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COMPARISON OF AMINO ACID CONTENT IN FROZEN *P. OSTREATUS* AND *A. BISPORUS* MUSHROOMS*

Emilia Bernaś, Grażyna Jaworska
Agricultural University of Krakow

Background. The present work compares the content of basic chemical constituents as well as amino acids in frozen *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. and *Agaricus bisporus* (Lange) Sing. mushrooms.
Material and methods. The material investigated comprised of frozen *P. ostreatus* and *A. bisporus* mushrooms after 8 months' storage, having been soaked and blanched in a solution 0.5% citric acid, 0.5% lactic acid and 0.1% L-ascorbic acid prior to freezing.
Results. Compared with frozen *A. bisporus*, frozen *P. ostreatus* contained significantly higher levels of total carbohydrates (32% higher), raw fat (38%) and most endogenous (6-163%) and exogenous (10-200%) amino acids, but lower levels of ash (45% lower), total nitrogen (50%) and protein nitrogen (40%). In both species, asparagine and glutamine were the most abundant endogenous amino acids (respectively 10-12% and 12-19% of total amino acids), and leucine and lysine the most abundant exogenous amino acids (7-9% and 6-7%). The least abundant were glycine and proline (both 5%), and cysteine and methionine (1% and 2%).
Conclusions. Frozen mushrooms of both species are potentially good sources of protein in the diet as no limiting amino acids were found when compared with the FAO/WHO reference protein pattern. However, it is important to determine the digestibility of protein in the mushrooms model studies using animals and people. The conversion coefficient from total nitrogen to protein was 5.23 in *P. ostreatus* and 2.46 in *A. bisporus*.

Key words: amino acid, mushrooms, *P. ostreatus*, *A. bisporus*, frozen

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Corresponding author – Adres do korespondencji: Dr inż. Emilia Bernaś, Department of Raw Material and Processing of Fruit and Vegetables of Agricultural University of Krakow, Balicka 122, 30-149 Cracow, Poland, e-mail: e.bernas@ur.krakow.pl

INTRODUCTION

According to statistics, *A. bisporus* is the most widely cultivated edible mushroom [Chang and Miles 2004]; however, *P. ostreatus* is becoming increasingly popular among consumers [Ques and Liu 2000].

Although edible mushrooms owe their popularity chiefly to their unique sensory traits, they are increasingly valued for their chemical composition, which includes biologically active compounds such as vitamins, polysaccharides and nitrogen compounds. The basic constituent in the dry matter of mushrooms is protein. Protein compounds make up more than half of total nitrogen, and their content, which varies between 19 and 39 g/100 g dry matter [Brenne 1990, Coşkuner and Özdemir 2000], depends, among other factors, on the composition of the substrate; size of the fruiting body; time of harvest; and species of mushroom.

Mushroom protein contains considerable amounts of endogenous amino acids, mostly alanine, arginine, glycine, histidine, glutamic acid, aspartic acid, proline and serine [Guo et al. 2007, Manzi et al. 1999, Mdachi et al. 2004, Shah et al. 1997], as well as all exogenous amino acids. However, it is difficult to ascertain protein quality in edible mushrooms from previous studies since the data in the literature is not consistent in this regard. For both species methionine and cysteine are most frequently mentioned as limiting amino acids, together with valine in the case of *A. bisporus* [Dabbour and Takruri 2002, Shah et al. 1997].

Nitrogen compounds other than protein are also found in mushrooms, such as: free amino acids; chitin; amines; nucleic acids and urea. In view of the fact that a large proportion of nitrogen in mushrooms is made up of non-protein compounds, the conversion coefficient from total nitrogen to protein, which ranges from 3.45 do 4.38 [Vetter and Rimoczi 1993] but is most frequently taken to be 4.38 [Tsai et al. 2007], is considerably lower than the generally accepted norm for most food products of 6.25. The presence of non-protein nitrogen compounds also affects the assimilability of mushroom protein, which averages about 70%. Dabbour and Takruri [2002] found that protein in fresh *P. ostreatus* is 74% assimilable, while according to Shah et al. [1997], assimilability in dried *P. ostreatus* and *A. bisporus* amounts to 84% and 77% respectively.

