose was determined by spectrophotometry (UV-Vis spectrophotometer, UV-2600, Shimadzu, Kyoto, Japan) at 510 nm with a glucose oxidase/peroxidase (GOPOD) reagent. The β-glucan content was *calculated* using a Mega-CalTM excel sheet (Megazyme International, Wicklow, Ireland).

Antioxidant Properties

Sample Preparation

The preparation of the native-MR and MAE-precipitate samples for antioxidant activity analysis followed Heleno *et al.* (2015) with modifications. A 100 mg of native-MR and MAE-precipitates (5-min irradiation time) were mixed with 2 mL methanol at 25°C and stirred at 300 rpm for 2 h. The extracts were filtrated with Whatman filter paper no. 4 and retained at -20°C for further analysis.

Total Phenolics by Folin-Ciocalteu Assay

The total phenolic content was determined by the Folin-Ciocalteu method (Heleno *et al.*, 2015) with minor modifications. A 0.2 mL sample was mixed with 2 mL Folin-Ciocalteu reagent and 0.6 mL Na₂CO₃ (20.0%). The mixtures were vortexed, covered with aluminum foil, and allowed to stand for 30 min in the dark before measurement at an absorbance of 765 nm. The results were expressed as mg of gallic acid per g dry weight sample (mg gallic acid/g sample, db).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The DPPH radical scavenging activity was measured following Heleno *et al.* (2015) with modifications. The DPPH solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and stored at 4°C. The working solution was prepared by diluting the DPPH solution with methanol to obtain an absorbance of 1.10 ± 0.06 at 515 nm. A 75 µL of the sample solution and standard solutions (Trolox, BHA, and BHT of 5, 20, 25, and 50 mg/L) were mixed with 200 µL DPPH radical solution in a 96-well plate. The mixture was shaken and retained in the dark for 30 min. Absorbance was then measured against the blank at 515 nm using a UV-spectrophotometer (Multiskan GO, Thermo Fisher Scientific Waltham, MA, USA). All results were expressed in µmol Trolox, BHA, and BHT equivalent DPPH radical scavenging activity per g dry weight sample (µmol Trolox, BHA, and BHT/ g sample, db).

2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) Radical Scavenging Assay

An ABTS radical scavenging assay was carried out following Adebayo *et al.* (2018) with modifications. The ABTS solution was prepared by reacting ABTS (7 mM) with potassium persulphate (140 mM) and incubated at room temperature in the dark for 12–16 h. Prior to use, the ABTS reagent was diluted with methanol for an absorbance of 0.70 ± 0.02

at 734 nm. A 0.2 mL of the sample solution and standard solutions (Trolox, BHA, and BHT of 5, 20, 25, and 50 mg/L) were mixed with 2 mL ABTS solution. The mixture was allowed to react at room temperature in the dark for 10 min, and the absorbance at 734 nm was measured using the UV-spectrophotometer. The results were expressed in μmol Trolox, BHA, and BHT equivalent ABTS radical scavenging per g dry weight sample (μmol Trolox, BHA, and BHT/ g sample, db).

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM, JSM-6010LV, Jeol Ltd., Tokyo, Japan) was used to examine the effect of MAE on the morphology of the native-MR, AE-residue at 120°C for 5 min, and MAE-residues at 80-140°C for 5 min. In this research, the SEM images of the MAE- precipitates showed no distinction between the samples and were thus excluded.

Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectra of the native-MR and MAE-precipitates (5-min irradiation time) were measured by Fourier transform infrared spectroscopy (FTIR, Tensor 27, Bruker Corp., Billerica, MA, USA) with OPUS 7.0 software. The spectra were scanned at room temperature with the wave number range of 4,000 to 400 cm^{-1} at 64 scans and 4 cm^{-1} resolution, and referenced against the air. The analysis was carried out in triplicate.

