



Effects of Heat Treatment on Free Radical Scavenging Capacities and Phenolic Compounds in *Tylopilus alboater* Wild Edible Mushrooms

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ABSTRACT

The bitter mushrooms (*Tylopilus alboater*) are generally found in eucalyptus forest in the northeastern part of Thailand. It contains a bitter taste and need to be heated by boiling and steaming in order to reduce the bitterness. Therefore, this research is to focus on the effects of heat treatment on antioxidant compounds, total polyphenolic compounds and flavonoids, along with radical scavenging activity of the mushroom. In addition, proximate analysis was also investigated. The results showed that the proximate chemical compositions of dried bitter mushroom (100 g) contained 20.63-21.60 g of crude protein, 0.91-1.00 g of crude fat, 16.23-17.99 g of fiber and 6.62-6.98 g crude ash. Moreover, it contained 1.65 g of beta glucan/100 g dried mushroom. The fresh bitter mushrooms (100 g) included 20.83 mg of GAE/100 g mushroom and 28.19 mg of catechin/100 g of mushrooms. After boiled the mushroom at 15 and 30 min, the total polyphenolic and flavonoid contents were obviously decreased due to leaching process whereas the contents of steamed mushroom were slightly reduced. However, the DPPH radical scavenging activity of heated mushrooms was higher than fresh mushrooms. The boiled mushrooms exhibited the highest activity. These suggested that the boiling method was a better way to get more benefits from bitter mushroom rather than steaming.

Keywords: *Tylopilus alboater*, mushroom, beta glucan, total phenol, flavonoid, DPPH radical scavenging

1. INTRODUCTION

The edible mushrooms are high in proteins, dietary fibers, minerals, together with vitamins but low in calories, fats, and essential fatty acids [1-3]. Moreover, mushrooms have been used as traditional medicines in Asia such as Shittake

mushrooms [4]. There have been reported that mushrooms can constrain platelet aggregation [5], reduce of blood cholesterol [6] and blood glucose levels [7], prevent heart disease [7], and prevent infections of bacterial, viral, fungal and parasitic pathogens [8].

Mushrooms also contain many phenols, which are very capability to scavenge peroxy radicals and have been found to be an excellent antioxidant and synergist that is not mutagenic [9-10]. Phenolic compounds, including flavonoids and phenolic acids, are major groups that have been linked with the inhibition of atherosclerosis and cancer [11-13]. The bioactivities of phenolic compounds may be related to their capability to chelate metals, inhibit lipoxygenase and scavenge free radicals [14]. Besides these, phenolic compounds can modulate enzymatic activity, affect signal transduction and activate the transcription factors and gene expression [15].

Mushrooms are also a potential source of dietary fiber such as chitin, other hemicelluloses, mannans and beta glucans [7]. Beta glucan is the most interesting functional components and plays a key role in some healthy properties of mushrooms, such as improvement of macrophage function and host resistance to many bacterial, viral, fungal and parasitic infections, activation of a non-specific immune stimulation, reduction of blood cholesterol and blood glucose levels [16-17].

The heat processed foods are considered to have a lower healthy when compared to the fresh one because the bioactive compounds are relatively unstable to heat and can be lost during the thermal processing. However, some studies have been reported that thermally processed foods, especially fruits and vegetables, may improve biological activities due to chemical changes during heat treatment [18-20].

Tylopilus alboater mushroom, a member of *Boletus griseipurpureus* Corner, is widely found in eucalyptus forest in the northeastern part of Thailand. Its fruit and ear are purple and show bitter taste. Thus, it is called as bitter mushroom or "Hed Kom" in Thai. It is

believed that it can reduce blood pressure and blood glucose levels but there was no report to be grown up due to its taste. Generally, the bitter mushroom has been used as traditional food and needs to be heated for reducing its bitter taste. Consequently, this study was focus on the effects of heat treatment (boiling and streaming) on total phenolic contents, flavonoid contents and free radical scavenging activity of bitter mushroom.

2. MATERIALS AND METHODS

2.1 Materials

The bitter mushrooms were collected at the eucalyptus forest with the same age and size. They are about 4-5 cm in height and 2-4 cm in diameter. Moreover, they are no physical damage and kept at -18°C.

