Seasonal Variations of Free Amino Acids in the Muscle and Viscera of Small Abalone *Haliotis diversicolor*

Deng-Fwu Hwang, Wen-Pin Liang, Chyuan-Yuan Shiau,
Tze-Kuei Chiou, and Sen-Shyong Jeng

Department of Marine Food Science, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.

(Received October 2, 1996)

Seasonal variation in the level of free amino acids was investigated in the muscle and viscera of cultured small abalone *Haliotis diversicolor*. Among free amino acids in the small abalone, the predominant one was taurine, followed by arginine, glycine, glutamic acid and alanine. The muscle contained higher amounts of total free amino acids, taurine, arginine, and glycine than the viscera, but the latter had more glutamic acid than the former. The levels of total free amino acids, taurine, and taste-active amino acids including arginine, glycine, glutamic acid, and alanine in the muscle of small abalone sampled from October to January were higher than those from other seasons. The result of free amino acid analysis was generally consistent with the fact that the small abalone was more tasty in the autumn and winter seasons.

Key words: *Haliotis diversicolor*, small abalone, free amino acids, seasonal variation, taurine

The small abalone *Haliotis diversicolor* is an aquacultural species with a high commercial value in Taiwan. According to consumer preference, the small abalone is the most acceptable for Taiwanese in the autumn and winter seasons. One of the possible reasons could be that the small abalone in these seasons is more palatable than those in other seasons. As described previously,¹⁻³ free amino acids have been found to evoke a marked taste sensation together with some 5'-nucleotides in muscle extracts of the marine invertebrates. The levels of free amino acids in the marine invertebrates, such as clam,^{4,5} ascidian,^{6,7} oyster^{4,8} and disk abalone,^{9,10} are well known to show significant seasonal variation. Among them, oyster was reported to be less palatable in the summer due to having lower levels of free amino acids including glycine.^{4,8} The total amount of free amino acids in disk abalone was the highest in September and decreased to almost one half of the amount in other months.⁹

It is an interesting subject to assess what free amino acid may possibly be associated with the taste preference of cultured small abalone. In this study, the seasonal variation of free amino acid level in the muscle and viscera of small abalone was investigated in detail throughout the year.

Materials and Methods

Samples

Live cultured small abalone *Haliotis diversicolor* specimens were collected from cultural farms in Taipei Prefecture, Northern Taiwan, and Pingtung and Kaohsiung Prefecture, Southern Taiwan in 1994-1995. The samples were transferred within 6 h to our laboratory in Keelung. The size of adult specimens collected was as follows: shell length 3.69-6.82 (5.08 ± 0.51 , mean $\pm$ S.D.) cm and body weight 4.73-28.22 (14.78 ± 4.43) g. For each collection the muscle and viscera from 10 specimens were separately pooled, cut into small pieces and mixed well for extrac-

tion.

Preparation of Extract

The sample was extracted according to the method described by Konosu *et al.*¹¹ A 10 g tissue sample was homogenized with 50 ml of 7% cold trichloroacetic acid (TCA) using a Polytron PT-3000 homogenizer for 2 min. The homogenate was centrifuged at $4000 \times g$ (4°C) for 20 min. The supernatant was filtered through Advantec Toyo No. 2 filter paper. The precipitate was extracted twice with

Fig. 1. Seasonal and regional variations of the level of total free amino acids in the muscle of small abalone *Haliotis diversicolor*.

TCA by the same procedure. The supernatants were combined and made up to 200 ml with 7% TCA. Forty ml of the TCA-extract was mixed with an equal amount of ether to remove TCA and fat. This procedure was repeated 5 times successively for removal of TCA. The aqueous solution was evaporated to dryness *in vacuo* at a temperature below 40°C. The dried matter was diluted with re-distilled water and made up to 25 ml for free amino acid analysis.

