Kasetsart J. (Nat. Sci.) 44 : 664 - 670 (2010)

Quality and Quantity of Protein in Certain Kinds of Edible Mushroom in Thailand

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ABSTRACT

The chemical composition and nutritional value were determined in four edible mushroom species from Thailand, namely Nang Rom mushroom (*Pleurotus sajorcaju*) Pao Heo mushroom (*Pleurotus ostreatus*) Hom mushroom or Shitake (*Lentinus edodes*) and Kra dang mushroom (*Lentinus lepidus*). Protein, fat, crude fiber and carbohydrate were 19.59-24.68, 0.25-1.42, 12.29-18.52 and 49.42-55.42% dry weight, respectively. The corrected protein efficiency ratio (C-PER), net protein utilization (NPU), biological value (BV) and true digestibility (TD) were 0.03-1.29, 30.32-40.20, 63.72-77.18 and 47.32-52.16, respectively, compared to values in casein of 2.50, 75.27, 85.98 and 87.49, respectively. Amino acid analysis showed that the protein contained nutritionally useful quantities of essential amino acids, while tryptophan was the limiting amino acid in all the samples. The protein digestibility corrected amino acid score (PDCAAS) of the four mushroom species ranged between 0.32 and 0.45. The results showed that the Hoom mushroom or Shitake had better nutritional protein qualities than the other species studied.

Keywords: mushroom, nutritional value, protein digestibility corrected amino acid score (PDCAAS), corrected protein efficiency ratio (C-PER), net protein utilization (NPU), biological value (BV), true digestibility (TD)

INTRODUCTION

More than 2,000 species of mushrooms exist in nature. However, less than 25 species are widely accepted for human consumption and only a few have attained commercial status (Lindequist *et al*., 2005). Mushrooms are a good source of vitamins and minerals and are preferred due to their special flavor and aroma. Different mushrooms have been studied by the scientific community in searching for new therapeutic alternatives and the results have proven their bioactive properties (Mattila *et al*., 2000). Mushrooms are rich sources of nutraceuticals (Elmastas *et al*., 2007; Ribeiro *et al*., 2007) that are responsible for their antioxidant, antitumor (Wasser and Weis, 1999), and antimicrobial properties (Hatvani, 2001; Lindequist *et al*., 2005; Barros *et al*., 2007a; Turkoglu *et al*., 2007)

Besides their pharmacological features, mushrooms are becoming more important due to their nutritional value, related to high protein, low fat and energy contents (Diez and Alvarez, 2001; Barros *et al*., 2007b). Several studies have been carried out on the chemical composition and nutritional quality of edible mushrooms from

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Received date : 18/11/09 Accepted date : 03/03/10

different countries, particularly Spain (Diez and Alvarez, 2001) and Italy (Manzi *et al*., 2001; Manzi *et al*., 2004).

However, there are no reports on the protein quality of mushrooms in Thailand in terms of a protein digestibility corrected amino acid score (PDCAAS), protein efficiency ratio (PER), net protein utilization (NPU), biological value (BV) and true digestibility (TD). Thus, the objectives of this study were to: 1) determine the chemical composition and amino acid pattern of four edible mushroom species, namely *Pleurotus sajorcaju, Pleurotus ostreatus, Lentinus edodes* and *Lentinus lepidus* ; and 2) evaluate the protein quality of these mushrooms in terms of PDCAAS, C-PER, NPU, BV and TD.

MATERIALS AND METHODS

Mushroom sample

The four types of mushroom (Nang Rom mushroom (*Pleurotus sajorcaju*) Kra Dang mushroom (*Lentinus lepideus*) Pao Heo mushroom (*Pleurotus ostreatus*) and Hoom mushroom or Shitake (*Lentinus edodes*)) were purchased from the Saphan Mai market in Bangkok. All samples were prepared by cleaning, washing, boiling for 10 min and drying in an oven at 60°C for 6 hr. The dried mushroom samples were ground in a pin mill.

Chemical analysis

The samples were analyzed for moisture fat, protein, ash and crude fiber contents using the standard methods of AOAC (2000).

Amino acid analysis

The composition of amino acids was determined by the Food Quality Assurance Service Center, Kasetsart University, using high-performance liquid chromatography (HPLC), and the method described by Petritis *et al*. (2002).

