FRACTIONATION AND ANTIOXIDANT ACTIVITIES OF THE PROTEIN HYDROLYSATE FROM BOLETUS MUSHROOM (*BOLETUS COLOSSUS*)

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ABSTRACT Mushrooms have been used for a tradition food and medicine in many Asian countries because of their desirable aroma, taste and high nutritional content. Boletus mushroom (*Boletus colossus*) are known as local edible mushrooms in Thailand. This mushroom contains high protein (32.06% dry weight basis), and low fat content (1.94% DW), which could be a good source for protein hydrolysate production. In this study, Boletus mushroom was hydrolyzed with 0, 5, 10, 15 and 20% (w/w) bromelain at 0.5, 1, 3, 6 and 12 h. The optimum hydrolyzed condition to produce enzymatic bromelain Boletus mushroom protein hydrolysate (eb-BPH) was 15% bromelain with hydrolysis time of 6 h, which gave the greatest degree of hydrolysis (68.01%) and low salt content (3%). The eb-BPH was further fractionated into four fractions, namely, eb–BPH-1 (>10 kDa), eb–BPH-2 (10–3 kDa), eb–BPH-3 (3-1 kDa), and eb–BPH-4 (below 1 kDa) by membrane ultrafiltration. The eb-BPH and its fractions were evaluated antioxidant activity by 1,1-diphenyl-2-picryhydrazyl ($DPPH^{\cdot}$) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) radical - scavenging activity assays. Results showed that eb–BPH-4 had the greatest efficiency to scavenge $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radicals (12.08 and 94.14 mg Trolox/L, respectively). Generally, eb-BPH-4 fraction had the highest of surface hydrophobicity (S^0) (1061/ 5 mg protein). The high activity of eb-BPH-4 in these antioxidant assay systems may be related to the high levels of surface hydrophobicity because the hydrophobic residues was an important characteristic associated with their ability to trap free radicals. It was suggested that Boletus mushroom protein hydrolysate could potentially used as antioxidant agents in functional foods and nutraceuticals applications.

1. INTRODUCTION

Thailand is located in tropical region characterized by a warm and humid environment is a great habitat for the growth of mushrooms. Boletus mushroom (*Boletus colossus*) is a local edible mushrooms in Thailand which one of the most consumed because of their desirable aroma, taste and high nutrition values. This mushroom contains high protein, but low fat content, which could be a good source for protein hydrolysate production. Enzymatic hydrolysates of various food proteins have been reported to exhibit antioxidant potential because they contained short chain peptides that are had higher nutritive values and may also be utilized more efficiently by the body than mixture of free amino acids (Grimble et al., 1987). Antioxidant peptides are considered natural antioxidant resources because of their potential health benefits associated with low molecular weight, high activity, good absorption, and no negative side effects (Razali et al, 2015). A literatures review of the previous work are considered molecular weight of peptides that play the important role in the antioxidant power. Therefore, the objective of this study was to investigate the relationship between the molecular weight of peptides and antioxidant activity of Boletus mushroom protein hydrolyzed by bromelain. The ultrafiltration membrane was used to isolate the high antioxidant activity peptide fractions.

2. MATERIALS AND METHODS

2.1 Materials

Boletus mushroom was purchased from a local market in Phra Nakhon Si Ayutthaya Province. Stem bromelain (E.C. 3.4.22.32; 119,325 U/g) was kindly provided by Hong Mao Biochemicals Co., Ltd. located

in Rayong, Thailand. All reagents were of analytical grade (Mallinckrodt Chemicals, St Louis, MO).

2.2 Preparation of Boletus mushroom Protein Hydrolysate (BPH)

Boletus mushrooms were analyzed of their chemical compositions such as protein, carbohydrate, ash, fiber and lipid content by AOAC (2000), then hydrolyzed by 5, 10, 15, 20% (g of bromelain/ g of sample) for 0.5, 1, 3, 6 and 12 h. The reaction was stopped by heating at 95°C for 15 min. Enzymatic bromelain Boletus mushrooms protein hydrolysates (eb-BPH) were filtered through filter paper (Whatman No. 1). The filtrate of eb-BPH was collected and analyzed for as following; Degree of hydrolysis (DH) was determined as described by the Flavia et al (1998) method which defined of DH as the percentage of soluble protein in trichloroacetic acid (TCA) and total salt content by salinity refractometer.

