Food Chemistry 125 (2011) 936–943

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of production process on the amino acid content of frozen and canned *Pleurotus ostreatus* mushrooms

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ARTICLE INFO

Article history:
Received 28 April 2010
Received in revised form 6 August 2010
Accepted 20 September 2010

Keywords:
Amino acid
Mushrooms
Freezing
Canning
Pre-treatment

ABSTRACT

The aim of the work was to determine the effects of the freezing and canning processes, followed by a 12-month storage, on the amino acid content of *Pleurotus ostreatus* mushrooms. The pre-treatment involved blanching, or soaking and blanching, in mushrooms in water or in solutions containing sodium metabisulphite, citric acid, L-ascorbic acid and/or low-methylated pectin. Freezing and canning resulted in significant decreases in the levels of alanine, glutamine, cysteine and tyrosine (6–39%), and, in the case of canned mushrooms arginine, glycine, serine, histidine, methionine and threonine (1–31%). Frozen products obtained from blanched mushrooms had significantly higher levels of 12 out of the 17 amino acids examined (4–28%) whereas, in canned mushrooms, only 5 amino acids showed higher levels (3–20%), than those obtained from soaked and blanched mushrooms. With the exception of samples blanched in water, frozen mushrooms had higher levels than canned mushrooms of all the investigated amino acids. Limiting amino acids were not found in mushrooms.

1. Introduction

Pleurotus ostreatus is among the most widely cultivated mushroom species globally, including Europe and Poland. This species owes its popularity mainly to a relatively simple method of cultivation and high nutritional value. In view of their high content of protein (containing all exogenous amino acids) (Manzi, Gambelli, Marconi, Vivanti, & Pizzoferrato, 1999; Shah, Khalil, & Jabeen, 1997), B vitamins and minerals, *P. ostreatus* mushrooms are a valuable foodstuff, especially for vegetarians. Apart from these compounds, *P. ostreatus* mushrooms have also been found to contain dietary fibre, in particular soluble β-glucans with β (1 → 3), β (1 → 4) and (1 → 6) glycosidic bonds, which have anti-carcinogenic properties and which lower blood cholesterol. According to Manzi, Aguzzi, and Pizzoferrato (2001), 100 g of fresh matter of *P. ostreatus* contain around 139 mg of β-glucans.

Mushrooms contain substantial amounts of proteins, free amino acids, amines, nucleic acids, urea and chitin. A considerable proportion of the nitrogen in mushrooms is in the form of non-protein nitrogen. Analysing 52 species of mushrooms, Bauer-Petrovska (2001) found that, on average, they contained 33% of total nitrogen. It is difficult to establish a coefficient to convert total nitrogen to protein. The values most generally accepted range from 3.45 to 4.38 (Braaksma & Schaap, 1996; Shah et al., 1997), the latter value being applied to *P. ostreatus* (Braaksma & Schaap, 1996). The assimilability of mushroom protein depends, to a large extent, on the species, ranging from 34% to 89%. Dabbour and Takruri (2002) found 74% in fresh *P. ostreatus* mushrooms while, according to Shah et al. (1997), 84% of protein in dried *P. ostreatus* and 77% in dried *Agaricus bisporus* is assimilable.

Edible mushrooms are highly perishable, largely due to their high water content (approximately 90%), high level of enzyme activity and the presence of microflora (Burton & Noble, 1993). On the basis of well documented data for *A. bisporus*, it may be stated that, among the changes in the quality of edible mushrooms occurring during storage, are darkening of the tissue, lengthening of the stipes, opening of the caps and hardening of the flesh (Burton & Noble, 1993) and, according to Yen (1992), a rapid increase in biogenic amino acids in *Volvariella volvacea*.

Preserving allows mushrooms to be used beyond the time when they are harvested. The method of preservation depends, among other factors, on the intended use of the products and the anticipated period of storage. Drying is the most frequently used method; however, two other methods are becoming increasingly common: freezing and preserving in hermetically sealed containers by sterilizing or pasteurising. Moreover, advances in food processing technology have led to the development of convenience foods, especially ready-to-cook products, which simply require thermal treatment, and ready-to-heat and ready-to-eat foods (Sloan, 2005). The methods of preservation mentioned above are part of this trend.

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doi:10.1016/j.foodchem.2010.09.084

The aim of this work was to determine the effects of the type of pre-treatment, preservation method (freezing or canning), and a 12-month storage on the contents of total nitrogen, protein nitrogen and amino acids in *P. ostreatus* (Jacq.: Fr.) Kumm. mushrooms. The different pre-treatments applied were blanching in water, and blanching or soaking and blanching in aqueous solutions containing sodium metabisulphite, citric acid, L-ascorbic acid and/or low-methylated pectin.

2. Materials and methods

2.1. Materials

The experimental material was fresh *P. ostreatus* (Jacq.: Fr.) Kumm. mushrooms, as frozen and canned products after 12 months of storage. The frozen and canned product was prepared from mushrooms blanched in water, blanched and soaked and blanched in solutions containing additional substances.

2.2. Treatments

2.2.1. Preliminary treatment

Fresh *P. ostreatus* were sorted, cleaned, and washed in cold running water. Next, the caps, 5.0–7.5 cm in diameter were cut in half; those over 7.5 cm in diameter were quartered. The preliminary processing, freezing and canning processes were conducted under laboratory conditions, enabling the precise control of parameters at every stage of technological treatment. Each batch of mushrooms was then divided into six parts. Four parts were blanched: in water (product code – BW); in an aqueous solution of sodium metabisulphite (0.2%) and citric acid (0.5%) (product code – BSM); in an aqueous solution of 0.5% citric acid and 0.1% L-ascorbic acid (product code – BCA); in an aqueous solution of low methoxyl pectin (0.5%), citric acid (0.5%) and L-ascorbic acid (0.1%) (product code – BPE). The remaining two parts were soaked and blanched in the same solutions as were products BCA and BLA and given the codes SBCA and SBLA, respectively. 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 5 mm thick strips before being preserved (freezing or canning).