Free Amino Acid analysis

Free amino acids were analyzed by a Hitachi L-8500 high-speed amino acid analyzer with a Hitachi 2622 SC packed column (4.6 × 60 mm). The eluents used were the standard lithium citrate buffers. Postcolumn derivatization with ninhydrin yielded amino acid derivatives, the absorbance of which was measured at 570 and 440 nm. Analytical conditions and guaranteed operations including

reproducibility of peak area and separation rate were performed according to the manual provided by the manufacturer (Hitachi, Ltd., Tokyo, Japan). The levels of free amino acids were estimated on the basis of peak areas of the known concentration of a standard (Wako, Ltd., Osaka, Japan) by a Hitachi D-2850 chromato data processor.

Results

Free Amino Acids in the Muscle of Small Abalone

The range of the moisture content in small abalone was 74.67–77.96 (76.39 ± 1.10, mean ± S.D.)% for Taipei's specimens, 74.50–76.47 (75.15 ± 0.76)% for Kaohsiung ones, and 74.60–76.68 (75.40 ± 0.71)% for Pingtung's ones (data not shown). Because the differences in the moisture content among samples were not substantial, the level of free amino acid was expressed in terms of mg in

Fig. 2. Seasonal variation of the levels of major free amino acids in the muscle of small abalone *Haliotis diversicolor* cultured in Taipei Prefecture (A), Kaohsiung Prefecture (B) and Pingtung Prefecture (C).

100 g wet weight of the tissue. The levels of total free amino acids in the muscle of small abalone are shown in Fig. 1. The small abalone sampled from October 1994 to January 1995 had higher levels of total free amino acids in the muscle than those collected from May to September 1995. The range of total free amino acids in the muscle was 1,137–2,407 ($1,760 \pm 484$) mg/100 g for Taipei's specimens, 1,512–2,255 ($1,894 \pm 333$) mg/100 g for Kaohsiung's ones, and 1,551–2,328 ($1,898 \pm 291$) mg/100 g for Pingtung's ones. Among all samples examined, the highest and lowest levels of total free amino acids were found in the specimens collected respectively at the same site in Taipei Prefecture in January and May. The seasonal variation of the samples collected from the Northern area of Taiwan, Taipei Prefecture was more significant ($p < 0.05$) than those from Kaohsiung and Pingtung Prefecture in Southern Taiwan.

The seasonal variations of the major free amino acids in the muscle of small abalone are shown in Fig. 2. Taurine, arginine, and glycine were presented in levels exceeding 100 mg/100 g throughout the whole year, in which taurine was the most dominant one. The highest level of taurine (1,456 mg/100 g) was found in the sample from Taipei Prefecture in January. Regardless of sampling site, the small abalone had a higher level of taurine in the period from October to January than those sampled during other seasons. The lowest level was found in May samples obtained from each collecting site. Arginine and glycine levels were also higher in winter time, but the variation was not remarkable. In addition, glutamic acid and alanine were abundant in the muscle of small abalone. Glutamic acid, alanine, and glycine are well known to have large effects on the taste of seafood.^{1–3} Arginine has also been reported as the taste-active component in scallop^{12,13} and short-necked clam.¹⁴ It was found that the total level of four taste-active amino acids including glycine, glutamic acid, alanine, and arginine in the samples collected from October to January were higher than those of the samples from May to September.

Free Amino Acids in the Viscera of Small Abalone

The levels of total free amino acids in the viscera of small abalone are shown in Fig. 3. The level of total free amino acids in the viscera was lower than that in the muscle. Seasonal variation was also found in the viscera. The range of total free amino acids in the viscera was 1,187–1,850 ($1,508 \pm 255$) mg/100 g for Taipei's specimens, 1,349–1,793 ($1,568 \pm 141$) mg/100 g for Kaohsiung's ones, and 1,472–1,935 ($1,679 \pm 168$) mg/100 g for Pingtung's ones. The highest level of total free amino acids was found in November in a Pingtung sample, but the lowest one was found in May in a Taipei one.