Nuclear Magnetic Resonance Spectroscopy (NMR)

^{13}C NMR spectra were characterized using NMR Spectroscopy 500 MHz (AVANCE III HD, Bruker Corp.). ^{13}C NMR (125.8 MHz) analysis was performed at 70°C on the MAE-precipitates (5-min irradiation time) dissolved in DMSO- d_6 (10 mg in 500 mL DMSO- d_6). ^{13}C NMR chemical shifts (δ) were expressed in parts-per-million (ppm). In this research, the ^{13}C NMR of the native-MR was not characterized since it failed to dissolve in DMSO- d_6 .

Atomic Force Microscopy (AFM)

The sample preparation for AFM followed Wang *et al.* (2018) with modifications, whereby the native-MR and MAE-precipitates (5-min irradiation time) were dissolved in distilled water by stirring at 320 rpm at 25°C for 48 h and filtered with Whatman paper no. 1 for 0.01 g/mL stock solution. The stock solution was diluted into 10 $\mu\text{g}/\text{mL}$ with distilled water and dropped onto glass slides and air-dried. All samples were characterized by an atomic force microscope (AFM, XE-120, Park Systems Corp., Suwon, South Korea) in non-contact mode at 256×256 pixels with a scanning rate at 0.3 Hz under the ambient environment.

Statistical Analysis

In this research, experiments were carried out in triplicate and results expressed as means $\pm$ standard deviations. One-way analysis of variance was used to statistically assess the extraction efficiency of the AE, MAE, and bioactive compounds in the MAE-precipitate, and a Tukey-HSD multiple comparison was used to compare means, given a 5% significance level ($p < 0.05$). The statistical analysis was carried out using Minitab® 17 (Minitab LLC, State College, PA, USA).

Results and Discussion

Effect of AE and MAE on Residue and Precipitate Yields of *P. sajor-caju*

Figure 2(a-c), respectively, illustrates the effect of the AE and MAE extraction temperatures and times on the residue yield of *P. sajor-caju*. The content of water-soluble compounds in the extract increased as the AE temperatures rose from 100°C to 120°C and as MAE temperatures increased from 100°C to 140°C, as evidenced by lower residue recovery ($p < 0.05$). This is consistent with Wang and Weller (2006) and Smiderle *et al.* (2017), who reported that higher temperatures facilitated the desorption of chemicals from the matrix and increased extraction yields. Specifically, elevated MAE temperatures enhanced the

extraction of compounds from the mushroom matrix by accelerating cell rupture and diffusion of extractives into the solvent (Li *et al.*, 2017; Smiderle *et al.*, 2017). According to Afshari *et al.* (2015), higher temperatures (>95°C) improved the extraction efficiency of polysaccharides from *Hibiscus rosa-sinensis* due to increased solubility of polysaccharides.

However, extended AE and MAE extraction times (from 5 to 15 min) insignificantly increased the extraction efficiency ($p>0.05$). Meanwhile, under elevated temperatures and long extraction time, the MAE residue recovery was significantly reduced ($p<0.05$).

This fact is confirmed by Wang and Weller (2006), who documented that, given a short extraction time, MAE significantly improved the extraction yield, compared with the conventional extraction methods.

After extraction, polysaccharides were recovered from the extract using 95% ethanol. Figure 3(a-c), respectively, illustrates the effect of the AE and MAE extraction temperatures and times on the precipitate yield of *P. sajor-caju*. The MAE temperatures were positively correlated to the MAE-precipitate yields ($p<0.05$), especially at temperatures above water boiling point (120°C and 140°C).