The aim of the present work was to compare the basic chemical composition, in particular the content of total nitrogen, protein nitrogen and amino acids, in frozen *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. and *Agaricus bisporus* (Lange) Sing. mushrooms.

MATERIAL AND METHODS

The material investigated comprised of frozen *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. and *Agaricus bisporus* (Lange) Sing. mushrooms after 8 months' storage, having been soaked and blanched in a solution containing citric, lactic and L-ascorbic acids prior to freezing.

The fresh mushrooms used in the study were classed as "premium quality" in the case of *Pleurotus ostreatus* [PN-R-75079 1996], and "first quality" in the case of *Agaricus bisporus* [PN-75/R-75078 1975]. Mushrooms were obtained from a specialist farm and frozen approximately 4 hours after being harvested. The pre-treatment prior to freezing involved: sorting (rejecting any unsound specimens); cleaning the mushrooms of any remaining litter; and washing in cold, running water. Next, in the case of the caps of *P. ostreatus* 5.0-7.5 cm in diameter, were cut in half; over 7.5 cm diameter were quartered. Then mushrooms were soaked and blanched in 0.5% citric acid, 0.5% lactic acid and 0.1% L-ascorbic acid water solution. Soaking lasted 1 h, the proportion by weight of mushrooms to solution being 1:2. Blanching was carried out at a temperature of 96-98°C, the proportion by weight of mushrooms to water or solution being 1:5. The blanching time, which was determined experimentally, was 3 min. The mushrooms were cut into strips 5 mm thick before being freezing. The mushrooms were placed in unit packages and frozen at a temperature of –35°C. When the temperature at the thermal centre of the frozen products reached –25°C (after approximately 120 min, starting from the moment when the mushrooms were put in blast-chiller), they were transferred to storage compartments and stored for 8 months under this temperature. The chemical composition of the frozen products was evaluated after the entire thawing for about 12 h at 4°C.

The dry matter, ash, total nitrogen, ash crude fat content were determined using AOAC [1995] methods, and protein nitrogen content using Awolumate [1983] method with trichloroacetic acid.

The total carbohydrates content was calculated as follows:

$$\text{Total carbohydrates content} = 100 - (\text{water} + \text{ash} + \text{crude protein} + \text{crude fat})$$

were crude protein = total nitrogen × 4.38 [Braaksma and Shaap 1996].

Liquid-phase hydrolysis of powdered samples was performed in 6M HCl containing 0.5% phenol (for tyrosine protection) at 110°C for 24 h under an argon atmosphere. The hydrolysates were lyophilised, dissolved in an appropriate volume of dilution buffer (sodium citrate buffer pH 2.2) and filtered through a 0.45 μm syringe filter before applying to the amino acid analyzer. Sulphur-containing amino acids were analysed as oxidation products obtained by performic acid oxidation followed by standard hydrolysis procedure with HCl. Amino acids were determined by ion-exchange chromatography with post-column derivatization with ninhydrin using an automatic amino acid analyzer (Ingos, Czech Republic) according to standard protocol of manufacturer.

In the literature, protein quality is usually determined by comparing the levels of exogenous amino acids with the FAO/WHO [1991] reference protein pattern for preschool children; however, since dietary recommendations advise against feeding mushrooms to pre-school children under the age of 3, protein quality was also related to the FAO/WHO [1991] and FAO/WHO [2007] patterns for adults. The CS index was calculated using the Mitchell and Block method [Osborne and Voogt 1978], and the integrated EAA index using the Oser [1951] method.

Total protein in mushrooms was determined on the basis of the molar mass of individual amino acids. In the case of tryptophan, which was not determined in the work, the average content as given in the source literature was accepted [Guo et al. 2007, Manzi et al. 1999, Shah et al. 1997]. Total protein having been determined and compared with total nitrogen, the conversion coefficient from total nitrogen to protein for the analyzed material was then calculated.

