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Effect of Autoclave and Microwave-assisted Extractions of *Pleurotus sajor-caju* (Fr.) Sing on Polysaccharides Yields and Microstructural Characteristics

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Abstract

The objective of this study was to investigate the efficiency of autoclave extraction (AE) and microwave-assisted extraction (MAE) of *Pleurotus sajor-caju* (Fr.) Sing on the yield of polysaccharides and microstructural properties. The mushroom sample was subjected to the AE and MAE at 120˚C for 15, 30 and 45 min using water as an extraction solvent. Extract was separated from solid residue by centrifugation. Solid precipitate was recovered by adding three volumes of ethanol into the extract. The results showed that extraction effectiveness was affected by the extraction method. The yields of solid precipitate, mainly polysaccharides, obtained from the MAE were significantly higher than those of the AE ($p<0.05$). However, the extended extraction time of the AE and MAE did not influence the yields of solid precipitate. Scanning electron microscopy (SEM) revealed that the MAE induced the structural changes in the cells to enhance the extractability. These results indicate that the short extraction time of the MAE (15 min) is highly effective and operationally viable for the polysaccharide extraction from the *Pleurotussajor-caju* (Fr.) Sing.

Keywords: Autoclave extraction, Microwave-assisted extraction, Mushroom, Polysaccharides

1 Introduction

Mushroom has been consumed for centuries because it is a good taste and rich source of nutrition. Mushroom contains high amount of protein, vitamins and minerals, but it is low in sodium, calories and fat. In Thailand, the consumption of mushroom; especially, *Pleurotussajor-caju* (Fr.) Sing, is average 10 kilograms per person per year (Prachachat, 2555: online). Many researchers have been reported the biological functions of mushroom including antinociceptive and anti- inflammatory activity (Silveira et al., 2015), antimicrobial, antimitogenic and antiproliferative (Ngai and Ng, 2004), and antioxidant (Kanagasabapathy et al., 2011). The important bioactive component found in mushroom is beta-glucan, which is a polysaccharide. The benefits of beta-glucan have been reported as follows; protection against cancer (Cha et al., 2012), modulation the immune system, antiviral and antibacterial properties (Moradali et al., 2007), antioxidant activity (Deng et al., 2012), reduction of blood glucose and cholesterol (Chen and Raymond, 2008), and immunobiological activities (Mizuno et al., 1995).

The polysaccharides can be extracted from the fruit bodies and mycelium of mushroom. The advanced extraction methods possess several advantages over the traditional extraction method (i. e., solvent extraction), including the short extraction time, environmentally friendly and high polysaccharides yields. The high temperature and pressure parameters of the extraction are more effective regarding the enhancement of polysaccharides extraction (Smiderle et al., 2017). Autoclave extraction (AE) and Microwave-assisted extraction (MAE) are technologies provided high temperature above the boiling point of water and considered as environmentally friendly because of their high extraction efficiency and subsequent lower energy consumption. Therefore, the objective of this study was to investigate the effects of AE and MAE on the efficiency of solid extraction and microstructural characteristic of mushroom sample.

2 Materials and Methods

2.1 Raw material

Fresh fruit bodies of mushroom (*Pleurotus sajor-caju* (Fr.) Sing.) were purchased from the Phet Sima market (Muang, Naknon Ratchasima). The mushroom sample was cut in slices and lyophilized. It was then ground and sieved through 60-mesh. The mushroom powder was vacuum packed and stored in refrigerator at 4°C prior to further analysis.