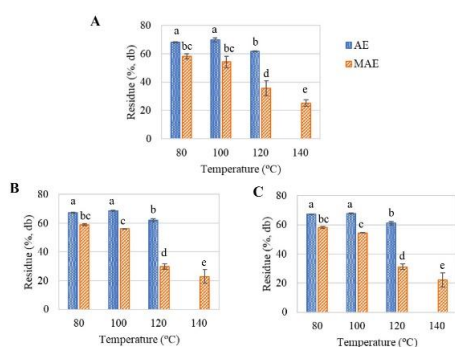


Figure 2. Effect of autoclave extraction (AE) and microwave-assisted extraction (MAE) on residue yields of *P. sajor-caju*, given extraction time: (A) 5 min, (B) 10 min, and (C) 15 min. The values are the mean of 3 replications $\pm$ standard deviation. Different letters represent significant differences between treatments ($p<0.05$)

Meanwhile, the extended irradiation time from 5 to 15 min had a minimal impact on the MAE-precipitate yields, consistent with Smiderle *et al.* (2017), who documented that the extended MAE time of *P. ostreatus* from 5 to 30 min, under 50, 115, and 180°C, insignificantly improved the polysaccharide yields. On the other hand, elevated temperatures and extended extraction time insignificantly improved the AE-precipitate recovery ($p>0.05$).

According to Santos-Neves *et al.* (2008) and Carbonero *et al.* (2012), conventional hot water extraction (100°C, 6 h) of *Pleurotus* spp. yielded approximately 6.0-16.0% polysaccharide crude extract (precipitate). The results were agreeable with the precipitate extraction efficiency (Figure 3), indicating that MAE achieved higher precipitate yields than the conventional hot water extraction. The MAE process effectively solubilized polysaccharides from the mushroom matrix by accelerating the cell rupture and diffusion of polysaccharides into the solvent (Li *et al.*, 2017; Smiderle *et al.*, 2017). Given that the extended irradiation time (10 and 15 min) had no effect on the MAE-precipitate yields, the 5-min irradiation time was adopted in subsequent experiments.

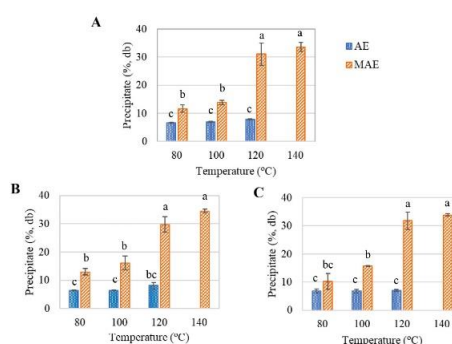


Figure 3. Effect of autoclave extraction (AE) and microwave-assisted extraction (MAE) on precipitate yields of *P. sajor-caju*, given extraction time: 5 min, (B) 10 min, and (C) 15 min. The values are the mean of 3 replications $\pm$ standard deviation. Different letters represent significant differences between treatments ($p<0.05$)

Figure 4(a-f) depicts the SEM images of the native-MR, AE-residue at 120°C for 5 min, and MAE-residues at 80, 100, 120, and 140°C for 5 min, respectively. In Figure 4(a), the morphology and surface of the native-MR was intact and smooth. Meanwhile, the AE residue (Figure 4(b)) and MAE residues (Figures 4(c-f)) exhibited a less dense and porous structure. By comparison, the MAE residues were considerably more porous than the AE residue. Elevated microwave temperatures (from 80°C to 140°C) induced structural disruption and the formation of voids and cracks on the surface as polysaccharides were removed. The formation of dented surfaces on MAE residue particles and increased porosity enhanced the extraction efficiency. The finding is consistent with Smiderle *et al.* (2017), who reported that polysaccharide yields were positively correlated to microwave extraction temperatures. Essentially, the SEM analysis (Figures 4(a-f)) confirmed that high MAE temperatures improved polysaccharide extraction efficiency.