2.2 Fractionation of eb-BPH by ultrafiltration

Eb-BPH solution was filtered using three ultrafiltration membranes with 10, 3 and 1 kDa molecular weight cut-off to obtain four fractions corresponding to molecular weights (MW) above 10 kDa (eb-BPH-1), between 10 to 3 kDa (eb-BPH-2), between 3 to 1 kDa (eb-BPH-3) and below 1 kda (eb-BPH-4). Four fractions were evaluated surface hydrophobicity (S_0) and antioxidant activities.

2.2.1 Surface hydrophobicity (S_0) : determined by using 1,8-anilinophthalenesulphonate (ANS), as a fluorescent probe, according to the method of Wu et al (1998). The sample was serially diluted 0.01 M phosphate buffer (pH 7.0) to obtain protein concentration ranging from 0.00125-0.03%. Twenty microliters of ANS (8x103 ANS in 0.01 M phosphate buffer, pH 7.0) added to 4 mL of diluted protein solutions. Fluorescence intensity (FI) was measured at 390 (excitation) and 470 nm (emission) at 20oC. The initial slope of the FI versus protein concentration plot was calculated by linear regression analysis and used as a surface hydrophobicity.

2.2.2 Antioxidant activity measurement

2.2.2.1 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical-scavenging activity : Samples (300 µL) were mixed with 900 µL of a 0.1 mM DPPH solution. The mixtures were incubated for 30 min in the dark at room temperature, and the reduction of DPPH radicals was measured at 517 nm. The control was prepared in the same manner, except that distilled water was used instead of the sample. The blank was the value of 300 µL of sample solution mixed with 950 µL of 95% ethanol (Kanu et al., 2009).

2.2.2.2 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) ($ABTS^{+\bullet}$) radical-scavenging activity : The stock solutions included 7.4 mM $ABTS^{+\bullet}$ solution and 2.6 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 mL $ABTS^{+\bullet}$ solution with 50 mL methanol, in order to obtain an absorbance of 1.1± 0.02 units at 734 nm using a spectrophotometer. Sample (50 µL) was mixed with 950 µL of ABTS+• solution and the mixture was left at room temperature for 2 h in the dark. The absorbance was then measured at 734 nm using a spectrophotometer. The control was prepared in the same manner, except that distilled water was used instead of the sample. The blank was the value of 50 µL of sample solution mixed with 950 µL methanol (Kanu et al., 2009).

2.3 Statistical Analysis

All results were conducted in triplicate and analyzed by analysis of variance (ANOVA) and Duncan's multiple-range (DMRT) using version 9 of the SAS program (SAS Institute Inc., Cary, NC).

3. RESULTS AND DISCUSSION

3.1 Chemical compositions of Boletus mushroom

The chemical compositions of Boletus mushroom were as follows (dry basis): 32.06 ± 1.64% protein, 61.53 ± 1.06% carbohydrate, 2.41 ± 0.04% ash, 2.06 ± 0.19% fiber, and 1.94 ± 0.57% lipid. Results showed that Boletus mushroom contains high protein and low fat content which could be a good source for protein hydrolysate production.

3.2 Optimization of hydrolysis condition for eb-BPH

It is well known that various parameters significantly affect the DH of protein and the bioactivities of hydrolysate (Zhang et al., 2012). Using DH (%) as the target, the effects of enzyme-to-substrate ratio (E/S) and hydrolysis time (t) were investigated (Table 1). The maximum DH of eb-BPH was E/S 15% and 6 h. Using the optimal results, confirmed the total salt content was 3% which low salt content.

Table 1 Degree of hydrolysis (%DH) of eb-BPH.