2.2.2. Freezing

The mushrooms were placed in unit packages and frozen in an air-blast tunnel in which the air temperature was −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 the blast-chiller), they were transferred to storage compartments and stored for 12 months at −25 °C. The chemical composition of the frozen products was evaluated, after the entire thawing, for about 12 h at 4 °C.

2.2.3. Canning

The 180 g of mushrooms were placed in 300 cm^3 "twist-off" glass jars. Then jars with mushrooms were treated with 100 cm^3 of hot solution containing 2% of salt, deaerated by immersion, sealed and sterilized. The process of sterilization was carried out as follows: elevation of temperature up to 100 °C – 5 min; a rise in temperature from 100 to 118 °C – 10 min; a sterilization at temperatures from 118 to 121 °C – 12 min; finally cooling to 30 °C – 10 min. Sterilization took place in a laboratory pressure sterilizer of USA manufacture. The canned mushrooms were kept in a storage chamber for 12 months at temperature of 8–10 °C.

2.3. Methods

2.3.1. Proximate composition

The dry matter, ash, total nitrogen and crude fat contents were determined using AOAC (1995) methods, and protein nitrogen content, using the Awolumate (1983) method, with trichloroacetic acid. The total carbohydrate content was calculated as follows:

Total carbohydrates content = 100 − (water + ash + crude protein + crude fat);
crude protein = total nitrogen × 4.38 (Braaksma & Schaap, 1996).

2.3.2. Amino acid composition

Liquid-phase hydrolysis of powdered samples was performed in 6 M 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 analyser. Sulphur-containing amino acids were analysed as oxidation products obtained by performic acid oxidation, followed by a standard hydrolysis procedure with HCl. Amino acids were determined by ion-exchange chromatography with post-column derivatization with ninhydrin, using an automatic amino acid analyser (Ingos, Czech Republic) according to standard protocol of the manufacturer.

The composition of amino acids was also expressed as g/16 g N to estimate the quality of the protein in mushrooms by comparing it with the FAO/WHO (1991, 2007) patterns. On the basis of the amino acid composition, the CS index was calculated, using the Mitchell and Block method (Osborne & Voogt, 1978), and the integrated EAA index using the Oser (1951) method.

2.4. Statistical analysis

Dry matter, ash, total nitrogen, protein nitrogen and crude fat content were calculated from four replicates, and amino acids from three replicates. 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 $\alpha = 0.05$.

3. Results and discussion

3.1. Proximate composition

Compared with the data in the literature (Manzi et al., 1999; Souci, Fachmann, & Kraut, 2000) fresh *P. ostreatus* mushrooms contained approximately 1.5 times less total nitrogen but almost twice as much crude fat (Table 1). Dry matter content was within the range given by Manzi et al. (1999) for this species. According to Yang, Lin, and Mau (2001), 100 g fresh *P. ostreatus* mushrooms

Table 1
Chemical evaluation of fresh *Pleurotus ostreatus* mushrooms (g/100 g).

Chemical constituents	Level
Dry matter (f.m.)	8.80 ± 0.01
Ash (d.m.)	6.70 ± 1.29
Total carbohydrates (d.m.)	70.9 ± 2.25
Total nitrogen (d.m.)	3.81 ± 0.25
Protein nitrogen (d.m.)	3.01 ± 0.24
Crude fat (d.m.)	5.45 ± 0.32

f.m. – fresh matter; d.m. – dry matter; mean ± standard deviation.

contained: 11.40 g of dry matter, of which total carbohydrates made up 61.1%, total nitrogen 7.59%, crude fat 2.16% and crude protein 23.9%. In the present work, crude protein was calculated as 16.69 g (using a conversion coefficient of 4.38) (Braaksma & Schaap, 1996), by which 79% was protein nitrogen.

The protein content (including amino acids) in edible mushrooms is liable to change during processing. Sasaki, Nakamura, Aoyagi, and Sugahara (1988) noted that rehydrating dried *Lentinula edodes* mushrooms led to a decrease in protein but an increase in free amino acids. Larousse (1986) and Martin-Belloso and Llanos-Barriobero (2001) found that canning *A. bisporus* caused a 10.4–11.0% decrease in protein content, whereas Nagy (1989) noted an average decrease of 13.9% following the canning of forest mushrooms, including such species as *Cantharellus cibarius* and *Boletus edulis*. Czapski (2003) observed no significant difference in protein contents between canned *A. bisporus* obtained from fresh and desalted mushrooms. Nagy (1989), on the other hand, found that, using desalted forest mushrooms in canning, resulted in an average decrease of 30% in protein content compared with using fresh forest mushrooms.

After 12 months of storage, frozen *P. ostreatus* contained less dry matter (0–10%), total nitrogen (7–16%) and protein nitrogen (4–15%) than did the raw material, whereas the canned product contained more dry matter (1–2%), but less total (9–17%) and protein nitrogen (13–33%). The frozen product had a significantly lower dry matter content (1–12%) than had the canned product, but higher levels of total and protein nitrogen (1–9% and 3–22%, respectively), but these differences were not statistically significant. In both frozen and canned mushrooms, protein nitrogen contents, as a proportion of total nitrogen, were similar among all the blanched products (product codes – BW, BSM and BCA), with the exception of product BPE; however, in soaked and blanched products (SBCA and SBPE) this proportion was 8–14% higher in frozen than in canned products (Table 2).