Chemical Composition of Native-MR and Precipitates

Table 1 tabulates the chemical composition of the native-MR and MAE-precipitates, on dry weight basis (db). Carbohydrates and proteins constituted 64.82% and 27.38% of the chemical composition of the native-MR, consistent with Ouzouni *et al.* (2009), who reported that the composition of wild edible mushrooms was predominantly carbohydrates (53.33-66.87%) including polysaccharides, particularly glucans and chitin, and proteins (21.57-34.77%). Meanwhile, the ash and fat

contents of *P. Sajor-caju* were 5.97% and 1.83%, respectively. The energy based on the nutritional composition was 385.27 kcal/100 g. The results were consistent with Finimundy *et al.* (2018), who reported that ash, fat, and energy of *P. sajor-caju* were 5.4%, 1.16 %, and 384 kcal/100 g, respectively. *P. sajor-caju* is thus a good source of carbohydrates, proteins, and minerals with a low fat content (Aguiló-Aguayo *et al.*, 2017).

The carbohydrate content (mainly polysaccharides) of the MAE-precipitates

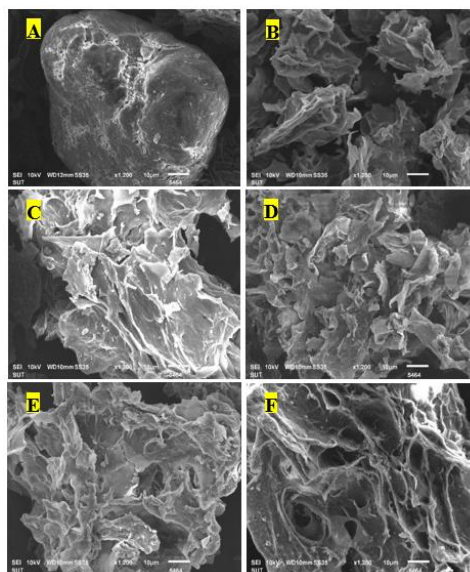


Figure 4. The 1200x SEM images of: (A) native-MR, (B) AE-R120 for 5 min, (C) MAE-R80, (D) MAE-R100, (E) MAE-R120, and (F) MAE-R140 for 5 min

Table 1. The chemical composition of native-MR and MAE-precipitates of *P. sajor-caju* on dry weight basis (db)

Sample	Composition (% db) ¹			
	Carbohydrates	Protein	Fat	Ash
Native-MR	64.82 ± 1.57 ^b	27.38 ± 1.60 ^a	1.83 ± 0.05 ^a	5.97 ± 0.01 ^a
MAE-P80	90.83 ± 3.98 ^a	5.66 ± 3.78 ^b	0.71 ± 0.15 ^b	2.79 ± 0.05 ^b
MAE-P100	90.21 ± 1.92 ^a	7.05 ± 1.89 ^b	0.56 ± 0.01 ^{bc}	2.18 ± 0.04 ^c
MAE-P120	95.65 ± 0.03 ^a	1.05 ± 0.09 ^b	0.33 ± 0.01 ^c	2.97 ± 0.06 ^b
MAE-P140	92.93 ± 0.32 ^a	4.46 ± 0.25 ^b	0.41 ± 0.00 ^c	2.20 ± 0.06 ^c

¹ The values are the mean of three replications ± standard deviation. Different letters in each column represent significant differences between treatments (p<0.05).

(90.21-95.65%) was significantly higher than that of the native-MR ($p < 0.05$), while the protein, fat, and ash contents of the MAE-precipitates were significantly lower than those of the native-MR ($p < 0.05$). The results indicated that MAE effectively solubilized carbohydrates (polysaccharides). Nevertheless, in the recovery of precipitates by ethanol, other undesirable solubilized compounds (protein, fat, ash) also co-precipitated. In addition, varying MAE temperatures between 80°C and 140°C minimally improved the recovery of carbohydrates (mainly polysaccharides) and proteins, whereas the ash recovery was varied. Ondruschka and Asghari (2006) and Tan *et al.* (2013) reported that microwave extraction temperatures affect the purity of extracted compounds. Typically, higher process temperatures enhance not only polysaccharide yields but also proteins and fats in the extract, giving rise to impurities in the precipitate. Thus, future research should focus on purification of polysaccharides.

