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Amino acids and antioxidant properties of the oyster mushrooms, *Pleurotus ostreatus* and *Pleurotus sajor-caju*

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ABSTRACT: The objective of this study was to investigate the amino acid composition and antioxidant properties of two oyster mushroom species, *Pleurotus ostreatus* and *Pleurotus sajor-caju*, commercially cultivated in NE Thailand. Proximate composition and dietary fibre of dried mushrooms were determined. Amino acid composition of fresh mushrooms was analysed by HPLC. Water and ethanol extracts were obtained from dried mushrooms and determined for total phenolic contents (TPC) by the Folin-Ciocalteu method, free radical scavenging efficacy by the DPPH method, and reducing power by the ferric reducing-antioxidant power method. Except for protein, proximate compositions of dried mushrooms were significantly different. Similar amino acid profiles in both mushrooms were obtained, with glutamic acid, aspartic acid, and arginine being the three most abundant. Water extracts contained higher TPC and possessed better antioxidant activities than did ethanol extracts for both mushrooms. Overall, *P. ostreatus* possessed more antioxidant than *P. sajor-caju*. The EC_{50} of *P. ostreatus* and *P. sajor-caju* water extracts were 11.56 and 13.38 mg/ml, respectively, while those of the ethanol extracts were 31.75 and 58.44 mg/ml, respectively.

KEYWORDS: DPPH, FRAP

INTRODUCTION

Mushrooms have been widely used as food or food ingredients in many food products for a long time. Some edible mushrooms have been used because of their antitumour, antifungal, and reducing hypercholesterolemia activities[1–3]. From a nutritional point of view, mushrooms contain high protein and low fat. In addition, high dietary fibre of mushrooms was reported to function as an antitumour and antiviral agent[4,5]. Moreover, mushrooms are recognized as a good source of amino acids which play an important role in their flavours[6].

Pleurotus mushrooms, commonly known as oyster mushrooms, grow wildly in tropical and subtropical areas, and are easily artificially cultivated. They are healthy foods, low in calories and in fat, rich in protein, chitin, vitamins and minerals[7–9]. They also contain high amounts of γ-amino butyric acid (GABA) and ornithine. GABA is a nonessential amino acid that functions as a neurotransmitter whereas ornithine is a precursor in the synthesis of arginine[8]. Jayakumar et al[10] revealed that an extract of *P. ostreatus* was able to alleviate the hepatotoxicity induced by CCl_4 in rats. Jayakumar et al[11] also reported that the extract from *P. ostreatus* appeared to protect major organs such as the liver, heart, and brain of aged rats against oxidative stress. There are about 40 species of *Pleurotus* mushrooms and they rank second among the important cultivated mushrooms in the world[2]. The objectives of this study were to investigate the amino acid compositions, and antioxidant properties of the two most commercially cultivated oyster mushrooms, *P. ostreatus* (Fr.) Kummer and *P. sajor-caju* (Fr.) Singer, in Thailand.

MATERIALS AND METHODS

Materials

P. ostreatus and *P. sajor-caju* were obtained from a local mushroom farm in Nakhon Ratchasima, Thailand. The mushrooms were dried in a tray dryer (NewWay, TD 372, Manufacturing Co., Ltd.) at 65 °C overnight to reduce the moisture to about 4–5%. The dried mushrooms were finely ground, immediately vacuum-packed in polyethylene bags, and kept in a freezer at −20 °C until use.

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu's reagent, gallic acid and 2,4,6-Tri(2-pyriayl)-s-triazine (TPTZ) were purchased from

Sigma Chemicals (St. Louis, MO). Amino acid standard mix was composed of L-alanine, L-arginine, L-aspartic acid, L-cystine, L-glutamic acid, L-histidine hydrochloride monohydrate, glycine, L-isoleucine, L-leucine, L-lysine hydrochloride, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine (Agilent PN 5061-3331), supplemental amino acids (L-glutamine, L-asparagine, L-tryptophan, L-norvaline, hydroxyproline, sarcosine) (Agilent PN 5062-2478), borate buffer (Agilent PN 5061-3339), *o*-phthaldialdehyde 3-mercaptopropionic acid (OPA-3MPA) (Agilent PN 5061-3335), and 9-fluorenylmethyl chloroformate (FMOC) (Agilent PN 5061-3337), all from Agilent Technologies (Wilmington, DE). Acetronitrile and methanol were HPLC grade, and other chemicals used were reagent grade.