Enzyme con[c]	Times (h)	%DH
5% E/S ratio	0.5	19.50[g]
	1	38.10[e]
	3	46.07[de]
	6	50.71[c]
	12	51.33[c]
10% E/S ratio	0.5	23.72[f]
	1	39.64[e]
	3	50.18[d]
	6	54.98[c]
	12	55.88[c]
15% E/S ratio	0.5	37.06[d]
	1	45.90[cd]
	3	57.39[c]
	6	66.61[a]
	12	63.24[b]
20% E/S ratio	0.5	39.11[d]
	1	52.99[cd]
	3	61.27[b]
	6	65.04[ab]
	12	61.81[bc]

[a, b, c, d, e, f,] and [g]. Mean in the same column with the different letters are significantly different ($p \leq 0.05$)

3.4 Surface hydrophobicity (S_0) of eb-BPH and membrane fractions.

The S_0 values of eb-BPH and membrane fractions are shown in Fig. 3, eb-BPH-4 was the highest (1061/ 5 mg protein). The results suggested that in native protein molecules, hydrophobic groups are buried inside the core of the folded structure, but after partial hydrolysis, some of these groups would be exposed, resulting in the increased S_o (Paraman et al., 2007).

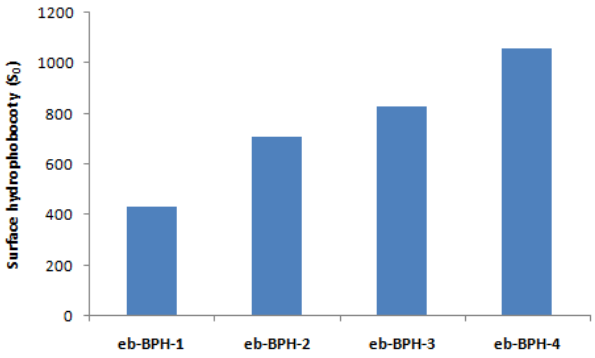

Fig 3 Surface hydrophobicity (S_0) of eb-BPH and membrane fractions.

3.3 Antioxidant activities of eb-BPH and membrane fractions.

The antioxidant activities of eb-BPH and membrane fractions determined using DPPH$^{\bullet}$ and ABTS$^{+\bullet}$ radical scavenging method, were compared with Trolox solutions. Among the four fractions, the highest antioxidant activities were found in eb-BPH-4 (below 1 kDa), which exhibited strongest scavenging activities on DPPH$^{\bullet}$ and ABTS$^{+\bullet}$ radical were 12.08 and 94.14 mg Trolox/L, respectively (Fig. 2). Result agreed with the previous reports, which showed that low molecular weight peptide had higher antioxidant activities than high molecular weight peptides because smaller peptides make the peptides more hydrophilic. The increased polarity of the low molecular weight peptides had a greater probability of engaging in more effective hydrogen or electron donors interactions with free radicals (Chi et al., 2015 and You et al., 2010).

The antioxidant activities of the hydrolysates may be correlated with their S_0 because hydrophobic characteristic played important roles in antioxidant activity (Shu et al., 2011). Hydrophobic residue at the C terminal was shown to be a preferred structural arrangement for antioxidants associated with their ability to trap free radicals (Jimenez-Ruiz et al., 2013). It was demonstrated that the antioxidant activities of peptides depends not only on their MW, but also on other factors, such as surface hydrophobicity, amino acid composition, and their sequences.

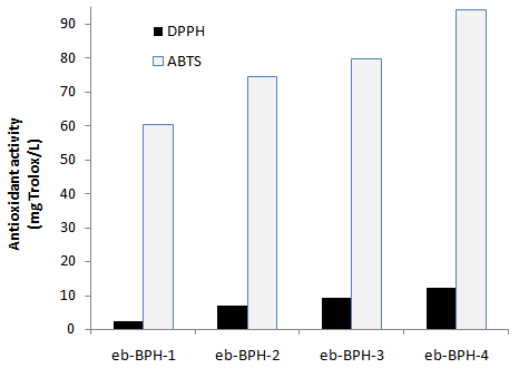

Fig 2 Antioxidant activities of eb-BPH and membrane fractions.

CONCLUSION

The eb-BPH was produced under optimal conditions using 15% E/S for 6 h. After fractionation, small peptides (eb-BPH-4, below 1 kDa) had the highest antioxidant assay and presence of high level of surface hydrophobicity. The high activity of eb-BPH-4 in these antioxidant assay systems may be related to the high levels of surface hydrophobicity because the hydrophobic residues was an important characteristic associated with their ability to trap free radicals. It was suggested that Boletus mushroom protein hydrolysate could potentially used as antioxidant agents in functional foods and nutraceuticals applications.

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