Dry matter, total nitrogen and protein nitrogen content were calculated from four replicates, amino acids from three replications. The results were statistically evaluated using the F-Snedecor and t-Student tests (Statistica 6.1 Pl program). The least significant difference was calculated for $\alpha = 0.05$.

RESULTS AND DISCUSSION

The dry matter content of frozen *Pleurotus ostreatus* and *Agaricus bisporus* was 8.49 and 7.95 g in 100 g fresh matter respectively. Compared with *A. bisporus*, frozen *P. ostreatus* had 32% more total carbohydrates and 38% more raw fat; however, it contained 45% less ash, 50% less total nitrogen and 40% less protein nitrogen (Table 1). Using a conversion coefficient of 4.38, raw protein was calculated as 15.6 g/100 g dry matter in *P. ostreatus*, and 31.3 g in *A. bisporus*; with a coefficient of 3.45, however, it amounted to 12.2 g and 24.6 g respectively. Protein nitrogen comprised 74% of total nitrogen in *P. ostreatus* and 62% in *A. bisporus*. Braaksma and Shaap [1996] found levels of total carbohydrates in *A. bisporus* similar to those obtained in the present study, while total nitrogen in fresh *P. ostreatus* as determined by Manzi et al. [1999] was higher at 4.55-7.93 g/100 g dry matter. The level of raw protein found by Bauer-Petrovska [2001] in fresh *A. bisporus* was also higher.

Table 1. Chemical composition of frozen mushrooms

Chemical constituents	Mushroom species	g/100 g dry matter	*p*
Total carbohydrates	*P. ostreatus*	76.97 ±0.62	$2.8 \cdot 10^{-7}$
	A. bisporus	58.11 ±1.38	
Ash	*P. ostreatus*	4.09 ±0.22	$2.3 \cdot 10^{-6}$
	A. bisporus	7.45 ±0.31	
Crude fat	*P. ostreatus*	4.34 ±0.08	$1 \cdot 10^{-4}$
	A. bisporus	3.14 ±0.25	
Total nitrogen	*P. ostreatus*	3.55 ±0.12	$2.6 \cdot 10^{-7}$
	A. bisporus	7.14 ±0.26	
Protein nitrogen	*P. ostreatus*	2.62 ±0.11	$1.2 \cdot 10^{-6}$
	A. bisporus	4.40 ±0.15	

p – level of probability.

The sum of all amino acids analysed in frozen *P. ostreatus* amounted to 1674 mg/100 g fresh matter, which was 87 mg (5%) greater than in frozen *A. bisporus*. According to Guo et al. [2007], the sum of 18 amino acids in dried *Pleurotus djamor* and *Pleurotus ferulae* was 8440 mg and 19 200 mg/100 g dry matter respectively.

The sum of endogenous amino acids per 100 g fresh matter was 60 mg (6%) lower in frozen *P. ostreatus* than in frozen *A. bisporus* (Table 2), which was probably due to the fact that total nitrogen in *P. ostreatus* was less than half that in *A. bisporus* (Table 1). Endogenous amino acids comprised 52% and 59% of total amino acids in *P. ostreatus* and *A. bisporus* respectively. According to Guo et al. [2007], the sum of endogenous amino acids in *Pleurotus djamor* was 4481 mg/100 g dry matter and 10291 mg in *Pleurotus ferulae*, making up 53% and 54% respectively of total amino acids.

