Note

Proximate and Free Amino Acid Composition during Development of Fruit Body of Kuroawabitake (*Pleurotus abalonus* Han, Chen et Cheng)

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Received May 13, 2003; Accepted October 21, 2003

Kuroawabitake (*Pleurotus abalonus*) were divided into two parts during development of fruit body: coremia (black-headed coremioid) and fruit body. The proximate compositions and free amino acids were determined. There was no marked difference between the coremia and fruit body in proximate composition of protein and carbohydrate (dry weight). The fruit body was high in moisture and fat, whereas the ash was high in coremia. The fruit body contained higher amounts of total free amino acids than the coremia. The contents of monosodium glutamate-like components, including aspartic and glutamic acids, were high in fruit body, and the two parts were considerably different tasting in their amino acids.

Keywords: Kuroawabitake (*Pleurotus abalonus*), coremia, fruit body, proximate composition, free amino acid

The Kuroawabitake mushroom (*Pleurotus abalonus* (Han, Chen et Cheng)) is also called an abalone mushroom. Morphologically, *P. abalonus* is characterized by the formation of black coremia at the base of the stem, black pileus, and brownish lamellae (Miller, 1969; Jong & Peng, 1975; Neda & Furukawa, 1987).

The strain of *P. abalonus* was introduced from Taiwan and the sawdust-based cultivation was started in Okinawa Pref. Research on the cultural characteristics (Kinjo *et al.*, 1992; Takayama *et al.*, 1993; Ohga, 2000) and biological activities (Tamaki *et al.*, 1997, 2002) of this mushroom has been reported.

P. abalonus have gradually become popular in Japan recently due to their delicious and unique texture.

In many parts of Japan, mushrooms have been part of the human diet for a long time. Mushrooms are nutritionally very important since most of them have a very high protein content, in comparison to vegetables (Kagakugijutsu Shigen-chousakai, 2001). It is also known that free amino acids such as glutamic and aspartic acids in mushrooms play an important role in the overall taste. Numerous papers describe the constituent changes in the stages of fruit body formation of the edible mushroom (Kazuno & Miura, 1984, 1985; Muratsubaki *et al.*, 1986; Yoshida *et al.*, 1986, 1987; Tabata & Yamasaki, 1995; Mau *et al.*, 1998). However, the change in components during development of the fruit body of *P. abalonus* is unknown.

Our objective was to examine the proximate and free amino acid compositions in the parts of coremia (note: coremia is characterized by its black-headed coremioid imperfect state which is produced abundantly in culture) and fruit body of *P. abalonus* as shown in Figs. 1–3.

Materials and methods

Cultivation of *P. abalonus* The strain of *P. abalonus* was purchased from Onuki Kinjin Co., Utsunomiya-shi.

The sawdust mixture was composed of 75% (w/w) hardwood sawdust from beech, 20% (w/w) rice bran, and 5% (w/w) corn flour. Tap water was added to the mixture to adjust the water content to about 70%. Substrate (1.2 kg, wet weight) was packed into a heat resistant polyethylene bag, which was then sealed with a cap.

Bags containing substrate were sterilized at 121°C (1.1 atm) for 2 h, then allowed to cool overnight. Each substrate block was inoculated with 10 g of solid sawdust spawn. Inoculated bags of substrate were incubated at 25°C in the dark. When the substrate was fully colonized by the fungal mycelia, that is, after a period of 4 to 5 weeks, they were transferred into a cultivation chamber and the bag was opened. In order to facilitate primordial induction, the temperature was maintained at 23–25°C, the relative humidity was adjusted at 90%, and the light intensity at 300–400 lux for 24 h.

The samples taken as specimens were divided into two parts, coremia and fruit body. Each sample was dried in a forced air-oven at 50–60°C for 70 h. Dried samples were ground in a stainless steel mill to obtain a homogeneous sample.