Table 2. β -glucan content of native-MR and MAE-precipitates of *P. sajor-caju*

Sample	β -glucan content (% db) ¹
Native-MR	32.43 $\pm$ 0.36 ^b
MAE-P80	31.14 $\pm$ 3.89 ^b
MAE-P100	49.29 $\pm$ 0.32 ^a
MAE-P120	48.70 $\pm$ 0.78 ^a
MAE-P140	44.14 $\pm$ 3.41 ^a

¹ The values are the mean of three replications $\pm$ standard deviation. Different letters in column represent significant differences between treatments ($p < 0.05$).

Effect of MAE on β -glucan Content of Precipitates

Table 2 tabulates the β -glucan content of the native-MR and MAE-precipitates of *P. sajor-caju*. At 80°C, the β -glucan content in the MAE-precipitate (31.14%) was insignificantly different from that of the native-MR (32.43%), consistent with Lee and Kim (2005), who reported that the β -glucan levels in edible mushrooms ranged from 4.7 to 46.2%. However, increased MAE temperatures (100, 120, and 140°C) significantly increased the β -glucan content in MAE-precipitates (44.14-49.29%), compared with the native-MR ($p < 0.05$). This could be attributed to elevated MAE temperatures facilitating the solubilization of β -glucan in the mushroom cell. Similarly, Temelli, (1997) reported that β -glucan extraction yields from oat and barley increased linearly with extraction temperatures. Interestingly, around half of the carbohydrate in MAE-P100, -P120, and -P140 precipitates was β -glucan (Table 1), suggesting that high β -glucan extraction efficiency could be achieved with elevated MAE temperatures (100, 120, and 140°C).

Effect of MAE on Total Phenolic Content and Antioxidant Properties of MAE-Precipitates

Table 3 tabulates the total phenolic content (TPC) and antioxidant properties of the native-MR and MAE-precipitates. The results showed that the highest TPC, DPPH, and ABTS radical scavenging activities were realized at 140°C MAE temperature (MAE-P140), nearly twice as high as those of the native-MR. However, the DPPH and ABTS

Table 3. Total phenolic content and antioxidant properties of native-MR and MAE-precipitates of *P. sajor-caju*, on dry weight basis

Sample code	Phenolics content ¹ (mg gallic acid/g db)	DPPH ²			ABTS ³		
		μ mol Trolox/g	μ mol BHA/g	μ mol BHT/g	μ mol Trolox/g	μ mol BHA/g	μ mol BHT/g
Native-MR	2.35 ^c $\pm$ 0.10	3.67 ^b $\pm$ 0.03	4.63 ^b $\pm$ 0.04	4.63 ^b $\pm$ 0.04	4.63 ^b $\pm$ 0.04	4.32 ^b $\pm$ 0.04	4.25 ^c $\pm$ 0.03
MAE-P80	1.48 ^d $\pm$ 0.14	1.74 ^d $\pm$ 0.02	2.04 ^c $\pm$ 0.01	2.04 ^c $\pm$ 0.01	2.04 ^a $\pm$ 0.01	1.54 ^d $\pm$ 0.03	2.33 ^d $\pm$ 0.02
MAE-P100	2.62 ^c $\pm$ 0.51	3.01 ^c $\pm$ 0.25	2.65 ^c $\pm$ 0.49	2.65 ^c $\pm$ 0.49	2.65 ^a $\pm$ 0.49	2.41 ^c $\pm$ 0.36	4.21 ^c $\pm$ 0.25
MAE-P120	3.53 ^b $\pm$ 0.26	3.77 ^b $\pm$ 0.02	3.99 ^b $\pm$ 0.08	3.99 ^b $\pm$ 0.08	3.99 ^b $\pm$ 0.08	3.51 ^b $\pm$ 0.03	4.96 ^b $\pm$ 0.02
MAE-P140	5.11 ^a $\pm$ 0.06	7.21 ^a $\pm$ 0.07	8.63 ^a $\pm$ 0.38	8.63 ^a $\pm$ 0.38	8.63 ^a $\pm$ 0.38	8.46 ^a $\pm$ 0.10	8.38 ^a $\pm$ 0.07

