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PROXIMATE COMPOSITION AND FREE NITROGEN-CONTAINING COMPOUNDS IN RAW HYBRID ABALONE (HALIOTIS DISCUS HANNAI x H. DIVERSICOLOR DIVERSICOLOR), COMMERCIAL PROCESSED ABALONE AND ABALONE ANALOGUE PRODUCTS

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PROXIMATE COMPOSITION AND FREE NITROGEN-CONTAINING COMPOUNDS IN RAW HYBRID ABALONE (*HALIOTIS DISCUS HANNAI* × *H. DIVERSICOLOR DIVERSICOLOR*), COMMERCIAL PROCESSED ABALONE AND ABALONE ANALOGUE PRODUCTS

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Key words: abalone, taurine, free amino acids, nucleic acids, abalone analogue, flavor additives.

ABSTRACT

The proximate composition, pH, free amino acids, ammonia and nucleotide-related compounds of raw abalone, commercial processed abalone and abalone analogues were investigated. Taurine was the dominant free amino acid (FAA), accounting for 61.4% of the total FAA in raw hybrid abalone and 29.9% in processed abalone. Abalone analogue products had a high amount of free amino acids, but only 0.3-2.5% taurine. The proximate composition and pH value of abalone changed only slightly after blanching and processing, while the taurine and glycogen content decreased by 50%. FAA profiles were significantly different between commercial processed abalone and analogue products, both of which had lower levels of taurine and arginine than raw abalone. Abalone analogue product made from fish surimi contained large amounts of proline and glycine, accounting for 53.9% of FAA. The profiles of adenosine 5'-triphosphate (ATP) related compounds, which were introduced as flavor additives during processing, were different between commercial processed abalone and abalone analogue products.

I. INTRODUCTION

Abalone is an expensive shellfish used in gourmet cuisines. It is usually consumed either fresh-cooked, canned or dehydrated. Abalones (*Haliotis* spp.) are marine gastropods in the family Haliotidae. They contain abundant vitamins and protein (Kim et al., 2006). Abalone muscle contains a high amount of taurine, which is an essential growth factor in the prevention of certain pathological ailments and also essential for bile acid synthesis in humans (Kim et al., 2006). Taurine is important for functional regulation of the brain, central nervous system, eyes, heart, and muscles in humans (Kim et al., 2006). It can also reduce fatigue, and ameliorate severe allergic reactions (Choi et al., 2003; Kim et al., 2006). Abalone contains carotenoids including α- and β-carotene, loroxanthin, lutein, siphonanthin, siphonein fucoxanthin and zeaxanthin (Ahn, 1974).

Small abalone (*Haliotis diversicolor*) are generally smaller in size and softer in texture than large forms called simply "abalone" herein. Abalone both large and small are often consumed by gently cooking with soy sauce, brown sugar, vinegar, spices, pepper, chili, herbs and food additives to enhance flavor. Processed abalone has been sold as frozen food in Taiwan in recent years. Inasmuch as surimi has been used as an intermediate-cost product for a variety of fabricated seafoods, a high demand exists for abalone analogues prepared from fish and squid surimi in Taiwan. The proximate composition, free amino acids and peptide content of small abalone have been studied (Hwang et al., 1997; Choi et al., 2003), but little is known regarding these and related chemical characteristics of abalone analogues compared to raw and processed abalone. The aims of the present investigation were to compare the proximate composition, glycogen, pH, ammonia level, free amino acids and nucleotide-related compounds of raw abalone, processed abalone and abalone analogues in Taiwan. This information can provide a better understanding of abalone and its application in the frozen food industry.

II. MATERIALS AND METHODS

1. Raw Materials and Chemicals

Cultured hybrid abalone (*Haliotis discus hannai* × *H. diver-*

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sicolor diversicolor) ranging in size (shell length) from 6.9 to

Table 1. Ingredients as indicated on product labels and manufacturers of abalone products used in this study.