Proximate analysis

Proximate composition of dried mushroom was determined according to Association of Official Analytical Chemists methods[12] with slight modifications. The moisture content was determined by drying in an oven at 105 °C for 24 h. The ash content was determined by ashing at 600 °C in a muffle furnace (Carbolite) for 6 h or until light grey or white ash was obtained. Total protein content was determined by the Kjeldahl method with a conversion factor of 6.25. Fat content was determined by the Soxhlet method (2050 Soxtec auto-extraction unit) and dietary fibre by the enzymatic gravimetric method. Carbohydrate content was calculated by difference.

Total amino acid analysis

Each fresh mushroom sample (0.2–1 g) was hydrolysed under nitrogen gas with 15 ml of 6 N HCl in an autoclave at 110 °C for 24 h and neutralized to pH 7.00 by 4 N NaOH. Alkaline hydrolysis for tryptophan determination was done according to the method of Wu and Tanoue[13]. Briefly, the fresh mushroom sample (1–3 g) was hydrolysed under nitrogen gas with 10 ml of 4 N NaOH and 200 µg of ascorbic acid as antioxidant in an autoclave at 110 °C for 16 h and adjusted to pH 9.00. The hydrolysate was filtered through a 0.45 µm cellulose acetate membrane filter before injection into the HPLC.

The amino acid composition was determined by reversed phase HPLC and gradient elution according to the method of Henderson et al[14]. The analysis was carried out using the Agilent 1100 HPLC system (Agilent Technologies) with autosampler, a Zorbax-Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm) with a Zorbax Eclipse-AAA guard column (4.6 × 12.5 mm, 5 µm) and fluorescence detector. Chemstation Rev.A.09.03 (1417) (Agilent Technologies 1990–2002) was used for data acquisition and analysis.

The sample was submitted to automatic pre-column derivatization with a combination of OPA-3MPA for primary amino acids and FMOC for secondary amino acids. The mobile phase A contained 40 mmol/l Na_2HPO_4 at pH 7.8 and B contained 45% acetonitrile, 45% methanol, and 10% deionized water. The chromatographic column temperature was set at 40 °C with a flow rate of 2 ml/min. The detector was set at 340/450 (Ex/Em) at 0 min and at 266/305 (Ex/Em) at 15 min.

Preparation of mushroom extracts

The mushroom extracts were prepared from dried mushrooms to evaluate the antioxidant property. The extractions were made using 95% ethanol and water following the method of Tsai et al[15]. The dried sample (10 g) was extracted with 100 ml solvent at room temperature on a shaker at 150 rpm for 24 h and filtered through No. 4 Whatman filter paper. The residue was re-extracted twice and the filtrates were combined. The extract was evaporated almost to dryness in a rotary evaporator (Rotavapor R-114, Buchi) at 40 °C and then subjected to freeze drier (LYOVAC, GEA). The dried extract was re-dissolved in the solvent to a concentration of 20 mg/ml and stored at 4 °C for antioxidant evaluation.

Total phenolic content

The total phenolic content was determined by Folin-Ciocâlteu method[16]. Sample solution of 100 µl was added to 2 ml of 2% sodium carbonate, mixed thoroughly and allowed to stand for 2 min. Then, 100 µl of Folin-Ciocâlteu reagent (Folin:Methanol, 1:1, v/v) was added and the mixture was mixed well. After incubation for 30 min, the absorbance was measured at 750 nm. A calibration curve was obtained using various concentrations of gallic acid. The total phenolic content of the sample was expressed as mg of gallic acid equivalents (GAEs) per gram of dry sample.

Free radical scavenging activity

The DPPH method was used to determine for radical scavenging activity of mushroom extracts according to the method of Sánchez-Moreno et al[17]. Various concentrations of samples (0.05 ml) were added to 1.95 ml of methanolic DPPH (0.1 mM) and mixed thoroughly. The mixture was left to stand for 45 min in the dark at room temperature and the absorbance was

measured at 515 nm. A lower absorbance represented a higher DPPH scavenging activity. The scavenging effect was expressed as scavenging activity $(1 - A_c/A_d)$, where A_c is the absorbance of solution when the extract is added at a particular concentration, and A_d is the absorbance of the DPPH solution.