2.2 Proximate Analysis

Proximate compositions of bitter mushroom including moisture content, crude protein content, crude fat, crude fiber, ash and nitrogen free extract were determined. The moisture content was obtained by a gravimetric method at 105°C. Total nitrogen content was determined by the Kjeldahl method and crude protein content was calculated by multiplying the nitrogen content with a factor of 4.38. Crude fat and crude fiber were determined by extraction with petroleum ether using a Soxhlet system and by acid digestion and alkali digestion, respectively. Ash content was obtained by gravimetric method using incineration at 550°C. All samples were analyzed for the above compositions in triplicate, in accordance with AOAC method [21].

2.3 Beta-glucan Contents

The bitter mushrooms were dried at 100°C for 3 hs and then ground. The beta glucan in bitter mushrooms was determined

according to Rhee *et al.* [22] with slightly modification. Powdered bitter mushroom samples (2 g) were suspended in a sodium carbonate-bicarbonate buffer (pH 10), stirred continuously at 45°C for 30 min and then centrifuged at 8940xg for 30 min. The supernatant were adjusted to pH 4.5 with 2 M of HCl and then centrifuged at 8940xg at 4°C for 30 min. After that, the supernatant was heated at 90°C for 10 min. The cooled supernatant was added with 250 mL of 1-propanol. The obtained precipitate was dried overnight at room temperature and then weighed.

2.4 Thermal Processing

The bitter mushroom was heated by boiling and steaming at 90°C for 15 and 30 min by using water bath.

2.5 Phenolic Compounds Extraction

The phenolic compounds and flavonoid contents of fresh and heated bitter mushroom were extracted according to Choi *et al.* [18] with some modification. The mushroom sample (25 g) was homogenized with 50 mL of 80% ethanol until it was homogeneous. The suspension was centrifuged at 8940xg for 30 min. The supernatant was concentrated by rotary evaporator at 40°C until approximately 10 mL. The extract was diluted with 25 mL of distilled water and stored at -18°C until analysis.

2.6 Total Phenolic Contents

The amount of total phenolic compounds in extracts from fresh and heated bitter mushroom were analyzed by the Folin-Ciocalteu method [23] with some modifications. The mushroom extract (0.4 mL) was mixed with 4 mL of 2% sodium carbonate solution and 0.2 mL of 50% Folin-Ciocalteu reagent. The solution was then incubated at room temperature for 30 min.

The absorbance of solution was measured at 750 nm. All extracts were analyzed in triplicate and the results were expressed as mg gallic acid equivalents per 100 g of mushroom.

2.7 Flavonoid Contents

The amount of flavonoid contents in fresh and heated bitter mushroom extracts were determined by a colorimetric method described by Jia *et al.* [24] with slightly modifications. The mushroom extract (0.5 mL) was mixed with 2.5 mL of distilled water and 0.15 mL of a 5% NaNO₂ solution and left to stand for 5 min. The mixed solution was added with 0.3 mL of 10% AlCl₃.H₂O solution and then mixed. After 6 min, 1 mL of 1 M NaOH and 0.55 mL of distilled water were added and mixed well. The absorbance of pink solution was measured at 510 nm. All extracts were analyzed in triplicate and the results expressed as mg (+)-catechin equivalents per 100 g of mushroom.

2.8 Free Radical Scavenging Activity

The free radical scavenging activity of the fresh and heated bitter mushroom extracts on DPPH radical was determined according to the method of Cheung *et al.* [25] with some modifications. The mushroom extract (1 mL) was mixed with 4 mL of 0.1 mM DPPH ethanolic solution and left to stand for 10 min in dark room. The absorbance of solution was then measured at 520 nm. The free radical scavenging activity (%) was calculated as following: Radical scavenging activity (%) = $(1 - A_{\text{sample}} / A_{\text{control}}) \times 100$; where A_{sample} is the absorbance of sample and A_{control} is the absorbance of blank. All extracts were determined in triplicate.

2.9 Statistical Analysis

All measurements were analyzed in triplicate. Statistics on a completely

randomized design (CRD) were performed with the analysis of variance (ANOVA) and Duncan's multiple range test ($P < 0.05$) was used to detect differences among the mean values.