In-vitro protein digestibility (IVPD)

In vitro protein digestibility (IVPD) of all samples was determined by the extent to which the pH dropped from pH 8 when the samples were subjected to sequential digestion with a multienzyme mixture using a modification of the multienzyme technique according to Hsu *et al*. (1977) and Satterlee *et al*. (1979). The enzymes used in the *in-vitro* protein digestion study were purchased from Sigma Chemical Co. Ltd., St. Louis, Missouri, USA. These were: porcine intestinal peptidase, porcine pancreatic trypsin (type IX), bovine pancreatic chymotrypsin (type II) and peptidase with registry numbers 9031-95-3, 9002-07-7, 9004-07-3 and 9031-96-3, respectively. The IVPD of each sample was calculated using Equation 1:

$$\text{Digestibility } \% = 234.84 - 22.56\,K \quad (1)$$

where: K is the pH recorded after a total digestion period of 10 min.

The multienzyme solution was freshly prepared before each series of tests. All analyses of each sample were done in triplicate.

Protein efficiency ratio (PER) and nitrogen balance studies

The standard methods of AOAC (2000) for the assessment of the protein efficiency ratio (PER) and nitrogen balance studies were followed. Three-week-old weanlinged male Sprague-Dawley rats were obtained from the National Laboratory Animal Center, Mahidol University. The experimental protocol was developed according to the guidelines of the Committee on the Care and Use of Animals for Scientific Purposes, National Research Council of Thailand. The rats had a mean initial weight of 50-60 g, with the mean body weight range within a group of not more than 10 g and between groups of not more than 5 g.

All rats were housed in individual, stainless steel, metabolic cages in an experimentally controlled environment at 20-22°C and

60% relative humidity, with a 12-hour, light-dark cycle. Rats were assigned randomly to five groups, with eight rats per group. Rats were given free access to their diet and water throughout the 28-day feeding period. Daily food intake and weekly body weight were recorded. The diet was prepared by the AOAC method (2000) and casein was the reference protein. The protein efficiency ratio (PER), net protein utilization (NPU), biological value (BV) and true digestibility (TD) were determined.

Amino acid score (AAS) and protein digestibility-corrected amino acid score (PDCAAS)

The amino acid score (AAS) is a measure of the actual amount of individual amino acids in a foodstuff, or in the diet relative to the need for this amino acid. This ratio is defined according to Equation 2:

$$\text{AAS} = \frac{\text{AA content (mg/g of protein) of food protein}}{\text{AA content of FAO/WHO/UNU (1985) pattern for 2 - 5 year old child}} \quad (2)$$

This ratio does not evaluate whether the protein is digestible or not. However, FAO/WHO has adopted a new scale called the protein digestibility corrected amino acid score (PDCAAS). It is much better and more accurate in relation to the true needs of humans and the scoring of food. PDCAAS is defined by the concentration of the limiting amino acid in the food protein, which is expressed as the proportion or percentage of the limiting amino acid concentration in a standard or reference amino acid pattern using Equation 3:

$$\text{PDCAAS} = \frac{\text{AA content (mg/g of protein) of food protein} \times \text{digestibility}}{\text{AA content of FAO/WHO/UNU (1985) pattern for 2 - 5 year old child}} \quad (3)$$

Statistical analysis

Data were analyzed using ANOVA and Duncan's new multiple range test.

RESULTS AND DISCUSSION

The results of the chemical composition analysis are shown in Table 1. Protein was found in high levels and varied between 19.59 g/100 g in Pao Heo mushroom (*Pleurotus ostreatus*) and 24.68 g/100 g in Hoom mushroom (*Lentinus edodes*). Fat ranged from 0.25 g/100 g in Nang Rom mushroom (*Pleurotus sajorcaju*) to 1.42 g/100 g in Hoom mushroom. Fiber ranged from 12.29 to 18.52 g/100 g. These high protein and low fat characteristics of mushrooms have been previously reported by many workers (Aletor, 1995; Longvah and Deosthale, 1998; Diez and Alvarez, 2001).

The amino acid composition of the mushrooms is shown in Table 2. The amino acid results showed that the protein contained nutritionally useful quantities of most of the essential amino acids, while tryptophan was the limiting amino acid in all the samples.

Table 3 show the proximate analysis of four mushroom diets and one control (casein). According to AOAC (2000), the diet must contain 10 ± 0.3% protein, which is present as a single source of protein.

Table 1 Chemical composition (%) of mushrooms and casein.