Ferric reducing-antioxidant power

Ferric reducing-antioxidant power (FRAP) assay was determined according to the method of Katalinic et al[18] with some modification. A 1.5 ml of FRAP reagent containing TPTZ (10 mM in 40 mM HCl), $FeCl_3$ (20 mM) and acetate buffer pH 3.6 (300 mM) in a ratio of 10:1:1 (v/v/v) was pre-incubated at 37 °C for 8 min and then mixed with 150 µl of deionized water and 50 µl of test sample. The mixture was left to stand at 37 °C for 8 min and the absorbance was measured at 593 nm. A calibration curve was obtained from various concentrations of $FeSO_4 \cdot 7\,H_2O$ (0.1–1 mM). The FRAP value of sample was expressed in mmol Fe^{2+}/l.

Statistical analysis

The experiment was carried out in duplicate and the composition analysis performed in triplicate. The data was statistically analysed in completely randomized design by Statistical Analysis System software[19]. Statistically significant differences ($p < 0.05$) among means of experiment results were evaluated by analysis of variance and means compared by Duncan's Multiple Range Tests.

RESULTS AND DISCUSSION

Proximate composition

The chemical composition of both dried oyster mushrooms is shown in Table 1. The original dried mushroom samples contained 4–5% moisture. Except for protein, proximate compositions of both dried oyster mushrooms were significantly different ($p < 0.05$). The protein, ash and carbohydrate contents of these oyster mushrooms were higher than previously reported[7,8,20]. *Pleurotus ostreatus* contained significantly higher ($p < 0.05$) dietary fibre (45.5%) than did *P. sajor-caju* (42.5%). The lipid content of both mushroom species were generally very low and less than 1% dry matter which were in agreement with the results of Akindahunsi and Oyetayo[7] and Manzi et al[8] for *P. ostreatus* and other species. In addition, similarly high amounts of dietary fibre were in agreement with the results of Cheung[21] who reported that the dietary fibre content of *P. sajor-caju* was about 42% dry matter. Oyster mushrooms, and in particular *P. sajor-caju*, have been reported to have antitumor and antiviral activity due to the high content of dietary fibre[22].

Table 1 Proximate composition of dried oyster mushrooms.

Composition (%)	*P. ostreatus*	*P. sajor-caju*
Moisture	4.46 ± 0.27^b	5.32 ± 0.30^a
Protein	20.82 ± 0.45	21.30 ± 0.29
Fat	0.56 ± 0.03^a	0.29 ± 0.02^b
Ash	5.81 ± 0.05^b	7.95 ± 0.05^a
Dietary Fibre	45.50 ± 0.05^a	42.50 ± 0.09^b
Carbohydrate	68.35^a	65.14^b

Values in the same row with different letters are significantly different ($p < 0.05$, $n = 6$).

Table 2 Amino acid content of fresh oyster mushrooms, (mg/g fresh weight).

Amino acid	*P. ostreatus*	*P. sajor-caju*
Alanine	1.90 ± 0.015^a	1.80 ± 0.014^b
Arginine	3.26 ± 0.015^a	2.52 ± 0.006^b
Aspartic acid	2.04 ± 0.020	2.01 ± 0.014
Glutamic acid	5.01 ± 0.015^a	4.22 ± 0.027^b
Glycine	0.83 ± 0.019^b	0.96 ± 0.014^a
Histidine*	0.55 ± 0.012^a	0.52 ± 0.013^b
Isoleucine*	0.62 ± 0.007^b	0.75 ± 0.016^a
Leucine*	1.13 ± 0.008^b	1.30 ± 0.016^a
Lysine*	0.71 ± 0.007^b	0.78 ± 0.013^a
Methionine*	0.28 ± 0.006^b	0.34 ± 0.009^a
Norvaline	0.03 ± 0.001^a	0.02 ± 0.002^b
Phenylalanine*	0.73 ± 0.006^b	0.86 ± 0.016^a
Proline	0.29 ± 0.008^b	0.33 ± 0.009^a
Serine	1.09 ± 0.009^a	0.99 ± 0.011^b
Threonine*	1.01 ± 0.010	0.98 ± 0.0102
Tryptophan*	0.15 ± 0.001^a	0.09 ± 0.005^b
Tyrosine	0.57 ± 0.008^b	0.67 ± 0.014^a
Valine*	0.91 ± 0.010^b	0.98 ± 0.013^a
Total amino acid	21.10	20.12

Values in the same row with different letters are significantly different at $p < 0.05$, $n = 6$
* Essential amino acids

Amino acid compositions

A total of 18 amino acids were recorded in *P. ostreatus* and *P. sajor-caju* (Table 2). However, cystine which is one of the essential amino acids for humans, was not detected due to the fact that it cannot give fluorescence under the conditions used. Cystine has been detected by diode array detectors only[14].