Sample code	Product type	Ingredients	Manufacturers
P1	abalone	abalone, soybean sauce, sucrose, salt, vinegar	Fubao (Gonglian District, New Taipei City, Taiwan)
S1	squid cubes	squid, water, sucrose, salt, monosodium L-glutamate, mirin, hydrolyzed animal protein, soybean hydrolyzed protein, seafood extract, sodium 5'-inosinate, sodium 5'-guanylate, glycine, DL-alanine, abalone flavor: succinate, disodium succinate, methionine, silicon dioxide, caramel, medium chain triglycerides, spices	Bai Xia Wu Enterprise Co. Ltd. (Qianzhen District, Kaohsiung City, Taiwan)
S2	squid slices	squid, water, sucrose, salt, monosodium L-glutamate, mirin, hydrolyzed animal protein, soybean hydrolyzed protein, seafood extract, sodium 5'-inosinate, sodium 5'-guanylate, glycine, DL-alanine, abalone flavor: succinate, disodium succinate, methionine, silicon dioxide, caramel, medium chain triglycerides, spices	Bai Xia Wu Enterprise Co. Ltd. (Qianzhen District, Kaohsiung City, Taiwan)
S3	squid slices	squid, salt, soybean sauce, monosodium L-glutamate, D-sorbitol, spices	Chin Chi Shun Industrial Co. Ltd. (Fengshan, Kaohsiung City, Taiwan)
S4	fish surimi	fish surimi, potato starch, wheat starch, soybean sauce, sucrose, salt, abalone extract, spices, vinegar, mirin, monosodium L-glutamate, glycine, disodium succinate, sodium 5'-inosinate, sodium 5'-guanylate	Shin Ho Sing Ocean Enterprise Co Ltd. (Chen District, Kaohsiung City, Taiwan)

7.9 cm and in body weight from 38.4 to 61.2 g, and with an average foot content of 39%, were obtained in three batches from October, 2015, to January, 2016, from a commercial aquaculture facility in Gonglian District, New Taipei City, Taiwan. All had been fed with *Gracilaria lemaneiformis* for at least 1 year, and before collection they were starved and kept in flow-through seawater for 1 day in a plastic cage. The raw abalone were blanched, cooked with sauce, packed in zipper bags and frozen (as sample P1) and stored at -20°C until analysis. Vacuum-packed frozen abalone analogue products (samples S1, S2 and S4, 180-200 g each) were purchased from a supermarket in New Taipei City, Taiwan, and supplemented by frozen sample S3 (180 g) from a different supermarket in Keelung, Taiwan. All chemicals used in this work were of analytical grade. The lists of ingredients of all samples and their manufacturers are shown in Table 1. For analysis, all samples were thawed in running tap water.

2. Proximate Composition, pH and Glycogen Analyses

Moisture content was measured following AOAC (2000) by drying the samples in an oven at 105°C for 24 h and recording the percent loss of weight from start to finish. Ash was measured after combustion at 530°C for 24 h. Lipid content was determined by the ether extraction method (AOAC, 2000). Crude protein (N × 6.25) was measured using the Kjeldahl method. Crude carbohydrate was obtained by 100 − (moisture + crude protein + lipid content + total ash percentages). Five g of each sample was added to 45 g of distilled water, blended and filtered with Whatman No. 1 filter paper (Sigma-Aldrich Co., St. Louis, Missouri, USA) before pH analysis (Mettler Toledo MP-220, Zurich, Switzerland).

Glycogen in abalone and abalone analogue products was measured according to the method of Carroll et al. (1956) with slight modification. A 5 g sample was homogenized with 15 ml pre-cooled 7% trichloroacetic acid (TCA) for 2 min at 4°C, then centrifuged for 20 min at 4200 *g* (Hitachi GR21, Tokyo, Japan). The precipitate was extracted twice with 7% TCA by the same procedure. The supernatants were combined and made up to 100 ml with 7% TCA. One ml of this extract was transferred into a tube with 5 ml of 95% ethanol and mixed thoroughly. The tubes were put in a water bath at 37-40°C for 3 hours. After precipitation was complete, the tubes of extract were centrifuged at 1100 *g* for 15 min. The supernatant was decanted from the glycogen precipitant, which was then dissolved by the addition of 2 ml of distilled water. Aliquots of this glycogen solution were taken up for suitable dilution and 5 ml of anthrone reagent (Sigma-Aldrich, St. Louis, MO, USA) were added to each tube, which was immediately cooled in an ice bath. The tubes were then shaken well and immersed in boiling water for 15 min, then removed to a cold water bath and cooled to room temperature. Each sample was read at 620 nm after adjusting the colorimeter (Hitachi U-1900, Tokyo, Japan) with a water blank treated in a similar manner. Standard glucose solution was also treated similarly. Glycogen concentration was expressed as glucose equivalent. It was estimated from the standard curve multiplied by 0.9.