^{1,2,3} The values are the mean of three replications $\pm$ standard deviation. Different letters in each column represent significant differences between treatments ($p < 0.05$).

radical scavenging activities of MAE-P80 and P-100 were significantly lower than those of the native-MR ($p < 0.05$). Meanwhile, an elevated MAE temperature enhanced the DPPH and ABTS scavenging radical activities of MAE-P140. The findings are consistent with Sulistiany *et al.* (2016), who documented that wild *Pleurotus* mycelial and its fruiting body contained TPC of 2.02 and 4.62 mg gallic acid/g extract, respectively, and exhibited high DPPH radical scavenging activity. According to Kullu *et al.* (2014), microwave irradiation disrupts the bonds and enhances the penetration of solvent into the matrix, allowing the dissolution of components to be extracted. Similar to the β -glucan extraction (Table 2), higher MAE temperatures (100, 120, and 140°C) liberated phenolic and antioxidant compounds from the mushroom cells.

In this research, the antioxidant properties of MAE-precipitates were strongly correlated with TPC, consistent with Khatun *et al.*, (2015) and Sulistiany *et al.*, (2016). According to Boonsong *et al.*, 2016, phenolics possess antioxidant properties and act as free radical scavengers. As a result, high TPC contributes to effective antioxidant properties. In essence, at 140°C MAE temperature, TPC and the antioxidant properties of MAE-precipitate were significantly enhanced, compared with the native-MR ($p < 0.05$).

FTIR Analysis of MAE-precipitate

FTIR was used to analyze the presence of polysaccharide, particularly β -glucan, in the MAE-precipitates (5-min irradiation time). The FTIR results were compared against previous research on purified β -glucan from *P. Sajor-caju* (Satitmanwiwat *et al.*, 2012; Jantaramanant *et al.*, 2014; Sermwittayawong *et al.*, 2018). Figure 5 illustrates the FTIR spectra of the native-MR and MAE-P80-P140 in the range of 4,000–400 cm^{-1} . Each sample showed the characteristic absorptions for typical polysaccharides. The broad bands at 3,298 cm^{-1} and small bands at around 2,880–2,923 cm^{-1} were attributed to the O-H and C-H stretching vibrations of polysaccharides, respectively (Sermwittayawong *et al.*, 2018; Zhang *et al.*, 2018).

The absorption peaks at 1,631–1,647 cm^{-1} were attributable to binding water (Zhang *et al.*, 2018; Satitmanwiwat *et al.*, 2012) and proteins (Klaus *et al.*, 2011; Yin *et al.*, 2018), due to the overlapping of water and proteins (Jantaramanant *et al.*, 2014). The results (1,631–1,647 cm^{-1}) indicated that the native-MR and MAE precipitates (MAE-P80-P140) contained some proteins, consistent with the chemical composition analysis (Table 1). The finding also suggested that MAE was unable to completely remove polysaccharide-binding proteins.

The absorptions peaks at 1,000–1,200 cm^{-1} were attributed to characteristic peaks of corresponding sugar residues (pyranose ring) due to the stretching vibration of C-O-C and C-OH (Kozarski *et al.*, 2011; Satitmanwiwat *et al.*, 2012; Yin *et al.*, 2018). The weak absorption peaks at 1,072–1,078 cm^{-1} were attributable to the presence of (1 $\rightarrow$ 3)- β -D-glucan (Sermwittayawong *et al.*, 2018; Kozarski *et al.*, 2011). However, the peaks at 993–994 cm^{-1} corresponding to (1 $\rightarrow$ 6)- β -D-glucan could not be observed. The findings were consistent with Jantaramanant *et al.* (2014) and Sermwittayawong *et al.* (2018), who documented the presence of (1 $\rightarrow$ 3)- β -D-glucan (1,073–1,078 cm^{-1}) and the absence of (1 $\rightarrow$ 6)- β -D-glucan (993–994 cm^{-1}) in polysaccharides from *P. sajor-caju*. Except for the native-MR, the weak peaks at 885–891 cm^{-1} were characteristic of α - and β -glycosidic linkages, indicating the presence of α - and β -glucan (Klaus *et al.*, 2011; Kozarski *et al.*,