Table 2
Dry matter, total nitrogen and protein nitrogen contents in frozen and canned *Pleurotus ostreatus* mushrooms.

Kind of preliminary treatment	Kind of product	Chemical constituents			
		Dry matter (g/100 g f.m.)	Total nitrogen (g/100 g d.m.)	Protein nitrogen	
				g/100 g d.m.	% of contribution in total N
BW	FM	8.31 ± 0.09	3.55 ± 0.09	2.71 ± 0.09	76
	CM	8.80 ± 0.08	3.41 ± 0.13	2.62 ± 0.16	77
BSM	FM	8.76 ± 0.11	3.31 ± 0.32	2.57 ± 0.08	78
	CM	8.82 ± 0.02	3.29 ± 0.16	2.50 ± 0.10	76
BCA	FM	8.48 ± 0.03	3.36 ± 0.08	2.70 ± 0.17	81
	CM	8.97 ± 0.04	3.23 ± 0.13	2.63 ± 0.08	81
BPE	FM	8.78 ± 0.01	3.36 ± 0.08	2.68 ± 0.20	80
	CM	8.98 ± 0.04	3.45 ± 0.02	2.51 ± 0.08	73
SBCA	FM	8.40 ± 0.03	3.21 ± 0.17	2.56 ± 0.09	80
	CM	8.93 ± 0.03	3.31 ± 0.02	2.44 ± 0.12	74
SBPE	FM	7.95 ± 0.11	3.46 ± 0.09	2.89 ± 0.16	84
	CM	8.69 ± 0.22	3.17 ± 0.08	2.36 ± 0.08	74
LSD, $\alpha = 0.05$		0.081	ns	ns	

FM – frozen mushrooms; CM – canned mushrooms; f.m. – fresh matter; d.m. – dry matter; mean ± standard deviation; ns – not significant; BW – blanching in water; BSM – blanching in solution of sodium metabisulphite (0.2%) and citric acid (0.5%); BCA – blanching in an aqueous solution of 0.5% citric acid and 0.1% L-ascorbic acid; SBCA – soaking and blanching in an aqueous solution of 0.5% citric acid and 0.1% L-ascorbic acid; BPE – blanching in an aqueous solution of citric acid (0.5%), L-ascorbic acid (0.1%) and low methoxyl pectin (0.5%); SBPE – soaking and blanching in an aqueous solution of citric acid (0.5%), L-ascorbic acid (0.1%) and low methoxyl pectin (0.5%).

3.2. Amino acid composition

Total amino acids in fresh *P. ostreatus* amounted to 1860 mg/100 g fresh matter. Processing and a 12-month storage resulted in this figure being reduced by 2–16% in frozen mushrooms and 12–19% in canned mushrooms to 1564–1825 mg and 1512–1628 mg/100 g fresh matter, respectively. Bernaś and Jaworska (2010) found, respectively, 15–34% and 25–34% higher values for total amino acids in frozen and canned *B. edulis* mushrooms pretreated by soaking and blanching in solutions containing lactic acid, citric acid and L-ascorbic acid. According to Guo, Lin, and Lin (2007), totals for 18 amino acids in dried *Pleurotus djamor*, *Pleurotus ferulae*, *Pleurotus nebrodensis* and *Pleurotus sapidus* mushrooms were 8440, 19,200, 11,300 and 11,900 mg/100 g dry matter, respectively.

Total endogenous amino acids in fresh *P. ostreatus* mushrooms amounted to 1008 mg/100 g fresh matter, comprising 56% of total amino acids (Table 3). Asparagine and glutamine were dominant among endogenous amino acids, comprising 17% and 27% of total endogenous amino acids, and 9% and 15% of total amino acids, respectively. Glycine and proline had the lowest percentage share at 9% and 8%, and 5% and 4%, respectively. In fresh *Pleurotus sajor-caju* mushrooms, Mdachi, Nkunya, Nyigo, and Urasa (2004) found asparagine and glycine in the highest quantities and glutamine in the lowest.

Production and storage brought about a reduction in total endogenous amino acids of 4–17% in frozen mushrooms and 15–21% in canned mushrooms. Guo et al. (2007) reported that total endogenous amino acids in dried *P. djamor* and *P. ferulae* mushrooms amounted to 4481 and 10,291 mg/100 g dry matter, respectively. After 12 months of storage, the greatest changes in total endogenous amino acids were noted for products soaked and blanched in solutions containing low-methylated pectin (SBPE), and the least for frozen product BPE and canned product BCA. Products obtained from blanched mushrooms had higher levels of total endogenous amino acids (6–17% for frozen products; 4% for canned products) than those obtained from soaked and blanched mushrooms. In both frozen and canned products, endogenous amino acids constituted 52–53% of total amino acids. According to Guo et al. (2007), total endogenous amino acids comprised 53% and 54%, respectively, of total amino acids in dried *P. djamor* and *P. ferulae* mushrooms, which are very similar to the results obtained in the present work.

In general, the production process, followed by 12 months of storage, had a significant effect on the level of all the endogenous amino acids investigated (Table 3). Compared with the raw material, both frozen and canned *P. ostreatus* mushrooms showed significant decreases in the levels of alanine (21–33% and 30–35%, respectively) and glutamine (21–31% and 29–33%, respectively), and, canned mushrooms showed decreases of arginine (12–16%); glycine (8–16%) and serine (1–9%). For the remaining endogenous amino acids examined, the changes were also significant and generally ranged from −14% to +22% in frozen products, and from −10% to +7% in canned products. The reduction in amino acid levels may have been the result of non-enzymatic browning of mushroom tissue (Maillard reaction), which involves condensation between amino groups of amino acids in the protein with sugars; subsequent reactions are not reversible and amino acids are destroyed (Baxter, 1995; Candela, Astiasaran, & Bello, 1997). According to Klein and Mondy (1981), heat treatment may lead to compositional changes in nitrogenous compounds, depending on the mechanism of heat transfer and the particular tissue under treatment. During preparation of food, the side chains of some protein-bound amino acids can react chemically with each other. Those reactions can result in a changing of the composition of amino acids (Sherr, Lee, & Jelesciewicz, 1989).