Table 2. Endogenous amino acid content in frozen mushrooms

Kind of amino acid	Mushroom species	mg/100 g fresh matter	*p*	g/100 g protein	*p*
Alanine	*P. ostreatus*	116.7 ±4.9	0.00020	6.35 ±0.06	0.04602
	A. bisporus	109.8 ±5.3		6.24 ±0.03	
Arginine	*P. ostreatus*	132.0 ±4.6	0.00049	8.08 ±0.33	0.00106
	A. bisporus	101.1 ±2.3		6.46 ±0.04	
Asparagine	*P. ostreatus*	170.9 ±2.0	0.01261	10.08 ±0.07	$7.8 \cdot 10^{-6}$
	A. bisporus	186.6 ±6.0		11.29 ±0.11	
Glutamine	*P. ostreatus*	197.6 ±1.9	$7.7 \cdot 10^{-7}$	11.82 ±0.12	$8.3 \cdot 10^{-8}$
	A. bisporus	296.6 ±2.7		18.51 ±0.02	
Glycine	*P. ostreatus*	87.0 ±3.7	$1.2 \cdot 10^{-5}$	4.51 ±0.04	$1.6 \cdot 10^{-5}$
	A. bisporus	72.5 ±2.6		3.92 ±0.06	
Proline	*P. ostreatus*	79.2 ±8.7	0.71568	4.55 ±0.40	0.77402
	A. bisporus	77.2 ±2.7		4.64 ±0.12	
Serine	*P. ostreatus*	92.3 ±2.3	0.64661	5.22 ±0.05	0.00159
	A. bisporus	91.9 ±4.9		5.42 ±0.14	
Sum	*P. ostreatus*	876		50.61	
	A. bisporus	936		56.48	

p – level of probability, mean ±standard deviation.

The most abundant endogenous amino acids in both species were asparagine and glutamine, while the least abundant were glycine and proline. In *P. ostreatus* these amino acids comprised 10%, 12%, 5% and 5% respectively of total amino acids, and 20%, 23%, 10% and 9% of total endogenous amino acids, while in *A. bisporus* the corresponding figures were 12%, 19%, 5% and 5%; and 20%, 32%, 8% and 8%. Guo et al. [2007] found that in dried *Pleurotus djamor* mushrooms aspartic acid was the most abundant endogenous amino acid, comprising 19% of total endogenous amino acids. Similar findings were recorded by Shah et al. [1997] in respect of dried *P. ostreatus*, according to which aspartic acid made up 33% of total endogenous amino acids, with glycine (9%) and serine (9%) being the least abundant. By contrast, in dried *Agaricus blazei* glutamic acid (45%) was the most abundant endogenous amino acid [Tsai et al. 2008].

Regardless of how the results were expressed, *P. ostreatus* contained significantly higher levels of alanine (2-6% higher), arginine (25-31%) and glycine (15-20%) than *A. bisporus*, but lower levels of asparagine (8-11%) and glutamine (33-36%).

Total exogenous amino acids in frozen *P. ostreatus* amounted to 798 mg in 100 g fresh matter, 147 mg (23%) more than in frozen *A. bisporus* (Table 3). In *P. ostreatus*

Table 3. Exogenous amino acid content in frozen mushrooms

Kind of amino acid	Mushroom species	mg/100 g fresh matter	*p*	g/16 g protein	*p*
Cysteine	*P. ostreatus*	24.3 ±1.1	0.00015	1.41 ±0.02	$2.4 \cdot 10^{-5}$
	A. bisporus	18.2 ±0.8		1.11 ±0.01	
Histidine	*P. ostreatus*	44.7 ±2.5	0.03777	2.90 ±0.20	0.01317
	A. bisporus	50.1 ±1.7		3.39 ±0.08	
Isoleucine	*P. ostreatus*	85.1 ±2.9	$3.6 \cdot 10^{-5}$	4.49 ±0.05	0.13276
	A. bisporus	57.6 ±2.2		3.54 ±0.01	
Leucine	*P. ostreatus*	144.2 ±1.2	$5.9 \cdot 10^{-5}$	8.49 ±0.08	$1.2 \cdot 10^{-5}$
	A. bisporus	117.6 ±2.3		7.23 ±0.03	
Lysine	*P. ostreatus*	113.2 ±3.4	0.00724	6.77 ±0.24	0.05932
	A. bisporus	102.7 ±1.1		6.41 ±0.05	
Methionine	*P. ostreatus*	40.4 ±2.4	$5.9 \cdot 10^{-5}$	2.42 ±0.03	$6.3 \cdot 10^{-6}$
	A. bisporus	31.1 ±0.8		1.95 ±0.02	
Phenylalanine	*P. ostreatus*	83.8 ±1.4	0.00145	5.09 ±0.10	0.01098
	A. bisporus	76.0 ±1.0		4.82 ±0.13	
Threonine	*P. ostreatus*	91.5 ±1.3	0.00049	5.30 ±0.13	$4.8 \cdot 10^{-5}$
	A. bisporus	82.7 ±0.8		5.00 ±0.11	
Tyrosine	*P. ostreatus*	67.3 ±2.7	$5 \cdot 10^{-5}$	4.13 ±0.04	$6 \cdot 10^{-7}$
	A. bisporus	46.0 ±1.9		2.95 ±0.03	
Valine	*P. ostreatus*	103.3 ±1.8	$1.4 \cdot 10^{-5}$	5.96 ±0.06	$8.3 \cdot 10^{-7}$
	A. bisporus	69.3 ±1.5		4.18 ±0.04	
Sum	*P. ostreatus*	798		46.96	
	A. bisporus	651		40.58	