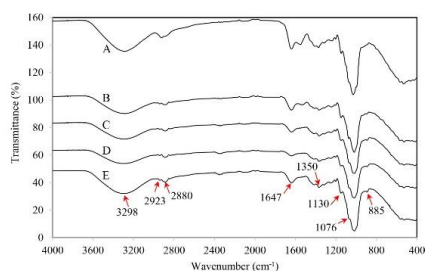


Figure 5. FTIR spectra of 4,000–400 cm^{-1} region of: (A) native-MR, (B) MAE-P80, (C) MAE-P100, (D) MAE-P120, and (E) MAE-P140

2011; Zhang *et al.*, 2018; Satitmanwiwat *et al.*, 2012).

¹³C NMR analysis of MAE-precipitate

¹³C NMR was used to characterize the structure of β -glucan in the MAE-precipitates (5-min irradiation time). Figure 6 illustrates the ¹³C NMR spectra of MAE- P80, P100, P120, and P140, and the analysis results were compared against those of Smiderle *et al.* (2017), Synytsya and Novak (2014), Fricova and Kovalakova (2013), and Carbonero *et al.* (2012). In the figure, the spectra revealed the chemical shifts (δ) of carbons (C1- C6), characteristic of (1 $\rightarrow$ 3,1 $\rightarrow$ 6)- β -D-glucan. The chemical shift at 103.03 ppm indicated the presence of (1 $\rightarrow$ 3)-linked C1 of glucan in β -configuration. The chemical shifts (δ) at 86.27-86.36 ppm belonged to C3 O-substituted of β -linkages ((1 $\rightarrow$ 3)-linked C3), and δ at 69.62 ppm and 60.90 ppm were associated with (1 $\rightarrow$ 6)-linked C6 and (1 $\rightarrow$ 3)-linked C6 of β -glucan, respectively (Smiderle *et al.*, 2017; Carbonero *et al.*, 2012). The chemical shifts at 76.4 ppm, 72.9 ppm, and 68.5 ppm indicated C5, C2, and C4 of glucans (Fricova and Kovalakova, 2013). The results indicated that the MAE-precipitates contained (1 $\rightarrow$ 3, 1 $\rightarrow$ 6)- β -glucan. The MAE temperatures had no effect on the structure of β -glucan. In addition, the small signals at δ of 100 ppm and 83.33 ppm indicated the presence of C1 and C3 of glucans in α -configuration (Smiderle *et al.*, 2017).

The ¹³C NMR analysis results were in good agreement with the FTIR results. According to Synytsya and Novak (2014), fruiting bodies of mushrooms contain 2 main types of glucans: branched (1 $\rightarrow$ 3, 1 $\rightarrow$ 6)- β -D-glucan and linear (1 $\rightarrow$ 3)- α -D-glucan.

AFM Analysis of MAE-precipitate

Figure 7 depicts the AFM molecular morphology (10 $\times$ 10 μ m) of water-soluble polysaccharides of the native-MR and MAE-precipitates (5-min irradiation time). Specifically, Figure 7(AI) shows the planar structure of native-MR polysaccharides which were varied in size, highly dispersed, and of small globular aggregates. In contrast, in Figure 7(BI- EI),