The seasonal variations of major free amino acids in the viscera of small abalone are shown in Fig. 4. Taurine, arginine, glycine, and glutamic acid were the major free amino acids, in which taurine was the most dominant one. The muscle contained higher amounts of taurine, glycine, and arginine than the viscera, but the latter had more glutamic acid than the former. The seasonal variations of arginine and glycine were much larger than other free amino acids. The level of arginine was much lower in January samples, but that of glycine was the lowest in the samples from May

Fig. 3. Seasonal and regional variations of the level of total free amino acids in the viscera of small abalone *Haliotis diversicolor*.

to July. It was found that the total levels of four taste-active amino acids in the viscera were lower than those in the muscle, and were higher in the period from September to November than in other seasons.

Discussion

The present study revealed that the muscle and viscera of the small abalone *H. diversicolor* are characterized by high concentrations of amino acids such as taurine, arginine, glycine, glutamic acid, and alanine, although their concentrations are variable according to the season and probably to the locality. These results were generally similar to those of Japanese abalone.^{9,10,15}

As described by Konosu and Yamaguchi,¹⁵ the concentration of taurine in the abalone was the highest among 60 species of aquatic animals. The accumulation of taurine in mollusk has been reported to be a function of the main cellular osmo-effector.^{16,17} Considerably high levels of taurine present in the muscle and viscera of small abalone throughout the year may also play a role in osmoregulation. As for the characteristic flavor of abalone and scallop, the contribution of taurine was not significant irrespective of a considerable amount.^{2,9} However, this amino acid has been identified as a taste-active component in the savoury soup of short-necked clam,³ in which the taurine level was relatively higher than other extractive components. Fuke³ proposed that not only combinations of taste-active components but also the relative amount of each component were important to produce the characteristic flavor of seafoods in which the kind of taste-active components resembled each other. In this connection, the high level of taurine might be related to the taste of small abalone. An investigation is necessary for further relevant information in this regard.

Fig. 4. Seasonal variation of the level of major free amino acids in the viscera of small abalone *Haliotis diversicolor* cultured in Taipei Prefecture (A), Kaohsiung Prefecture (B) and Pingtung Prefecture (C).

Several free amino acids are known to play an important role in seafood taste. For instance, glycine, alanine, and proline can contribute to sweetness;^{18,19} glutamic acid, glycine, alanine, arginine and serine intensify umami sensation like monosodium-glutamate;^{1,20} leucine, isoleucine, and valine can create bitterness and astringency.^{1,19} The small abalone were rich in glycine, glutamic acid, arginine, and alanine which are known as the taste-active components in abalone,^{9,10} snow crab,¹ scallop,^{12,13} and short-necked clam.¹⁴ These four amino acids may also play an important role for the flavor of small abalone.

The total level of taste-active amino acids was higher in the viscera of small abalone from September to November, and it was higher in the muscle from October to January. In these seasons, the muscle and viscera of small abalone are more tasty. This is consistent with the seasons when large amounts of these free amino acids are accumu-

lated. This finding suggests that the levels of free amino acids are related to the flavor of small abalone. The reason why the seasonal variations of free amino acids in the muscle and viscera of the small abalone are somewhat different should be studied in the future.

References

- 1) S. Konosu, T. Hayashi, and K. Yamaguchi: Role of extractive components of boiled crab in producing the characteristic flavor, in "Umami: A Basic Taste" (ed. by K. Kawamura and M. R. Kare), Marcel Dekker, New York, 1987, pp. 235-253.
- 2) S. Konosu, K. Watanabe, and K. Yamaguchi: Acceptance effects of taste compounds, sensory analysis of taste active compounds in the adductor muscle of scallop, in "Food Acceptance and Nutrition" (ed. by L. Solms, D. A. Booth, R. M. Pangborn, and O. Raunhardt), Academic Press, London, 1987, pp. 143-155.
- 3) S. Fuke: Taste-active components of seafoods with special refer-