p – level of probability, mean ± standard deviation.

exogenous amino acids comprised 48% of total amino acids; in *A. bisporus* the figure was 41%. According to Guo et al. [2007], the sum of exogenous amino acids in dried *Pleurotus djamor* and *Pleurotus ferulae* mushrooms was 3959 and 8909 mg/100 g dry matter respectively, comprising 47% and 46% of total amino acids. This proportion was similar to the results obtained in the present study, as were the findings of Shah et al. [1997] for dried *P. ostreatus*. However, according to the above authors, exogenous amino acids made up 49%, of total amino acids in dried *A. bisporus*, a higher proportion than found in the present study.

In both species leucine and lysine were the most abundant exogenous amino acids, while cysteine and methionine were found in the lowest amounts (Table 3). In frozen *P. ostreatus* these amino acids comprised 9%, 7%, 1% and 2% respectively of total amino acids, and 18%, 14%, 3% and 5% of exogenous amino acids; in frozen *A. bisporus* the corresponding figures were 7%, 6%, 1% and 2%; and 18%, 16%, 3% and 5%. Similarly to the results obtained in the present study, Shah et al. [1997] found that leucine (18% of total exogenous amino acids) and lysine (15%) were the most abundant exogenous amino acids in dried *P. ostreatus*; however, histidine (4%), methionine (4%) and tryptophan (3%) were found in the lowest amounts. According to the above authors, the dominant exogenous amino acids in dried *A. bisporus* were leucine (15%) and lysine (17%), with methionine (2%) being found in the lowest amount. According to Guo et al. [2007], cysteine and valine were most abundant in dried *Pleurotus djamor* mushrooms (15% and 14% respectively of total exogenous amino acids), while leucine (25%) dominated in *Pleurotus ferulae*. However, these authors found that histidine (5%) and methionine (3%) were found in the lowest amounts in *Pleurotus djamor*, and cysteine (3%) in *Pleurotus ferulae*.

With the exception of histidine, levels of all the exogenous amino acids analysed were significantly (6-49%) higher in frozen *P. ostreatus* than in frozen *A. bisporus*, whether expressed in terms of amino acid content per 100 g fresh matter or per 100 g protein.

Compared with the FAO/WHO [1991] and FAO/WHO/UNU [2007] reference protein patterns for adults and the FAO/WHO [1991] pattern for pre-school children, no limiting amino acids were found in either species; the CS index values were 181-486, 144-243 and 117-170 respectively for *P. ostreatus*, and 180-409, 107-226 and 110-178 for *A. bisporus*. In *P. ostreatus* products, the amino acids with the lowest CS values for each of the three above patterns in the order given were leucine, histidine and lysine respectively, while the highest values were recorded for phenylalanine with tyrosine (both adult patterns) and valine. In *A. bisporus* products, the lowest CS index values for the three above patterns in the order given were found for methionine with cysteine; valine; and both leucine and lysine, while the lowest values were found for phenylalanine with tyrosine; and histidine (for the last two patterns).