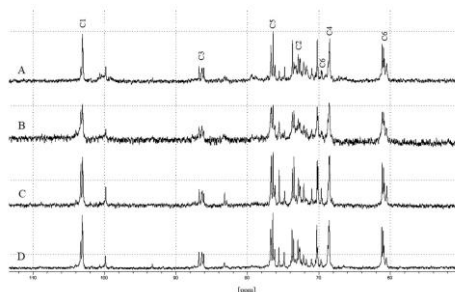


Figure 6. ¹³C NMR spectra of: (A) MAE-P80, (B) MAE-P100, (C) MAE-P120, and (D) MAE-P140

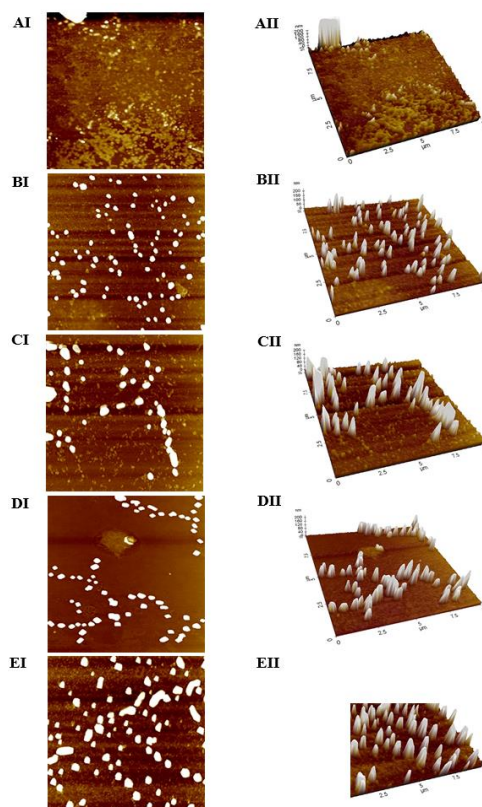


Figure 7. AFM images of: (A) native-MR, (B) MAE-P80, (C) MAE-P100, (D) MAE-P120, and (E) MAE-P140, where I and II represent AMF planar and three-dimensional images, respectively

polysaccharides of the MAE-precipitates were less dispersed with the formation of large particles. Figure 7(AII-EII) illustrate the three-dimensional structure of polysaccharides of the native-MR and MAE-precipitates, in which the surface topographies are rugged, comprising irregular and steep pointed protrusions, consistent with Wang and Nie (2018). By comparison, the heights of the MAE-precipitate protrusions were considerably greater than that of the native-MR, which could be attributed to more aggregation of polysaccharide fractions (Li *et al.*, 2017).

Conclusions

This research investigated the effect of MAE on the precipitate yields of *P. sajor-caju* and the bioactive compound contents: β -glucan, phenolics, and antioxidants. In the study, the MAE temperatures and irradiation times were varied between 80, 100, 120, and 140°C and 5, 10, and 15 min, respectively. The physical characteristics of the MAE-precipitates were also characterized, including FITR, ^{13}C NMR, and AFM. Additionally, autoclave extraction (without microwave) was carried out under variable extraction temperatures and times, and the results (residue and precipitate yield) compared with those of the MAE extraction. The experimental results revealed that the MAE-precipitate yields were positively correlated with the extraction temperatures, while an extended irradiation time had no effect on the MAE-precipitate yields and thus the 5-min irradiation time was adopted in subsequent experiments. The β -glucan content was insignificantly different beyond 100°C, while total phenolics and the antioxidant activities of the MAE-precipitates were significantly increased as the MAE temperatures increased from 80 to 140°C. The findings thus indicated that the optimal MAE condition of *P. sajor-caju* was under elevated extraction temperatures (120 or 140°C) with a short irradiation time (5 min). Furthermore, the physical characterization showed the presence of (1 $\rightarrow$ 3, 1 $\rightarrow$ 6)- β -glucan in the MAE-precipitates. Further research should focus on

β -glucan isolation from MAE-precipitates and characterization of the biological effects, including antioxidant, antimicrobial, and anti-inflammatory properties.

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