Local name	Moisture	Protein	Fat	Fiber	Ash	Carbohydrate
Nang Rom mushroom	7.57	21.22	0.25	16.68	3.09	51.19
Kra Dang mushroom	7.21	21.15	0.70	13.68	1.84	55.42
Pao Heo mushroom	7.58	19.59	0.62	18.52	3.66	50.03
Hoom mushroom or Shitake	7.45	24.68	1.42	12.29	4.74	49.42
Casein	10.46	81.35	1.00	1.64	3.95	1.60

Table 2 Essential amino acid composition of mushrooms, casein and FAO/WHO standard.

Essential amino acid / Sample	Amino acid mg/gm of protein					FAO/WHO[1] 1972
	Nang Rom mushroom	Kra Dang mushroom	Pao Heo mushroom	Hoom mushroom	Casein	
Isoleucine	38	36	36	37	41	40
Leucine	57	59	56	53	97	70
Lysine	40	38	38	41	71	55
Methionine+Cystine	55	54	50	60	44	35
Phenylalanine+Tyrosine	58	58	59	54	101	60
Threonine	49	43	48	50	44	40
Tryptophan	7 *(70)	6 *(60)	7 *(70)	6 *(60)	-	10
Valine	53	58	66	48	53	50

1 Source : Food composition table for use in East Asia (FAO, 1972).

$$*(\ )\ \text{Chemical score} = \frac{\text{amino acid content in diet}}{\text{amino acid content in FAO/WHO Standard}} \times 100$$

Table 3 Chemical composition (%) of mushroom diets and casein.

Test diet	Protein	Moisture	Fat	Ash	Crude fiber
Nang Rom mushroom	10.16	7.93	10.18	4.16	6.85
Kra Dang mushroom	9.89	8.68	10.99	3.68	7.85
Pao Heo mushroom	9.97	8.79	11.56	4.60	7.57
Hoom mushroom	10.12	9.31	11.32	4.69	5.58
Casein	10.00	10.83	8.98	3.49	2.00

The protein quality of mushrooms evaluated by the bioassay in rats as PER, NPU, BV and TD is shown in Table 4. Hoom mushroom (*Lentinus edodes*) had the highest C-PER value (1.29) and Kra Dang mushroom (*Lentinus lepideus*) had the lowest C-PER (0.03). Values for NPU, BV and TD ranged from 30.32 to 40.20, 63.72 to 77.18 and 43.38 to 52.16, respectively, compared to 75.27, 85.98 and 87.49, respectively in casein. True digestibility has been reported previously for two species of mushroom, namely *Schizoplrillum commune* (53.2%) and *Lentinus edodes* (76.3%) (Longvah and Deosthale, 1998).

However, all the parameters studied were significantly lower in these mushroom diets compared to casein. The low chemical score and deficiency of sulfur-containing amino acids in these mushrooms may be responsible for the poor performance of animals fed the mushrooms diets. Normally these mushrooms are dried, so that polyphenols and antinutritional factors, such as tannins, may be present, thereby affecting either or both of the digestion and absorption of nutrients (Huisman, 1991). Based on the in-vivo biological values examined, the protein quality of *L. edodes* appeared to be better than the others.

The essential amino acid composition of each individual mushroom with its limiting amino acid is shown in Table 5. The amino acid results showed that the protein contained nutritionally useful quantities of most of the essential amino acids, while tryptophan was the limiting amino acid in all the samples.

Table 4 Corrected protein efficiency ratio (C-PER), net protein utilization (NPU), biological value (BV) and true digestibility (TD).

Test diet	C-PER[1]	NPU[2]	BV[3]	TD[4]
		%		
Nang Rom mushroom	1.13 ± 0.50[b]	31.26 ± 3.49[b]	71.94 ± 2.76[b]	43.38 ± 3.47[b]
Kra Dang mushroom	0.03 ± 0.39[c]	30.32 ± 6.74[b]	63.72 10.66[b]	47.32 ± 3.02[b]
Pao Heo mushroom	1.02 ± 0.15[b]	35.34 ± 2.33[b]	74.82 ± 3.14[ab]	47.21 ± 1.31[b]
Hoom mushroom	1.29 ± 0.19[b]	40.20 ± 1.74[b]	77.18 ± 2.73[ab]	52.16 ± 3.43[b]
Casein	2.50 ± 0.26[a]	75.27 ± 4.32[a]	85.98 ± 3.63[ab]	87.49 ± 1.80[a]

Values are means of eight animals in each group and different letters indicate significant differences at $P < 0.05$.

1. Corrected protein efficiency ratio (C-PER)
   = weight gain/protein consumed (corrected PER as adjusted to 2.50 for casein.)
2. Net protein utilization (NPU) = (retained nitrogen/intake nitrogen) 100
3. Biological value (BV) = (retained nitrogen/absorbed nitrogen) 100
4. True digestibility = (absorbed nitrogen/intake nitrogen) 100

Table 5 Amino Acid Score of mushrooms and casein.