3. RESULTS AND DISCUSSIONS

3.1 Proximate Chemical Composition

The chemical composition of bitter mushrooms was proximately determined including crude protein, crude fat, crude fiber and ash while carbohydrate content was calculated by Carbohydrate = $100 \text{ g} - (\text{g of Protein} + \text{g of Fat} + \text{g of Fiber} + \text{g of Ash})$. Moreover, the amount of beta glucan in mushroom sample was also determined by alkaline extraction method. The proximate chemical composition of bitter mushrooms was shown in Table 1.

Table 1. Proximate chemical composition of bitter mushroom.

	Composition (g/100 g dry weight)
Crude Protein ($N \times 4.38$)	20.63-21.60
Crude Fat	0.97-1.00
Crude Fiber	16.23-17.99
Ash	6.62-6.98
Carbohydrate*	52.43-55.55
Beta glucan	1.65 g

*Carbohydrate = $100 \text{ g} - (\text{g of Protein, Fat, Fiber, Ash})$

The crude protein content of bitter mushrooms was 20.63-21.60 g/100 g dry weight which is higher than *Auricularia sp.* Philippine var. (4.2 % dry weight), *Flammulina velutipes* (17.6 % dry weight) and *Lentinula edodes* (13.4-17.5 % dry weight); however, it is close to *Pleurotus ostreatus* (10.5-30.4 % dry weight) [26]. The crude fat content of bitter mushrooms was the lowest 0.91-1.00 g/100

g dry mushroom compared to *Auricularia sp.* Philippine var. (8.3% dry weight), *F. velutipes* (1.9 % dry weight) and *P. ostreatus* (1.6-2.2 % dry weight) [26]. On the other hand, the amount of crude fiber and crude ash of bitter mushrooms showed high amount which was 16.23-17.99 and 6.62-6.98 g/100 g dried mushrooms, respectively which is near *Auricularia sp.* Philippine var. (19.8 and 6.1-9.8 % dry weight, respectively) [26]. Therefore, the bitter mushrooms indicated the high amount of protein, fiber and ash but low amount of crude fat.

For beta-glucan content in bitter mushrooms which was extracted by alkali showed that bitter mushrooms contained 1.65 g/100 g dried mushrooms which was lower than in Chaga mushroom (13.7 g/100 g dried mushroom) [22]. However, the beta glucan content in bitter mushrooms was higher than *L. edodes* (0.22 g/100 g on a dry basis), *P. ostreatus* (0.22-0.38 g/100 g on a dry basis) and *Pleurotus pulmonarius* (0.53 g/100 g on a dry basis) [7]. In conclusion, the bitter mushroom was a type of high nutritive mushrooms.

3.2 Effect of Heat Treatment on Total Polyphenolic Contents

Phenolics, including flavonoids and phenolic acids, are major groups of dietary components that have been shown antioxidant activity [1, 11]. Mushrooms also contained many phenols, which are highly capable to scavenge of peroxy radicals [9]. Accordingly, the total polyphenolic of fresh and heated bitter mushrooms were extracted and determined with the result expression as mg gallic acid equivalents per 100 g of mushrooms. Only free polyphenol were extracted and analysed because the bound phenol was little amount also found in shitake mushrooms [18].

The figure 1 exhibited total polyphenolic

contents in bitter mushrooms. The results showed that the fresh bitter mushrooms contained total polyphenolic as 20.83 mg of GAE/100 g mushroom which was lower than other mushrooms *P. ostreatus* (54.90 mg/g)

[1], *C. versicolor* (23.28 mg/g), *G. lucidum* (47.25 mg/g), *G. tsugae* (51.28 mg/g) and *G. lucidum* (antler) (55.96 mg/g) [27]. However, it was slightly lower compared to Shitake mushrooms (29.0 mg/100 g) [18].