Proximate composition The moisture, protein, fat, fiber and ash were estimated according to the conventional method (Sugahara, 1995). The use of common factor 6.25 in protein analysis for mushrooms containing more nonprotein may result in overestimation of protein content. Therefore, the averaged value of 3.99 derived from total nitrogen and amino acid nitrogen data of mushrooms was used (Ogawa, 1994; Fujihara *et al.*, 1995). The amount of carbohydrate was estimated by subtracting the amount of protein, fat and ash from 100%.

Analysis of free amino acid One gram of dried mushroom powder was shaken with 25 ml of 10% (w/v) sulfosalicylic acid

Fig. 1. Coremia of *Pleurotus abalonus* on sawdust medium.

Fig. 2. Coremia of *Pleurotus abalonus* on sawdust medium (a stereoscopic microscope photograph).

Fig. 3. Fruit bodies of *Pleurotus abalonus*.

66.8% on a dry basis (Kurasawa *et al.*, 1982). The carbohydrate contents in the two parts of *P. abalonus* were slightly higher than this range. However, the carbohydrate was estimated by subtracting the protein and others from the whole sample. Therefore, the observed high carbohydrate contents in this study are partly responsible for the lower protein estimated using the conversion factor of 3.99 (Table 1).

Fifteen free amino acid contents are shown in Table 2. The coremia and fruit body contained 23.37 and 27.91 mg/g of total free amino acids based on dry weight, respectively. The contents of total free amino acid in the two parts were notably higher than those found in Shiitake mushroom (*Lentinus edodes*) (16.65–21.12 mg/g) (Terashita *et al.*, 1990; Kiribuchi, 1991). The contents were similar to those in Enokitake (*Flammulina velutipes*) (27.85 mg/g) but much lower than those in Hiratake (*Pleurotus ostreatus*) (63.23 mg/g) (Kiribuchi, 1991).

The monosodium glutamate-like components, including aspartic and glutamic acid, were about 1.7 times (75%) higher in fruit body, 6.29 mg/g, than the coremia, 3.59 mg/g. The MSG-like components in fruit body were higher than those in Shiitake mushroom (4.78–4.81 mg/g) (Terashita *et al.*, 1990; Kiribuchi, 1991), whereas those in the coremia were lower than those in Shiitake, Hiratake (8.78 mg/g), and Enokitake (4.55 mg/g) (Kiribuchi, 1991).

The sweet taste components showed no marked differences in threonine, proline, glycine and serine between the coremia and fruit body. However, the content of alanine was much higher in the fruit body. Therefore, 1.1 times more of the sweet tasting amino acids were contained in the fruit body than in the coremia.

(Wako Pure Chemical Industries Co., Osaka) for 20 min at ambient temperature and filtered through Whatman No.4 filter paper. The filtrate was adjusted to pH 2.2 with 3 N NaOH and made up to 100 ml with sodium citrate buffer (Wako). One milliliter of the extract was diluted to 5 ml and filtered through a Millipore LH nonsterile filter (0.45 μ m). The diluted filtrate was mixed with *o*-phthalaldehyde reagent (Wako) in an Eppendorf tube, shaken to facilitate derivatization, and then immediately injected onto the high-performance liquid chromatograph (HPLC). A Shimadzu LC-6A equipped with fluorescence detector, with fluorescence excitation at 348 nm and emission at 450 nm, and a Shim-pack ISC-07/S 1504 Na column (Shimadzu) was used. Each amino acid was quantified by a calibration curve of the authentic amino acid.

Results and Discussion

Fruit body contained higher moisture and fat than coremia, although the ash was low. Coremia contained low amounts of fat but high amounts of ash. Both coremia and fruit body had the same degree of protein and carbohydrate contents. Generally, mushrooms are a good source of protein, and their protein contents range from 10 to 35% of dry weight (Kurasawa *et al.*, 1982). The protein content in the two parts was within this range. The lipid contents in other species of *Pleurotus* range from 1.7 to 4.5% on a dry basis (Kurasawa *et al.*, 1982). The lipid contents in the two parts of *P. abalonus* were within this range. The carbohydrate contents in other species of *Pleurotus* range from 56.9 to

Table 1. Proximate compositions in the coremia and fruit body of *P. abalonus*.