Table 3
Endogenous amino acid content in fresh, frozen and canned *Pleurotus ostreatus* mushrooms (mg/100 g fresh matter).

Amino acid	Fresh mushrooms	Kind of product	Kind of preliminary treatment						LSD, $\alpha = 0.05$
			BW	BSM	BCA	BPE	SBCA	SBPE	
Ala	150 ± 0.5	FM	99.8 ± 1.4	109 ± 2.5	113 ± 0.4	116 ± 3.0	118 ± 2.5	110 ± 4.7	3.23
		CM	102 ± 0.9	101 ± 2.2	105 ± 1.3	106 ± 1.2	100 ± 2.3	101 ± 3.4	
Arg	143 ± 1.2	FM	122 ± 3.1	137 ± 6.1	144 ± 3.1	149 ± 5.6	135 ± 2.2	124 ± 5.4	5.45
		CM	126 ± 3.0	124 ± 0.9	126 ± 5.1	124 ± 1.0	123 ± 10.3	120 ± 2.0	
Asp	176 ± 6.6	FM	182 ± 1.7	192 ± 6.4	193 ± 1.3	206 ± 4.8	176 ± 4.6	162 ± 6.4	5.78
		CM	176 ± 1.1	173 ± 1.7	183 ± 0.8	173 ± 2.3	166 ± 0.5	158 ± 3.5	
Glu	274 ± 1.7	FM	188 ± 2.6	204 ± 8.9	218 ± 1.7	215 ± 5.7	205 ± 3.4	189 ± 8.1	7.25
		CM	186 ± 2.8	180 ± 3.7	191 ± 1.1	180 ± 1.7	188 ± 0.7	179 ± 1.9	
Gly	89.9 ± 0.5	FM	77.9 ± 0.6	86.3 ± 2.5	90.0 ± 0.7	90.8 ± 2.3	86.7 ± 2.3	80.2 ± 3.6	2.69
		CM	78.9 ± 2.2	79.7 ± 1.1	82.4 ± 1.3	77.4 ± 1.2	81.2 ± 1.4	75.9 ± 0.3	
Pro	82.7 ± 9.2	FM	75.5 ± 9.3	84.7 ± 5.2	88.1 ± 0.8	93.4 ± 3.3	89.4 ± 1.9	82.7 ± 0.2	7.21
		CM	82.3 ± 3.2	79.6 ± 0.6	80.1 ± 1.4	80.4 ± 2.1	77.9 ± 4.5	76.6 ± 0.8	
Ser	92.7 ± 0.8	FM	88.6 ± 0.8	95.7 ± 2.6	99.7 ± 0.6	102 ± 2.3	94.7 ± 2.2	86.6 ± 4.0	2.74
		CM	89.0 ± 1.4	87.8 ± 3.1	91.8 ± 0.6	87.2 ± 1.3	87.6 ± 1.3	84.7 ± 2.4	
Sum	1002	FM	834	907	957	972	905	833	
		CM	841	825	859	829	823	795	

FM – frozen mushrooms; CM – canned mushrooms; mean ± standard deviation; BW – blanching in water; BSM – blanching in solution of sodium metabisulphite (0.2%) and citric acid (0.5%); BCA – blanching in an aqueous solution of 0.5% citric acid and 0.1% L-ascorbic acid; SBCA – soaking and blanching in an aqueous solution of 0.5% citric acid and 0.1% L-ascorbic acid; BPE – blanching in an aqueous solution of citric acid (0.5%), L-ascorbic acid (0.1%) and low methoxyl pectin (0.5%); SBPE – soaking and blanching in an aqueous solution of citric acid (0.5%), L-ascorbic acid (0.1%) and low methoxyl pectin (0.5%).

As was the case with the fresh product, in processed *P. ostreatus* products asparagine and glutamine were the most abundant (Table 3). In frozen mushrooms, they comprised 19–22% and 22–23%, respectively, of total endogenous amino acids and, in canned mushrooms, 20–21% and 22–23%. As a proportion of total amino acids, they comprised 10–12% and 12%, and 10–11% and 11–12%, respectively. Glycine and proline were the least abundant in both types of product, in each case comprising 9–10% of total endogenous amino acids and 5% of total amino acids. As in the present study, Bernaś and Jaworska (2010) found that, in frozen and canned *B. edulis* mushrooms pretreated by soaking and blanching in solutions containing lactic acid, citric acid and L-ascorbic acid, asparagine (17–18% of total endogenous amino acids) and glutamine (25–26%) were most abundant, while glycine (8–9%) and proline (8%) were found in the lowest quantities. Guo et al. (2007) reported that the dominant amino acid in dried *P. djamor* mushrooms was aspartic acid (19% of total endogenous amino acids) whereas, in dried *P. ferulae,* it was glutamic acid (32%). Shah et al. (1997), on the other hand, found that, in dried *P. ostreatus* mushrooms, glutamic acid (33%) was the dominant endogenous amino acid, with glycine (9%) and serine (9%) being present in the lowest quantities. Glutamic acid was also the dominant endogenous amino acid in dried *Agaricus blazei*; however, proline was not found at all (Tsai, Tsai, & Mau, 2008).