Overall, the amino acid profiles were similar in both mushroom species as they belong to the same

family of *Pleurotus*. Although significantly different concentrations ($p < 0.05$) of some amino acids did exist, both mushrooms had comparable amounts of total amino acids – 21.11 mg/g and 20.12 mg/g fresh weight, for *P. ostreatus* and *P. sajor-caju*, respectively. In terms of individual amino acids, the three most abundant amino acids found in both mushroom species were glutamic acid, aspartic acid, and arginine. This was in agreement with the reports of Manzi et al[8], Mattila et al[9] and Mendez et al[23] for *P. ostreatus*, Akindahunsi and Oyetayo[7] for *P. tuber-regium*, and Mdachi et al[24] for *P. sajor-caju*.

Leucine was found to be most abundant in both mushroom species, whereas threonine and valine were found to be the second and the third most abundant essential amino acids, respectively. The three most abundant amino acids of these mushrooms were consistent with the results reported by Manzi et al[8] and Mdachi et al[24]. On the other hand, Akindahunsi and Oyetayo[7] reported valine was the most abundant amino acid in other *Pleurotus* species while threonine and leucine were found to be the second and third most abundant, respectively. It is possible that the differences of amino acids found in the same mushroom species could be a consequence of the genetic variation and cultivation process applied in commercial practices which was also revealed by Chiu et al[25] and Mendez et al[23]. Tryptophan was found to be the least abundant essential amino acid in the *Pleurotus* mushroom which was in agreement with the reports of Manzi et al[8] and Mdachi et al[24].

Total phenolic contents

Phenolic compounds have been reported to be the major antioxidant components found in mushrooms, whereas other potential antioxidants such as ascorbic acid, β-carotene, lycopene, and γ-tocopherol have only been found in very small amounts[26–28]. The total phenolic contents (TPC) in water and ethanol extracts of both commercial oyster mushrooms were 42.47 ± 2.27 and 30.93 ± 1.92 GAEs/g dry weight for *P. ostreatus*, and 37.98 ± 1.88 and 29.30 ± 1.60 GAEs/g dry weight for *P. sajor-caju*, respectively. The TPC in water extracts were higher ($p < 0.05$) than in ethanol extracts for both mushrooms. Moreover, the water extract TPC of *P. ostreatus* differed from that of *P. sajor-caju* while the ethanol extract TPCs of both mushrooms were not significantly different (29.30–30.93 GAEs/g dry weight). However, with regard to solvents used, it was obvious that *P. ostreatus* contained higher TPC than *P. sajor-caju*. Higher amounts of phenolic compounds were obtained with increasing the solvent polarity, which was in agreement with reports by Cheung et al[29] and Puttaraju et al[30]. Hence, the higher total phenolic content of *P. ostreatus* might account for the better antioxidant activity found in its scavenging activity on DPPH radical and reducing power.

ANTIOXIDANT ACTIVITY

Radical scavenging activity on DPPH

Free radical scavenging is one of the mechanisms in inhibiting lipid oxidation commonly used to estimate antioxidant activity. The radical scavenging activity (RSA) of mushroom extracts was tested against the DPPH. Fig. 1 shows the RSA values of mushroom extracts in different concentrations, expressed as the ratio of sample absorbance decrease and the absorbance of DPPH solution in the absence of extract at 515 nm. The RSA of water extracts from both oyster mushrooms was found to be higher ($p < 0.05$) than those of ethanol extracts and water extract of *P. ostreatus* provided the highest ($p < 0.05$) RSA at the same concentration tested. The RSA results were consistent with the total phenolic contents of both oyster mushrooms, the higher phenolic content the better RSA. In addition, since both mushrooms contained similar total amino acid contents (Table 2), the RSA of these two oyster mushrooms might have a strong correlation with the phenolic content rather than their amino acid contents, although some amino acids were known to have antioxidant activity[31]. In addition,