Essential amino acid / Test diet	Uncorrected amino acid score mg/g of protein				
	Nang Rom	Kra Dang	Pao Heo	Hoom	Casein
Isoleucine	0.95	0.90	0.90	0.92	1.02
Leucine	0.81	0.84	0.80	0.76	1.39
Lysine	0.73	0.69	0.69	0.74	1.29
Methionine+cystine	1.57	1.54	1.43	1.71	1.26
Phenylalanine+Tyrosine	0.97	0.97	0.98	0.90	1.68
Threonine	1.22	1.08	1.20	1.25	1.10
Tryptophan	0.70	0.60	0.70	0.60	-
Valine	1.06	1.16	1.32	0.96	1.06

$$\text{Uncorrected amino acid score} = \frac{\text{amino acid in test}}{\text{amino acid in FAO/WHO Standard}}$$

In-vitro and *in-vivo* digestibility are shown in Table 6. In-vitro digestibility had higher values than for in-vivo digestibility, excepted for casein. There were significant differences between the in-vitro digestibility and in-vivo digestibility methods.

Using the amino acid scores, an approximate protein digestibility corrected amino acid score (PDCAAS) was calculated for assessing the overall protein quality according to the Nutrition Labeling Regulations of the Food and Drug Administration (FDA, 1993). PDCAAS is the product of the lowest uncorrected amino acid score and protein digestibility. The results of PDCAAS in this study varied between 0.38 and 0.45 compared to a value of 0.86 for casein. However, the amino acid content and protein quality in edible mushrooms varied widely between species and even depended on the environmental conditions where they were grown, with the protein qualities of these mushroom comparable to those of some selected vegetable proteins (Diez and Alvarez, 2001).

Table 6 *In-vitro* digestibility and *in-vivo* digestibility (%).

Test diet	*In-vitro* digestibility	*In-vivo* digestibility
Nang Rom mushroom	63.61 ± 2.48[b]	43.38 ± 3.47[b]
Kra Dang mushroom	66.09 ± 2.74[b]	47.32 ± 3.02[b]
Pao Heo mushroom	62.41 ± 2.05[b]	47.21 ± 1.31[b]
Hoom mushroom or Shitake	63.69 ± 5.31[b]	52.16 ± 3.43[b]
Casein	83.91 ± 7.86[a]	87.49 ± 1.80[a]

Means within a column with the same letter are not significantly different at P≥0.05.

Table 7 Calculation of protein digestibility corrected amino acid score (PDCAAS) of four kinds of mushroom compared to casein.

Test diet	*In-vitro* digestibility (%)	Lowest uncorrected amino acid score	PDCAAS *
Nang Rom mushroom	63.61 ± 2.48[b]	0.70	0.45[b]
Kra Dang mushroom	66.09 ± 2.74[b]	0.60	0.40[b]
Pao Heo mushroom	62.41 ± 2.05[b]	0.70	0.44[b]
Hoom mushroom or Shitake	63.69 ± 5.31[b]	0.60	0.38[b]
Casein	83.91 ± 7.86[a]	1.02	0.86[a]

Means within a column with the same letter are not significantly different at P≥0.05.

* PDCAAS = Lowest uncorrected amino acid score × digestibility/100

The results of a study by Henley and Kuster (1994) on the PDCAAS for many proteins showed that the PDCAAS of casein, beef, peanuts, whole wheat and wheat gluten was 1.00, 0.92, 0.52, 0.40 and 0.25, respectively. Thus, the PDCAAS values of mushrooms in the current study were comparable to whole wheat.

CONCLUSION

This study showed that Hoom mushroom or Shitake (*Lentinus edodes*) had a higher protein content (24.68%) than the others. Pao Heo mushroom (*Pleurotus ostreatus*) had the lowest protein content (19.59%). Protein quality evaluation showed that Hoom mushroom had the highest C-PER (1.29) and Kra Dang mushroom had the lowest C-PER (0.03). In conclusion, the chemical composition and nutritional value of these mushrooms clearly indicate that they provide key nutrients such as protein, fat, fiber, carbohydrate and could prove to be excellent foods that can be used in low calorie diets because of their low fat content and energy.

ACKNOWLEDGEMENTS

The author would like to acknowledge the Kasetsart University Research and Development Institute (KURDI) for providing research funds.

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