The EAA index values for frozen *P. ostreatus* calculated against the FAO/WHO [1991] and FAO/WHO/UNU [2007] reference protein patterns for adults and the FAO/WHO [1991] pattern for pre-school children were 343, 176 and 147 respectively, 14-15% higher than for frozen *A. bisporus*.

The conversion coefficient from total nitrogen to protein, calculated on the basis of the molar mass of individual amino acids and the total nitrogen content, was 2.46 for frozen *A. bisporus*, considerably lower than the levels stated in the literature [Vetter and Rimoczi 1993]. The coefficient for frozen *P. ostreatus* was over twice that for *A. bisporus*, reflecting the fact that *A. bisporus* contains more non-protein nitrogen compounds.

CONCLUSIONS

1. Frozen *P. ostreatus* contained significantly higher levels of total carbohydrates, raw fat and most endogenous and exogenous amino acids than frozen *A. bisporus*, but lower levels of ash, total nitrogen and protein nitrogen.

2. In both species, asparagine and glutamine were the most abundant endogenous amino acids and leucine and lysine the most abundant exogenous amino acids. The least abundant were glycine and proline, and cysteine and methionine.

3. Frozen mushrooms of both species are potentially good sources of protein in the diet as no limiting amino acids were found when compared with the FAO/WHO reference protein pattern.

4. Frozen *A. bisporus* contained considerably more non-protein nitrogen compounds than *P. ostreatus*, the conversion coefficient from total nitrogen to protein being less than half of that in *P. ostreatus*.

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PORÓWANANIE ZAWARTOŚCI AMINOKWASÓW W MROŻONYCH OWOCNIKACH *P. OSTREATUS* I *A. BISPORUS*

Wstęp. W pracy porównano zawartość podstawowych składników chemicznych oraz aminokwasów w mrożonych owocnikach *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. i *Agaricus bisporus* (Lange) Sing.
Materiał i metody. Materiałem badawczym były mrożone owocniki boczniaka ostrygowatego (*Peurotus ostreatus*) i pieczarki dwuzarodnikowej (*A. bisporus*) po ośmiu miesiącach składowania, otrzymane z grzybów poddanych przed mrożeniem moczeniu i blanszowaniu w roztworze wodnym 0,5-procentowego kwasu cytrynowego, 0,5-procentowego kwasu mlekowego i 0,1-procentowego kwasu L-askorbinowego.
Wyniki. Mrożone owocniki *P. ostreatus*, w porównaniu z mrożonymi *A. bisporus*, charakteryzowały się istotnie większym poziomem: węglowodanów ogółem o 32%, tłuszczu surowego o 38%, większości aminokwasów endogennych o 6-163% i aminokwasów egzogennych o 10-200%. Natomiast wyróżniały się mniejszą zawartością: popiołu o 45%, azotu ogółem o 50%, azotu białkowego o 40%. Wśród aminokwasów endogennych w obu gatunkach grzybów w największej ilości występowała asparagina i glutamina, a spośród aminokwasów egzogennych leucyna i lizyna, które stanowiły odpowiednio 10-12% i 12--19% oraz 7-9% i 6-7% sumy wszystkich aminokwasów. W najmniejszej ilości oznaczono glicynę i prolinę, odpowiednio po 5%, oraz cysteinę i metioninę, odpowiednio 1% i 2%.
Wnioski. Mrożonki z obu gatunków grzybów mogą być potencjalnie dobrym źródłem białka w diecie człowieka, ponieważ w porównaniu z wzorcami białka FAO/WHO nie stwierdzono w nich aminokwasów ograniczających. Istotne jest jednak określenie strawności białka grzybowego w badaniach modelowych z wykorzystaniem zwierząt i ludzi. Współczynnik przeliczeniowy azotu ogółem na białko wynosił 5,23 w *P. ostreatus* i 2,46 w *A. bisporus*.

Słowa kluczowe: aminokwasy, grzyby, *P. ostreatus*, *A. bisporus*, mrożenie

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