2.2 Autoclave extraction (AE)

A 0.5 g mushroom powder was dispersed in 30 mL deionized water (1:60, w/v) in an autoclave bottle. The bottle was then processed in an autoclave machine (SX-700, TOMY, Japan) at 120°C for 15, 30

and 45 min. After cooling, the extract was separated from the solid residue by centrifugation at 10,000 rpm for 20 min at 4°C. Ethanol (3:1; v/v) was added to the extract for solid precipitate at 4°C overnight. The solid precipitate was then obtained by centrifugation at 10,000 rpm for 20 min at 4°C. The solid precipitate and solid residue were the lyophilized for further physicochemical analysis as describe in section 2.4. The solid precipitate and solid residue yields, on dry weight basis (w/w), were calculated as per equation 1 and 2, respectively.

$$\text{Soild precipitat e yield (\%)} = \frac{\text{Mass of solid recovered from extract (g)}}{\text{Mass of original mushroom powder (g)}} \times 100 \quad (1)$$

$$\text{Solid residue yield (\%)} = \frac{\text{Mass of residue after extraction (g)}}{\text{Mass of original mushroom powder (g)}} \times 100 \quad (2)$$

2.3 Microwave-assisted extraction (MAE)

MAE was carried out using a microwave device (Multiwave 3000SOLV, Anton Paar, USA). Each extraction tube was filled with 0.25 g of mushroom powder, 15 mL of deionized water (1:60, w/v) and a small magnetic stirrer. Extraction was performed at 120°C for 15, 30 and 45 min and the system microwave power reached a maximum of 1,200 W. After extraction, the tube was cooled to 30°C and the extract was separated from the solid residue by centrifugation at 10,000 rpm for 20 min at 4°C. Ethanol (3:1; v/v) was added to the extract for solid precipitate at 4°C overnight. The solid precipitate was then recovered by centrifugation at 10,000 rpm for 20 min at 4°C. The solid precipitate and solid residue were then lyophilized for further physicochemical analysis as describe in section 2.4. The solid precipitate and solid residue yields, on dry weight basis (w/w), were calculated as per equation 1 and 2, respectively.