In general, the type of pre-treatment applied had a significant effect on the content of endogenous amino acids: blanching resulted in significantly higher levels than did soaking and blanching. In frozen products, arginine was 6–20% higher, asparagine 10–28%, glutamine 6–14%, glycine 4–13% and serine 5–17%. In canned products, the level of alanine was 5% higher, asparagine 9–11% and serine 3–5%.

With the exception of mushrooms blanched in water, frozen *P. ostreatus* products generally had higher levels of endogenous amino acids than had canned products: alanine was 7–18% higher, arginine 3–20%, asparagine 2–19%, glutamine 6–14%, glycine 6–17%, proline 6–16% and serine 2–17%, with the greatest differences being found in products blanched using pectin.

In fresh *P. ostreatus* mushrooms, total exogenous amino acids amounted to 852 mg/100 g fresh matter and comprised 46% of total amino acids (Table 4). The most abundant exogenous amino acids were leucine (18% of total exogenous amino acids and 8% of total amino acids) and lysine (14% and 6%); the least abundant were cysteine (4% and 2%), histidine (7% and 3%) and methionine (5% and 2%). According to Souci et al. (2000), total exogenous amino acids for this species amount to 920 mg/100 g of edible parts, with leucine (18%), lysine (16%) and valine (15%) being most abundant, while tryptophan (3%), histidine (5%) and methionine (5%) were found in the lowest quantities. Manzi et al. (1999) pointed out that leucine and lysine were the dominant exogenous amino acids in fresh *P. ostreatus* (SMR 122) mushrooms, comprising 6.5% and 6.3% of total amino acids. Yang et al. (2001) reported that the total of 10 free amino acids amounted to 89 mg/100 g of dry matter, with L-isoleucine, L-lysine and L-phenylalanine being the most abundant; L-leucine and L-threonine were not found at all.

Compared with fresh mushrooms, total exogenous amino acids fell by 0–14% in frozen products and 10–16% in canned products. Exogenous amino acids constituted 47–48% of total amino acids. Similar results were obtained by Shah et al. (1997), who determined exogenous amino acids as 47% of total amino acids in dried *P. ostreatus*. Guo et al. (2007), found total exogenous amino acid levels of 3959 and 8909 mg/100 g of dry matter, respectively, in dried *P. djamor* and *P. ferulae* mushrooms, comprising 47% and 46% of total amino acids.

Compared with the raw material, processing and 12-month storage of frozen and canned *P. ostreatus* brought about a significant reduction in the content of cysteine (6–20% and 25–39%, respectively) and tyrosine (8–17% and 16–20%), as well as a decrease in methionine of 20–31% in canned products. There were significant changes in the remaining exogenous amino acids investigated, but they were considerably smaller, ranging from −12% to +14% in frozen products, and from −7% to +11% in canned products.

As in fresh mushrooms, the dominant exogenous amino acids in frozen and canned *P. ostreatus* mushrooms were leucine and lysine, comprising 18–19% and 13–14% of total exogenous amino acids, and 8–9% and 6–7% of total amino acids, respectively. The lowest amounts were found for cysteine, methionine and histidine, comprising 2–3%, 4–5% and 6% of total exogenous amino acids, and 1–2%, 2–3% and 3% of total amino acids, respectively. According

Table 4
Exogenous amino acid content in fresh, frozen and canned *Pleurotus ostreatus* mushrooms (mg/100 g fresh matter).

Amino acid	Fresh mushrooms	Kind of product	Kind of preliminary treatment						LSD, $\alpha = 0.05$
			BW	BSM	BCA	BPE	SBCA	SBPE	
Cys	30.1 ± 0.6	FM	24.2 ± 0.3	27.4 ± 0.2	27.7 ± 0.4	28.2 ± 0.4	26.6 ± 0.3	24.7 ± 0.8	0.57
		CM	22.6 ± 1.1	21.9 ± 0.4	22.0 ± 0.2	20.9 ± 0.5	18.3 ± 0.1	19.7 ± 0.2	
His	56.5 ± 0.8	FM	40.8 ± 0.9	46.8 ± 0.7	47.9 ± 1.7	50.1 ± 1.2	45.5 ± 0.5	41.4 ± 1.6	1.19
		CM	43.5 ± 0.3	44.0 ± 0.2	44.6 ± 0.5	44.1 ± 0.2	43.1 ± 0.3	42.2 ± 0.2	
Ile	89.4 ± 0.9	FM	76.8 ± 0.9	84.9 ± 2.5	87.7 ± 0.6	91.6 ± 1.5	83.6 ± 1.8	78.7 ± 2.7	2.29
		CM	83.0 ± 0.6	81.2 ± 0.0	84.1 ± 0.8	81.8 ± 0.2	80.1 ± 0.3	78.9 ± 0.7	
Leu	149 ± 1.3	FM	130 ± 2.9	141 ± 3.5	147 ± 1.7	155 ± 4.0	140 ± 2.3	134 ± 5.6	4.22
		CM	139 ± 2.7	137 ± 2.4	140 ± 0.9	136 ± 1.6	139 ± 0.8	132 ± 1.8	
Lys	118 ± 1.2	FM	105 ± 0.8	116 ± 3.4	121 ± 2.8	113 ± 4.3	115 ± 1.0	108 ± 4.4	3.33
		CM	106 ± 2.2	105 ± 0.4	109 ± 1.2	101 ± 1.5	106 ± 3.1	101 ± 1.3	
Met	42.6 ± 0.8	FM	37.7 ± 0.6	40.8 ± 0.6	42.8 ± 0.4	45.7 ± 0.9	41.9 ± 0.7	38.4 ± 1.6	1.10
		CM	31.9 ± 0.8	32.6 ± 0.2	34.1 ± 0.3	30.2 ± 0.2	31.3 ± 0.3	29.2 ± 0.3	
Phe	89.2 ± 2.1	FM	75.6 ± 0.6	83.8 ± 2.8	86.7 ± 1.4	89.2 ± 3.6	81.6 ± 1.2	77.1 ± 4.4	3.33
		CM	79.7 ± 0.7	78.3 ± 0.4	81.0 ± 0.9	76.6 ± 0.7	78.7 ± 0.1	76.1 ± 0.7	
Thr	93.6 ± 0.2	FM	85.4 ± 0.9	93.0 ± 2.6	96.7 ± 0.6	98.9 ± 2.2	92.5 ± 1.9	84.8 ± 3.8	2.65
		CM	87.0 ± 0. 5	85.6 ± 0.2	89.6 ± 0.3	85.8 ± 0.3	87.2 ± 0.3	83.3 ± 0.3	
Tyr	75.7 ± 2.6	FM	62.6 ± 2.8	67.2 ± 4.1	74.1 ± 1.0	69.9 ± 3.4	70.7 ± 2.9	62.5 ± 5.6	4.53
		CM	63.3 ± 1.0	61.8 ± 0.8	63.5 ± 1.1	61.0 ± 0.3	62.7 ± 0.3	60.6 ± 1.3	
Val	107 ± 0.2	FM	93.0 ± 0.9	102 ± 3.4	105 ± 2.7	111 ± 2.3	102 ± 4.1	95.1 ± 4.2	3.60
		CM	98.7 ± 0.4	97.6 ± 1.4	101 ± 0.7	97.6 ± 3.3	99.4 ± 0.6	94.5 ± 0.7	
Sum	782	FM	731	804	837	852	799	744	
		CM	755	745	769	735	746	717	