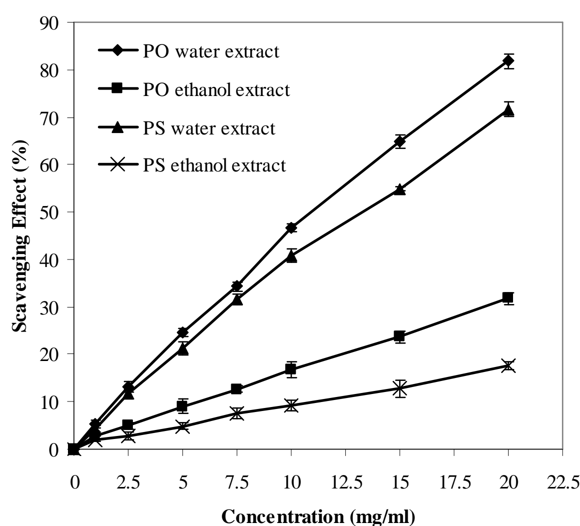

Fig. 1 Radical scavenging effect on DPPH of water and ethanol extracts of oyster mushrooms. In this and next figure: PO = *Pleurotus ostreatus*, PS = *Pleurotus sajor-caju*. Each value is expressed as mean with error bars indicating the standard deviation ($n = 6$).

the antioxidant capacity of these oyster mushroom species could also be contributed to from water-soluble dietary fibre[5,22] (nonstarch polysaccharides). Therefore, a higher antioxidant activity of *P. ostreatus* than that of *P. sajor-caju* could contribute to its higher ($p < 0.05$) dietary fibre content (Table 1).

The water extract of *P. ostreatus* gave the highest RSA followed by the extract of *P. sajor-caju* in the same solvent. The half effective concentration (EC_{50}) of *P. ostreatus* water extract was 11.56 mg/ml and that of *P. sajor-caju* was 13.38 mg/ml. Similarly, the ethanol extract of *P. ostreatus* was also found to be higher than that of *P. sajor-caju* with EC_{50} values of 31.75 mg/ml and 58.44 mg/ml, respectively. Overall, it could be concluded that between these two particular oyster mushrooms, the *P. ostreatus* had a better antioxidant activity in terms of RSA on the DPPH free radical.

Ferric reducing-antioxidant power

As with other studies on mushroom extracts[26,27,32], the reducing power abilities of mushroom extracts increased with concentration (Fig. 2). The antioxidant activities of both oyster mushroom extracts in terms of FRAP were similar to their RSA on DPPH radical in that both water and ethanol extracts of *P. ostreatus* gave higher ($p < 0.05$) activity than those of *P. sajor-caju*, and the highest ($p < 0.05$) FRAP value was obtained from the water extract of *P. ostreatus*. At 20 mg/ml the reducing power was as high as 1.00. *P. ostreatus* water extract (4.38 mmol Fe^{2+}/l) $>$ *P. sajor-caju* water extract (2.09 mmol Fe^{2+}/l) $>$ *P. ostreatus* ethanol extract (1.61 mmol Fe^{2+}/l) $>$ *P. sajor-caju* ethanol extract (1.07 mmol Fe^{2+}/l). Yang et al[28] reported that oyster mushrooms had a higher reducing power than shiitake and golden mushrooms. Puttaraju et al[30] also reported that water extracts of mushrooms revealed better antioxidative reducing power ability. From the FRAP values of both oyster mushroom extracts in this study, it could be noted that *P. ostreatus* had a higher reducing power due to the fact that it contained higher total phenolic content and higher dietary fibre (Table 1) than *P. sajor-caju* which was in agreement with the results of Zhang et al[22] and Zhang et al[5].

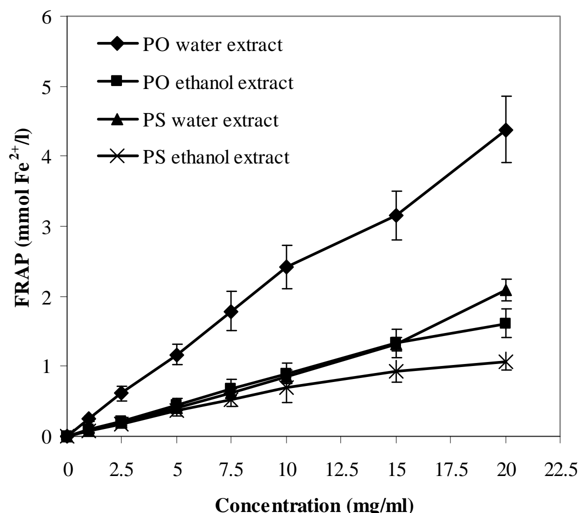

Fig. 2 Ferric reducing-antioxidant power of water and ethanol extracts of oyster mushrooms.

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