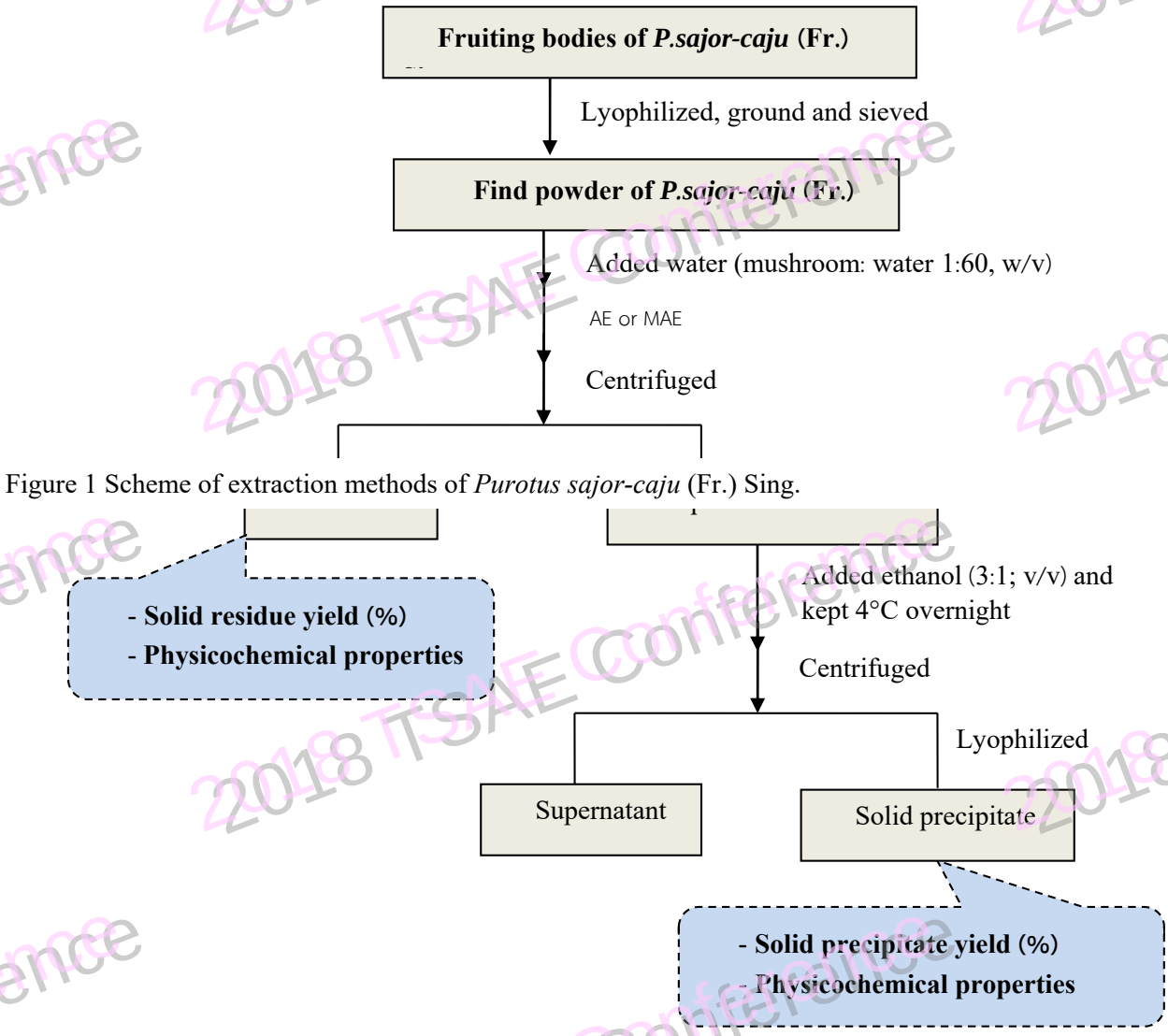

Figure 1 Scheme of extraction methods of *Purotus sajor-caju* (Fr.) Sing.

2.4 Physicochemical determination

The raw mushroom powder, solid precipitate and solid residue were analyzed for the content of crude protein, crude fat, crude fiber, ash and polysaccharides. Crude protein was determined by Kjeldahl method according to AOAC procedure (2000) using a digestion Apparatus (KB20S, Gerhardt, Germany) and a distillation system (KjeltecTM8100 Distillation Unit, Foss, Denmark). The protein content was calculated using 4.38 as a conversion factor. Crude fat content was determined by an automated Soxhlet extraction (Soxtec™ 8000, Foss, Denmark) using petroleum ether as a solvent (AOAC 2000). Crude fiber and ash values were determined following the procedure in AOAC method (1995), whereas the polysaccharides value was calculated according to the following equation 3:

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

2.5 Microstructural analysis

Scanning electron microscopy (SEM, JSM-6010LV, JEOL, Japan) was used to determine the effect of extraction methods and conditions on morphology of the mushroom samples. The raw mushroom powder and solid residue were mounted on metal stub and sputter-coated with thin gold film. Microstructure and surface characteristics of the samples were observed.

2.6 Statistical analysis

All analyses were undertaken in duplicate, and the results expressed as the mean ± standard deviation. Two-way analysis of variance (ANOVA) was used to assess the effects of extraction methods, extraction times, and their interaction on the physicochemical characteristics. The TurkeyHSD multiple comparison was used to compare the difference between means, given the 5% significance level ($p < 0.05$). The statistical analysis was carried out using the Minitab® 17 statistical software.

3 Results and Discussion

3.1 Chemical composition analysis

Chemical composition of raw mushroom sample (*Pleurotus sajor-caju*) presented in Table 1. The values of protein, fat, fiber, carbohydrate, and ash were 18.31, 2.05, 8.23, 71.57, and 8.07% (dry weight basis), respectively. The analysis results are consistent with Finimundy et al. (2018) and Bonatti et al. (2004). Moreover, Hung and Nhi (2012) reported that polysaccharides are a major component in edible mushroom varied between 52.3 - 88.6%. However, in this study, the fiber content (8.23%) was considerably lower than 22% in Alam et al. (2007). The variable composition was probably due to the factors (i.e., environmental conditions and species) affecting the growth of mushroom (Crisan and Sands, 1978).