FM – frozen mushrooms; CM – canned mushrooms; mean ± standard deviation; BW – blanching in water; BSM – blanching in solution of sodium metabisulphite (0.2%) and citric acid (0.5%); BCA – blanching in an aqueous solution of 0.5% citric acid and 0.1% L-ascorbic acid; SBCA – soaking and blanching in an aqueous solution of 0.5% citric acid and 0.1% L-ascorbic acid; BPE – blanching in an aqueous solution of citric acid (0.5%), L-ascorbic acid (0.1%) and low methoxyl pectin (0.5%); SBPE – soaking and blanching in an aqueous solution of citric acid (0.5%), L-ascorbic acid (0.1%) and low methoxyl pectin (0.5%).

to Bernaś and Jaworska (2010), the most abundant exogenous amino acids in frozen and canned *B. edulis* mushrooms were leucine (15–17% of total exogenous amino acids) and valine (14–15%); cysteine was the least abundant (3%). As in the present study, Shah et al. (1997) found that leucine (18%) and lysine (15%) were the dominant exogenous amino acids in dried *P. ostreatus*; however, they determined histidine (4%), methionine (4%) and tryptophan (3%) as the least abundant. According to Guo et al. (2007), cysteine and valine were dominant among exogenous amino acids in dried *P. djamor* mushrooms, comprising 15% and 14%, respectively whereas, in dried *P. ferulae*, leucine (25%) was dominant. The lowest quantities were found for histidine (5%) and methionine (3%) (*P. djamor*), and cysteine (3%) (*P. ferulae*).

In general, the type of pre-treatment applied had a significant effect on the content of exogenous amino acids. In the case of frozen mushrooms, the lowest amounts of most exogenous amino acids were found in the product blanched in water; among canned mushroom products, however, the smallest quantities were found in the product soaked and blanched in a low-methylated pectin solution. Furthermore, it was found that, after 12 months of storage, frozen products obtained from blanched mushrooms had 5–15% more total exogenous amino acids than had those obtained from soaked and blanched mushrooms, and significantly more in the case of cysteine (4–14%), histidine (5–21%), isoleucine (5–16%), leucine (5–16%), lysine (5–17%), phenylalanine (6–16%), and valine (4–17%). In the case of canned products, the difference was 3% for total exogenous amino acids, and 6–20% for cysteine and 4–5% for isoleucine. According to Hurrell (1984) processing and storage modify the nutritional value of proteins, decreasing the amino acid content and/or availability of certain essential amino acid through desulphuration, deamination and isomerisation reactions, with lysine, methionine, cystine and tryptophan being the most susceptible to damage.

With the exception of products obtained from mushrooms blanched in water, frozen *P. ostreatus* products generally had higher levels than had canned products of most of the investigated exogenous amino acids. In particular there were cysteine (25–45% more), isoleucine (4–12%), leucine (1–14%), lysine (6–12%), methionine (25–51%), phenylalanine (1–16%), threonine (2–15%), tyrosine (3–17%) and valine (1–14%). As in the case of endogenous amino acids, the greatest differences were found in products obtained from mushrooms blanched in a pectin solution.

The nutritional value of protein contained in food depends primarily on the amounts and mutual proportions of exogenous amino acids. Mushroom protein contains all of the exogenous amino acids. However, Manzi et al. (1999) and Shah et al. (1997) showed that the main limiting amino acids in mushrooms are the sulphur amino acids and, in certain species, leucine and valine. Mushrooms contain relatively high amounts of lysine, whereas the lysine content of grains is low; for this reason, it is recommended that mushrooms be eaten together with grain products in order to balance the intake of exogenous amino acids (Shah et al., 1997).

3.3. Chemical score (CS) and essential amino acid (EAA) indices

In the relevant literature, protein quality is most frequently defined by comparing the content of exogenous amino acids with the FAO/WHO (1991) pattern; however, in view of the fact that FAO/WHO issued a new pattern in 2007, the results of the present work are given in terms of both patterns.