	Content (%) ^{a)}	
	Coremia	Fruit body
Moisture	87.51 $\pm$ 1.61	90.96 $\pm$ 1.71
Protein ^{b)}	2.31 $\pm$ 0.01 (18.49)	1.71 $\pm$ 0.01 (18.81)
Fat	0.24 $\pm$ 0.03 (1.92)	0.33 $\pm$ 0.04 (3.65)
Carbohydrate	8.77 $\pm$ 0.42 (70.22)	6.37 $\pm$ 0.45 (70.46)
Ash	1.17 $\pm$ 0.03 (9.37)	0.64 $\pm$ 0.03 (7.08)

^{a)} Mean $\pm$ standard deviation ($n=3$). Numbers in parenthesis indicate the percentage on a dry basis. ^{b)} The nitrogen factor used for protein calculation was 3.99.

Table 2. Taste characteristics of free amino acids in the two parts of *P. abalonus*.

Taste characteristic	Content (mg/g, dry weight) ^{a)}	
	Coremia	Fruit body
MSG-like	(3.59±0.04)	(6.29±0.03)
L-Aspartic acid	1.16±0.03	1.81±0.02
L-Glutamic acid	2.43±0.04	4.48±0.03
Sweet	(4.70±0.02)	(5.16±0.01)
L-Alanine	1.35±0.04	2.49±0.01
Glycine	0.46±0.01	0.54±0.01
L-Proline	0.55±0.02	0.56±0.01
L-Serine	1.16±0.02	0.82±0.01
L-Threonine	1.18±0.01	0.75±0.01
Bitter	(13.98±0.03)	(14.69±0.03)
L-Arginine	10.82±0.12	12.50±0.08
L-Histidine	0.43±0.02	0.45±0.01
L-Isoleucine	0.62±0.01	0.27±0.01
L-Leucine	0.77±0.01	0.43±0.01
L-Phenylalanine	0.54±0.02	0.60±0.01
L-Valine	0.80±0.01	0.44±0.03
Tasteless	(1.10±0.02)	(1.77±0.03)
L-Lysine	1.10±0.02	1.11±0.03
L-Tyrosine	nd ^{b)}	0.66±0.03
Total	23.37±0.03	27.91±0.02

^{a)} Mean±standard deviation (n=3). ^{b)} not detected.

Much more arginine, a bitter tasting amino acid, was present in the fruit body, about 5.1% (14.69 mg/g), than in the coremia, 13.98 mg/g. The bitter tasting amino acids in these two parts was higher than those in Shiitake (9.53 mg/g) and Enokitake (9.98 mg/g), but lower than in Hiratake (27.02 mg/g) (Kiribuchi, 1991). However, the bitterness in coremia and fruit body of *P. abalonus* could be masked by the sweet components and also by soluble sugars (Mau *et al.*, 1998).

The coremia contained more of the essential amino acids threonine, isoleucine, leucine, and valine than the fruit body. On the other hand, no sulfur-containing amino acid was detected in this study. However, there remains a possibility that the amino acids which are not abundant in mushrooms were destroyed in the course of the experiment. In conclusion, the coremia and fruit body were similar in proximate composition but the two parts were considerably different in free amino acid compositions. Also, the protein contents in the two parts were similar in spite of the lower content of free amino acid in the coremia.

The taste of fruit body of *P. abalonus* might be more intense than Shiitake mushroom on the basis of total free amino acids and MSG-like amino acids shown in Table 2 (27.91 mg/g and 6.29 mg/g, respectively).

Acknowledgment The authors thank Mr. K. Otsuki, Special Forest Products Department, Forestry Experiment Station, Wakayama Research Center of Agriculture, Forestry and Fisheries, Japan, for supplying a photograph of *Pleurotus abalonus*.

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