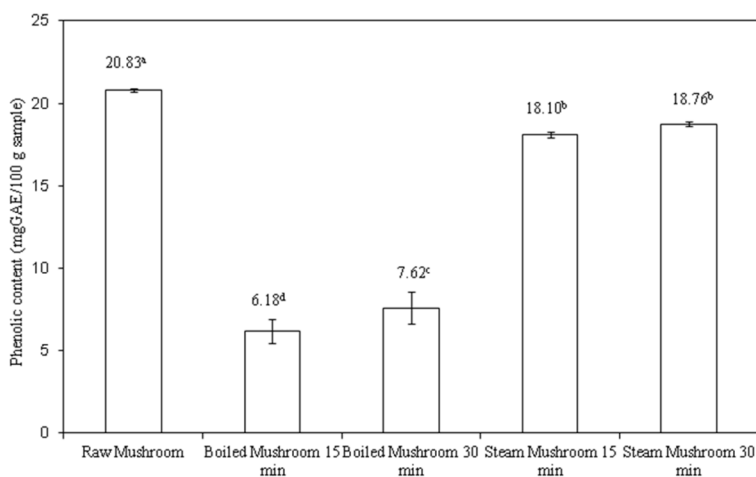


Figure 1. the total phenolic content in fresh and heated bitter mushroom.

* Means on the bar with different letters were significantly different ($P < 0.05$, ANOVA, DMRT).

Since the bitter mushrooms were generally heated in order to reduce the bitter taste in 2 ways, boiling and steaming. Therefore, we are interested in heating of bitter mushrooms by boiling and steaming at 90°C for 15 and 30 min. The total polyphenolic contents in heated mushrooms was significantly decreased to 6.18 and 7.62 mg/100 g for boiling at 15 and 30 min, respectively, while decreased to 18.10 and 18.76 mg/100 g for steaming at 15 and 30 min, respectively. However, the steamed mushrooms were slightly decreased when compared to boiled mushrooms. Moreover, cooking time also affected to the polyphenolic content in boiled sample while non significant was found in steamed sample. These results can be explained by the solubility of polyphenolic in mushrooms which leaching during heat treatment. The boiling water contained polyphenolic as 0.35 and 0.31 mg/100 g for boiling water at 15 and 30 min, respectively. However, the

heat treatment also changed their extractability by disruption of the plant cell wall thus polyphenolic would be released more easily [28] and found in the boiled water. Nevertheless, our results differ from Shitake mushrooms which the amount of total phenol was increased after heating [18]. This was probably due to the heating method.

3.3 Effect of Heat Treatment on Flavonoid Contents

The flavonoids are a class of plant secondary metabolites known to possess strong antioxidant properties [29]. The flavonoid contents of fresh and heated bitter mushrooms were also determined and expressed as mg (+)-catechin equivalents per 100 g of mushrooms which was shown in figure 2. The flavonoid content in fresh bitter mushroom was 28.19 mg/100 g of mushroom which was lower than Chrysin mushrooms (40 mg/100 g) and Rutin mushrooms (31.2 mg/100 g) [1]. However,

it was higher than in Shitake mushrooms (0.8 mg/100 g) [18].

The effect of boiling and steaming for 15 and 30 min on flavonoid content was shown in figure 2. In accordance with the polyphenolic content, the flavonoid content in heated mushroom was decreased. The flavonoid content in boiled mushroom for 15 and 30 min was 15.87 and 16.80 mg/100 g, respectively. However, the steamed mushroom was similar (23.33 and 28.00 mg/

100 g for 15 and 30 min, respectively). In addition, the cooking time has no effect on the flavonoid contents. These results can be also explained by leaching during heat treatment which the boiling water contains flavonoid 37.89 and 40.79 mg/100 g mushroom. These suggest that the heat treatment helps the extractability of flavonoid in the same as polyphenolic [28] and flavonoid contents were also found in the boiled water (data not shown).

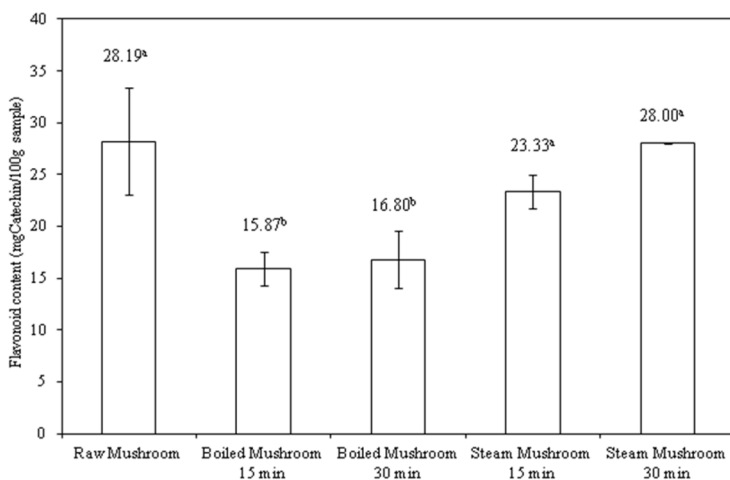


Figure 2. the flavonoid content in fresh and heated bitter mushroom.