3.2 Effects of AE and MAE on the solid residue and solid precipitate yields

Table 2 compares the effects of different extraction methods (AE and MAE), extraction times (15, 30 and 45 min), and their interaction on the solid residue yields and their chemical compositions of *Pleurotus sajor-caju*. By comparison, the solid residue yields obtained from the AE and MAE methods were in the range of 56.61-57.68% and 39.73-46.00%, respectively. The major solid extractable could be soluble polysaccharides (i.e., glucan, mono-, di- and oligosaccharides). This finding is agreeable with Bonatti et al. (2004), who reported that the soluble polysaccharides content of *Pleurotus sajor-caju* were about 43%. In this study, the results indicated that the MAE method significantly improved the efficiency of solid extraction compared with the AE method ($p<0.05$). However, varying extraction times (15, 30 and 45 min) of the AE and MAE insignificantly impacted the solid residue yields ($p<0.05$). More specifically, the extraction time was not correlated to the solid residue yields. The protein, fat, polysaccharides, and ash values of the mushroom samples were efficiently extracted by about 64-73%, 47-85%, 28-47%, and 88-92%, respectively. The extended extraction time of the MAE significantly lowered the protein and polysaccharides contents in the solid residue ($p<0.05$). Moreover, the interactions between the two factors (extraction method and time) were significant for the protein, fat, and polysaccharides values ($p<0.05$).

After the AE and MAE of mushroom samples, the solid precipitate, mainly polysaccharides was recovered from the extract or supernatant by addition of ethanol (Smiderle et al., 2006). In Table 3, the solid precipitate yields and their chemical composition values were presented. Interestingly, the solid precipitate yields recovered from MAE-extract were significantly higher than that from AE- extract ($p<0.05$) (under all conditions). However, varying extraction times (15, 30 and 45 min) of the AE and MAE insignificantly affected the solid precipitate yields ($p<0.05$). The higher solid precipitate yields could be attributed to the denaturation of the cell membranes by microwave irradiation resulting in enhancing the solid extraction. The chemical composition of solid precipitate showed in Table 3. The protein and polysaccharides values of solid precipitate recovered from MAE- extract were significantly higher than those from the AE-extract ($p<0.05$) (under all conditions). However, the interactions between the two factors (extraction

method and time) were insignificant for every response evaluated (p<0.05).

Table 1 Chemical composition of raw *Pleurotus sajor-caju*. All values are calculated as percent dry-weight of original mushroom sample.

Sample	Composition (%, dry basis)				
	Protein	Fat	Fiber	Polysaccharides	Ash
Pleurotus sajor-caju	18.31± 0.25	2.05±0.14	8.23±0.91	71.57±0.43	8.07±0.06

Table 2 Solid residue yields of and their chemical compositions. All values are calculated as percent dry-weight of original mushroom sample.

Treatment		Solid residue	Composition (%, dry basis)*			
			Protein	Fat	Ash	Polysaccharides
Autoclave 120ºC	15 min	57.68±0.56^{b}	5.12±0.14^{ab}	0.31±0.00^{a}	0.96±0.04^{ab}	51.29±0.18^{d}
	30 min	56.82±1.09^{b}	5.25±0.06^{ab}	0.77±0.10^{c}	1.04±0.08^{b}	49.76±0.11^{c}
	45 min	56.61±0.95^{b}	5.49±0.06^{b}	0.48±0.01^{ab}	0.90±0.15^{ab}	49.74±0.10^{c}
Microwave 120ºC	15 min	46.00±7.15^{ab}	6.33±0.14^{c}	1.04±0.05^{d}	0.81±0.01^{ab}	37.82±0.19^{b}
	30 min	39.73±4.59^{a}	5.33±0.03^{ab}	0.75±0.10^{c}	0.70±0.00^{a}	32.95±0.02^{a}
	45 min	39.77±0.21^{a}	4.89±0.22^{a}	0.65±0.02^{bc}	0.69±0.00^{a}	33.55±0.24^{a}
p-value of Interaction		0.512	0.000	0.000	0.224	0.000

*The values present the mean of duplicate± standard deviations. The different letters in the same column represent the difference among treatment at p<0.05.