- ences to *Umami* substance, in "Seafoods: Chemistry, Processing Technology and Quality" (ed. by F. Shahidi and J. R. Botta), Blackie Academic & Professional, Glasgow, UK, 1994, pp. 115-139.
- 4) I. Takagi and W. Simidu: Seasonal variation of chemical constituents and extractive nitrogens in some species of shellfish. *Nippon Suisan Gakkaishi*, **29**, 66-70 (1963).
- 5) T. K. Chiou, J. F. Lin, and C. Y. Shiau: Seasonal variation in chemical components of cultured hard clam (*Meretrix lusoria*). *Food Sci.*, **23**, 726-733 (1996).
- 6) K. Watanabe, H. Maezawa, H. Nakamura, and S. Konosu: Seasonal variation of extractive nitrogen and free amino acids in the muscle of the ascidian *Halocynthia roretzi*. *Nippon Suisan Gakkaishi*, **49**, 1755-1758 (1983).
- 7) K. Watanabe, H. Uehara, M. Sato, and S. Konosu: Seasonal variation of extractive nitrogenous constituents in the muscle of the ascidian *Halocynthia roretzi*. *Nippon Suisan Gakkaishi*, **58**, 1293-1298 (1985).
- 8) M. Sakaguchi and M. Murata: Seasonal variations of free amino acids in oyster whole body and adductor muscle. *Nippon Suisan Gakkaishi*, **55**, 2037-2041 (1989).
- 9) H. Watanabe, H. Yamanaka and H. Yamakawa: Seasonal variations of extractive components in the muscle of disk abalone. *Nippon Suisan Gakkaishi*, **58**, 921-925 (1992).
- 10) K. Hatae, H. Nakai, A. Shimada, T. Murakami, K. Takada, Y. Shirojo, and S. Watabe: Abalone (*Haliotis discus*): Seasonal variations in chemical composition and textural properties. *J. Food Sci.*, **60**, 32-35 & 39 (1995).
- 11) S. Konosu, K. Watanabe, and T. Shimizu: Distribution of nitrogenous constituents in the muscle extracts of eight species of fish. *Nippon Suisan Gakkaishi*, **40**, 909-915 (1974).
- 12) S. Konosu, K. Watanabe, T. Koriyama, T. Shirai, and K. Yamaguchi: Extractive components of scallops and identification of its taste-active components by omission test. *Nippon Shokuhin Kogyo Gakkaishi*, **35**, 352-358 (1988).
- 13) K. Watanabe, H. L. Lan, K. Yamaguchi, and S. Konosu: Role of extractive components of scallop in its characteristic taste development (taste-active components of scallop-Part 2). *Nippon Shokuhin Kogyo Gakkaishi*, **42**, 378-381 (1990).
- 14) S. Fuke and S. Konosu: Taste-active components of a few species of bivalves, in "Society for Research on *Umami* Taste '89 Forum" (ed. by Y. Kawamura), Society for *Umami* Research on *Umami* Taste, Tokyo, 1989, pp. 85-91.
- 15) S. Konosu and K. Yamaguchi: The flavor components in fish and shellfish, in "Chemistry & Biochemistry of Marine Food Products" (ed. by R. E. Martin, G. J. Flick and D. R. Ward), AVI Publishing, Westport, CT, 1982, pp. 367-404.
- 16) E. N. Powell, K. Margaret, E. Chen, M. Koenig and J. Pecon: Changes in the free amino acid pool during environmental stress in the gill tissue of the oyster, *Crassostrea virginica*. *Comp. Biochem. Physiol.*, **71A**, 591-598 (1982).
- 17) B. W. Heavers and C. S. Hammen: Fate of endogenous free amino acids in osmotic adjustment of *Crassostrea virginica* (Gmelin). *Comp. Biochem. Physiol.*, **82A**, 571-576 (1985).
- 18) N. R. Jones: Fish flavors, in "The Chemistry and Physiology of Flavors" (ed. by H. W. Schultz, E. A. Day and L. M. Libbey), AVI Publishing, Westport, CT, 1967, pp. 267-295.
- 19) T. Nishimura and H. Kato: Taste of free amino acids and peptides. *Food Rev. Int.*, **4**, 175-194 (1988).
- 20) J. Kirimura, A. Shimizu, A. Kimizuka, T. Ninomiya and N. Katsuya: The contribution of peptides and amino acids to the taste of foodstuffs. *J. Agric. Food Chem.*, **17**, 689-695 (1969).