* Means on the bar with different letters were significantly different ($P < 0.05$, ANOVA, DMRT).

3.4 Effect of Heat Treatment on Radical Scavenging Activity

Both polyphenolic and flavonoid have been reported to scavenge free radicals chelation of metals which many phenols in mushroom are highly scavenge peroxy radicals [1, 15, 30-31]. Therefore, the radical scavenging activity of the fresh and heated bitter mushrooms was determined by scavenging DPPH radical and the results was shown in figure 3.

The DPPH radical scavenging activity (%) of the fresh sample extract showed 62.97% which was higher than Shitake mushrooms (45%) [18]. After being heated, the DPPH

radical scavenging activity was increased in boiled sample (66.39 and 70.20% for 15 and 30 min, respectively). With the longer boiling time, the activity was significantly increased while the DPPH radical scavenging activity of steamed mushroom was significantly increased only in 30 min steaming (68.51%). However, the results showed that the heat treatment increased an activity in contrast with the amount of antioxidant compounds which was decreased. These results were probably due to Maillard reaction products which forming during heating that could also show antioxidant activity [32].

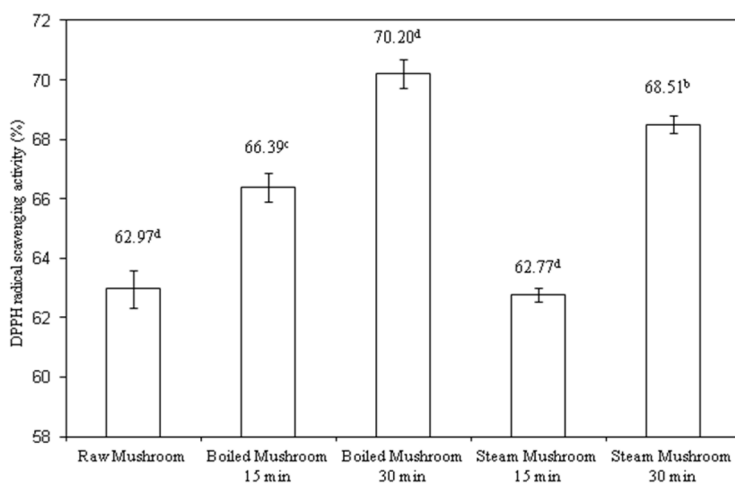


Figure 3. the DPPH radical scavenging activity (%) in fresh and heated bitter mushroom.
* Means on the bar with different letters were significantly different ($P < 0.05$, ANOVA, DMRT).

Moreover, the DPPH radical scavenging activity of boiling water showed 78.26 and 76.48% for boiling water for 15 and 30 min, respectively. This can support the explanation that the heat treatment help extractability of antioxidant compound from bound polyphenolic to free polyphenolic and show high activity in boiling water.

4. CONCLUSIONS

Bitter mushrooms are generally found in the northeastern part of Thailand. The proximate chemical composition showed that it was the one type of high nutritive mushroom with high amount of protein, fiber and ash including beta glucan content but low amount of crude fat. The antioxidant compound, polyphenolic and flavonoid contents, in bitter mushrooms was slightly lower than Shitake mushroom for polyphenolic content and higher than in Shitake for flavonoid content. The effect of heat treatment on antioxidant compounds by imitate knowledge cooking method, boiling and steaming for reducing the bitter taste showed that the steam method

slightly decreased antioxidant compounds. In the other hand, heat treatment increased the DPPH radical scavenging activity (%) of bitter mushrooms due to extractability of antioxidant compound and maillard reaction products. Thus, from our results suggested that the cooking method for bitter mushrooms in order to get more benefits should be boiling for 30 min. In further research, the effect of cooking temperature and other cooking method on antioxidant compounds should be studied.

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