Table 3 Solid precipitate yields of and their chemical compositions. All values are calculated as percent dry-weight of original mushroom sample.

Treatment		Solid precipitate	Composition (%, dry basis)*			
			Protein	Fat	Ash	Polysaccharides
Autoclave 120ºC	15 min	7.36± 0.37^{a}	0.38±0.04^{a}	0.03±0.01^{ab}	0.26±0.04^{ab}	6.68±0.01^{a}
	30 min	7.83±0.42^{a}	0.32±0.00^{a}	0.03±0.01^{a}	0.14±0.00^{a}	7.34±0.00^{a}
	45 min	9.36±0.31^{a}	0.45±0.01^{a}	0.03±0.02^{ab}	0.29±0.00^{ab}	8.59±0.01^{b}
Microwave 120ºC	15 min	28.74±1.45^{b}	1.61±0.12^{b}	0.21±0.08^{ab}	0.57±0.23^{b}	26.35±0.03^{c}
	30 min	30.17±0.04^{b}	1.57±0.03^{b}	0.20±0.04^{ab}	0.45±0.03^{ab}	27.95±0.05^{d}
	45 min	30.78±3.24^{b}	1.67±0.55^{b}	0.22±0.07^{b}	0.45±0.03^{ab}	28.44±0.65^{d}
p-value of Interaction		0.875	0.996	0.966	0.482	0.096

*The values present the mean of duplicate± standard deviations. The different letters in the same column represent the difference among treatment at p<0.05.