The protein in fresh *P. ostreatus* mushrooms is a complete protein since, compared with the FAO/WHO (1991, 2007) patterns for adults, the CS index values for the individual amino acids investigated were above 100. Compared with the 1991 pattern, the highest CS index was for histidine (200); but, compared with the 2007 pattern, the highest value was for phenylalanine with tyrosine (231) (Figs. 1 and 2). Regardless of the pattern, the lowest CS index values were found for leucine and lysine: 116 and 106 (FAO/WHO, 1991), and 130 and 137 (FAO/WHO, 2007), respectively; additionally, valine scored 138 compared with the 2007 pattern. Dabbour

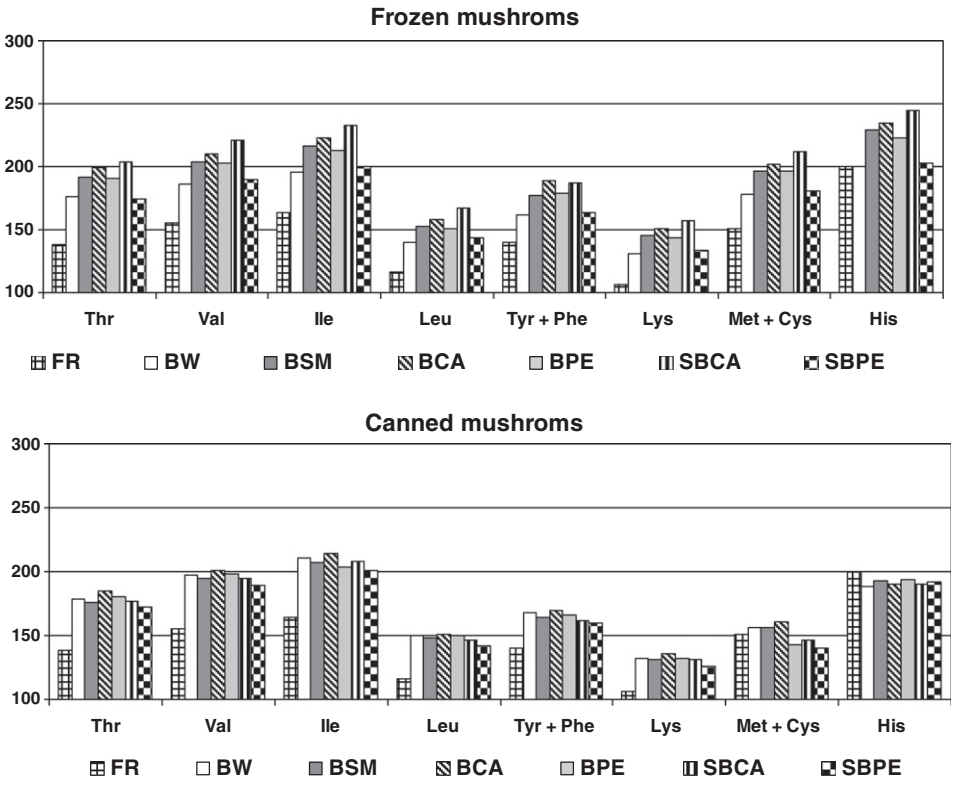

Fig. 1. Chemical score indices for fresh, frozen and canned mushrooms, based on the FAO/WHO (1991) pattern for adults. FR – fresh mushrooms, BW, BSM, BCA, BPE, SBCA, SBPE – see Table 2.

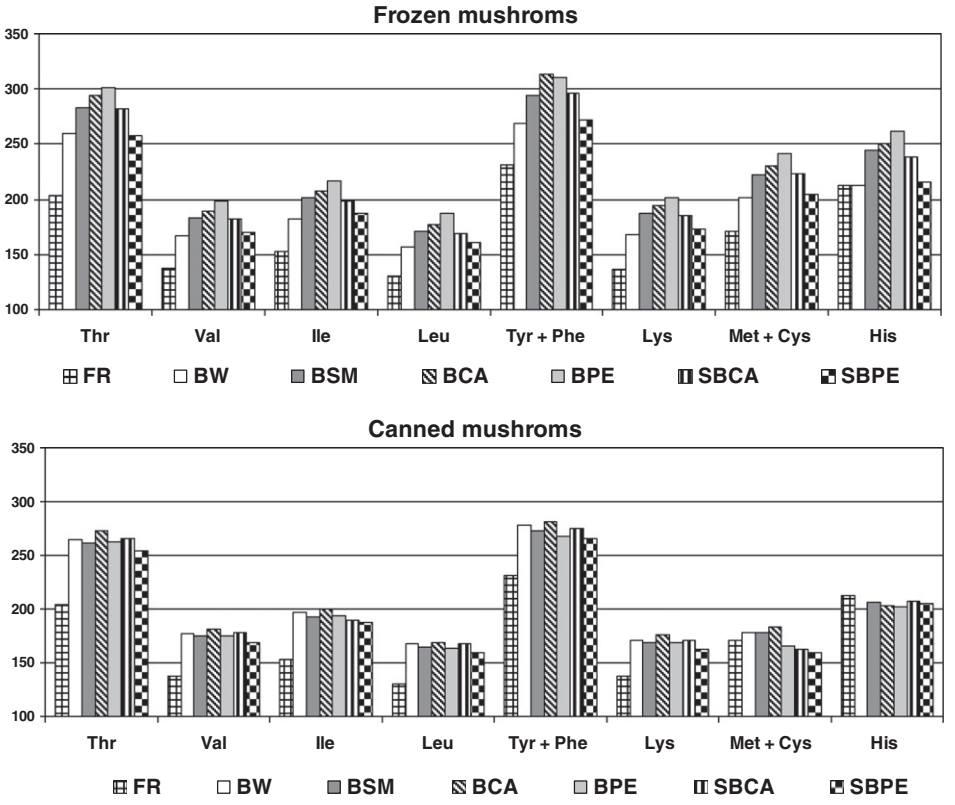

Fig. 2. Chemical score indices for fresh, frozen and canned mushrooms, based on the FAO/WHO/UNU (2007) pattern for adults. FR – fresh mushrooms, BW, BSM, BCA, BPE, SBCA, SBPE – see Table 2.

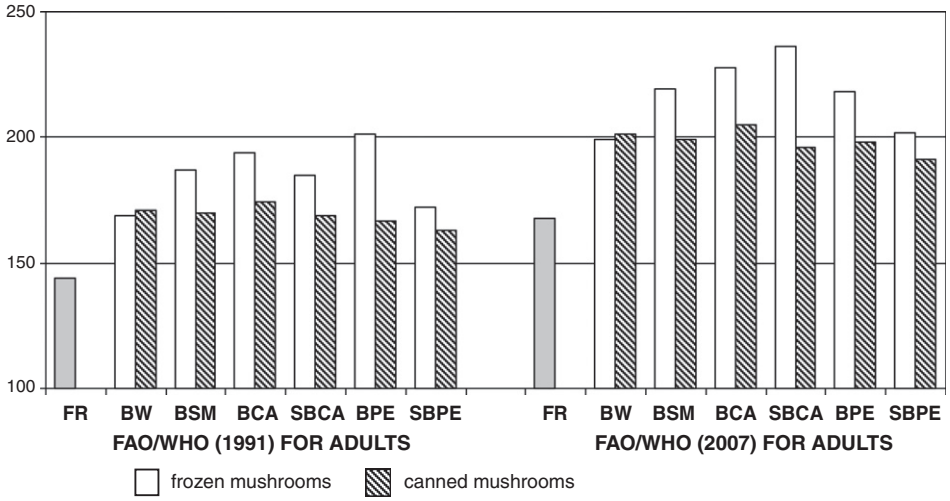

Fig. 3. Essential amino acid indices for fresh, frozen and canned mushrooms according to the reference protein patterns (FAO/WHO). FR – fresh mushrooms, BW, BSM, BCA, BPE, SBCA, SBPE – see Table 2.

and Takruri (1988) and Shah et al. (1997) showed that the amino acids limiting assimilability of protein in *P. ostreatus* are the sulphur amino acids, with a CS index of 61 (Dabbour and Takruri, 1988).

In finished products, as in the raw material, histidine scored the highest CS index value, based on the FAO/WHO (1991) pattern; the lowest values were noted for leucine and lysine and, in the case of canned products, methionine with cysteine. On the basis of the 2007 pattern, threonine, and phenylalanine with tyrosine scored highest on the CS index, while valine and lysine scored lowest, together with methionine and cysteine in the case of canned products. Compared with fresh mushrooms, finished products generally had higher CS index values, the increases ranging from 1% to 48% in frozen products and 15% to 34% in canned products. According to the 1991 pattern, the greatest changes among frozen products were in product BPE and the smallest were in product BW. In the case of canned products, the greatest changes were seen in product BCA and the smallest in product SBPE. Compared with the 2007 pattern, the products tended to be fairly similar, except that, in the case of frozen mushrooms, product SBCA showed the highest increase in the CS index.

With the exception of mushrooms blanched in water, frozen *P. ostreatus* products had higher CS index values than had canned products: histidine was 5–30% higher, isoleucine 4–12%, leucine 1–14%, lysine 6–20%, methionine with cysteine 25–45%, phenylalanine with tyrosine 2–16%, threonine 2–15% and valine 1–14%.

EAA index values for fresh *P. ostreatus* mushrooms varied according to the pattern applied: 144 (FAO/WHO, 1991) and 168 (FAO/WHO, 2007) (Fig. 3). Values for this index increased by 18–47% in frozen products and 13–22% in canned products, recording scores of 169–201 and 163–174 (FAO/WHO, 1991) and 199–236 and 191–205 (FAO/WHO, 2007), respectively. With the exception of products coded BW, frozen mushrooms scored 5–20% higher than did canned mushrooms on the EAA index. The type of pre-treatment applied had little effect on the quality of mushroom protein, as indicated by the EAA index, the main differences arising from the pattern used for comparison.

4. Conclusion

Fresh, frozen and canned *P. ostreatus* mushrooms are potentially a good source of protein in the diet since, on the basis of the FAO/WHO patterns of 1991 and 2007, they were found to contain no limiting amino acids. Of particular note are the high levels of isoleucine, phenylalanine with tyrosine, and threonine.

Freezing and canning, followed by 12 months of storage, brought about a significant reduction in the contents of alanine, glutamine, cysteine and tyrosine and, in the case of canned products, arginine, glycine, serine, histidine, methionine and threonine. The percentage share of endogenous and exogenous amino acids in total amino acids was practically the same for both frozen and canned products.

The type of pre-treatment applied had an effect on the levels of particular amino acids. Frozen products obtained from blanched mushrooms, compared with those obtained from soaked and blanched, had significantly higher levels of arginine, asparagine, glutamine, glycine, serine, cysteine, histidine, isoleucine, leucine, lysine, phenylalanine and valine.

In the case of canned mushrooms, products obtained from blanched mushrooms had higher levels of alanine, asparagine, serine, cysteine and isoleucine. With the exception of products blanched in water, frozen *P. ostreatus* mushrooms generally had significantly higher levels than had canned mushrooms of all the amino acids investigated, the greatest differences being found in products blanched using pectin.

Acknowledgement

The study was financed by the Ministry of Education and Science under research Project No. 2 PO6T 041 29.

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