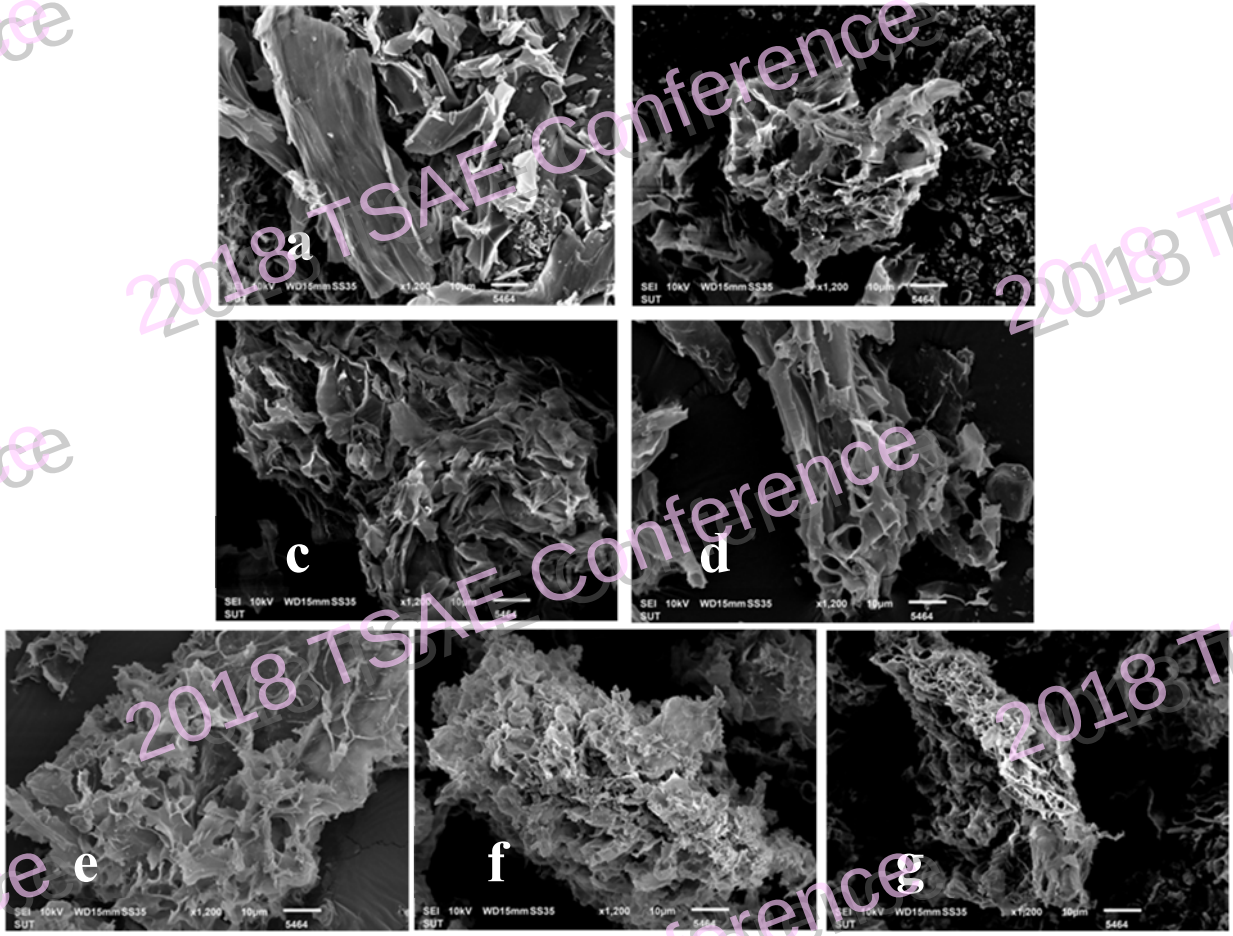

Figure 2 SEM images (1200x) of: (a) raw mushroom; (b, c, and d) AE-extracted mushroom at 15, 30, and 45 min, respectively; (e, f, and g) MAE-extracted mushroom at 15, 30, and 45 min, respectively

3.3 Microstructural analysis

SEM was used to investigate the microstructures of raw, AE-extracted and MAE-extracted mushroom samples (Fig. 1). Fig. 1 (a), the raw mushroom had a a somewhat complete structure, compact shape and smooth surface. In Fig. 1(b-d), the micro-factures, cracks and rough surface appeared on the AE-extracted mushroom. The MAE-extracted mushroom exhibited numerous micropores with fissured and rough surface (Fig. 1e-g). These observations indicated that the MAE method considerably induced the structural changes in the mushroom sample. The result is consistent with Chen et al. (2015), who documented that dynamic microwave-assisted extracted *Armillarialuteo-virens* showed some irregular-shape particles. The surface topology of polysaccharides might be affected by the extraction.

4 Conclusions

This study comparatively investigates the effects of extraction methods the autoclave extraction (AE) and microwave-assisted extraction (MAE) and extraction times of *Pleurotus sajor-caju* on the physiochemical and microstructural characteristics. In the study, the extraction temperature was 120ºC, while the extraction time was varied between 15, 30, and 45 min. The result showed that the MAE enhanced the solid precipitate yields. The extended extraction time of the AE and MAE insignificantly increased the solid residue and solid precipitate yields ($p<0.05$). The SEM images demonstrated that the MAE induced the structural changes, fissures, and cavities in the *Pleurotus sajor-caju*. Overall, the short extraction time of MAE (15 min) is highly effective and operationally viable for the extraction of *Pleurotus sajor-caju* because of the improved extractability. Further study is needed to evaluate the effects of extraction methods on antioxidant activity and beta-glucan yield of the solid precipitate.

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