3. Amino Acid and Ammonia Analyses

The free amino acid assay followed the method of Konosu et al. (1974). Abalone (5.0 g) was homogenized with 15 ml of 7% trichloroacetic acid (TCA) for 2 min, and the resulting mixture was centrifuged at 4°C for 20 min at 4200 *g* (Hitachi GR21, Tokyo, Japan). The supernatant was then filtered with Toyo No. 2 filter paper (Toyo Roshi Kaisha, Ltd., Japan). The sediment was blended with 7% TCA solution at 4°C and centrifuged as above. These procedures were conducted twice and then the solutions were filtered with Toyo No. 2 filter paper (125 mm) and made up to 100 ml with 7% TCA. All TCA-extracted super-

natants were mixed with an equal amount of ether to remove the TCA (Konosu et al., 1974). This procedure was repeated five times. The aqueous solution was then evaporated under vacuum (IKA Vacuum Decompression Concentrator RV 10 basic, Staufen, Germany) at 40°C. Each TCA extract was dissolved in 25 ml of double-distilled water. One ml of the resulting solution was taken up for suitable dilution in 0.02 N HCl, then filtered through a nylon filter (0.2 μm) and injected into an amino acid analyzer (Hitachi L-8900, High-Technologies Corp. Tokyo, Japan) for amino acid and ammonia analyses. A Hitachi 2622 SC packed column (4.6 mm I.D. × 60 mm) was used to separate the free amino acids and ammonia; standard lithium citrate buffers were used. Postcolumn derivatization with ninhydrin yielded amino acid derivatives, the concentration of which was measured by spectrophotometry (Hitachi U-1900, Hitachi Ltd., Tokyo, Japan) at 440 and 570 nm. For most amino acids, the resultant blue-violet color showed an absorption with λmax = 570 nm, proline yielded a yellow-colored compound with an absorption maximum at 440 nm.

Table 2. Proximate composition and glycogen concentration (%) of raw abalone, commercial processed abalone and abalone analogue products.

Sample		Moisture	Crude protein	Crude fat	Ash	Carbohydrate[1]	Glycogen
C1	Raw abalone	75.69 ± 1.66[2]	14.27 ± 0.35	0.18 ± 0.03	1.93 ± 0.03	7.93 ± 1.04	3.63 ± 0.11
P1	Processed abalone	75.00 ± 0.34	15.67 ± 1.08	0.16 ± 0.01	2.87 ± 0.04	6.29 ± 1.34	1.98 ± 0.05
S1	Abalone analogue	76.42 ± 0.27	17.56 ± 0.96	0.32 ± 0.04	2.27 ± 0.09	3.99 ± 1.27	-*
S2	Abalone analogue	78.09 ± 0.28	15.97 ± 1.30	0.26 ± 0.05	2.20 ± 0.17	3.49 ± 1.58	-
S3	Abalone analogue	68.46 ± 0.12	10.53 ± 0.17	0.13 ± 0.01	2.97 ± 0.03	17.91 ± 0.11	-
S4	Abalone analogue	71.03 ± 0.10	15.57 ± 1.11	0.16 ± 0.01	0.99 ± 0.03	12.24 ± 0.98	1.79 ± 0.04

* Not detected.

[1] Carbohydrate (%) = 100% − (Moisture + Crude protein + Crude fat + Ash) × 100%.

[2] Values are expressed as mean ± standard deviation, n = 3.

4. Adenosine 5'-Triphosphate (ATP) Related Compounds

ATP-related compounds were extracted from abalone and abalone analogue products with 6% perchloric acid (PCA). A 5.0 g portion of each sample was homogenized with 10 ml of 6% PCA for 2 min (Brinkmann Polytron PT 3000, American Laboratory Trading, San Francisco, CA, USA). The homogenized samples were then centrifuged at 3000 g for 20 min at 4℃ (Hitachi SCR 20 B, Tokyo, Japan). The supernatant was filtered with Toyo No. 2 filter paper, and the precipitant was extracted with 6% PCA as described above. All filtrates were combined and made up to 100 ml with 6% PCA solution. A Capcell Pak C_{18} AQ S5 stainless steel column (4.6 mm I.D. × 250 mm) was used for the separation of ATP-related compounds. Potassium dihydrogen phosphate (50 mM) and dipotassium hydrogen phosphate (50 mM) were dissolved in HPLC-grade water (Great Tide Instrument Co., Ltd., Taipei, Taiwan) and pH was adjusted to 6.5 for use as the mobile phase. The solution was filtered using a 13 mm PVDF 0.22 μm filter (Pure Tech, Changhua County, Taiwan) and sonicated (Ultrasonic Cleaner DC 200H, Macro Fortunate Co., Ltd., New Taipei City, Taiwan) for 30 min prior to injection into the column. Flow rate was maintained at 0.8 ml/min. Absorbance of the elutrient was measured at 260 nm.

Nucleotides and related compounds were measured using reverse phase high-performance liquid chromatography (Shimazu LC-10A, Nacalai Tesque Inc., Kyoto, Japan) in accordance with the method described by Shi et al. (2015). The identification of ATP, ADP, AMP, IMP, HxR and Hx was determined by comparing their retention times with a standard mixture (Sigma-Aldrich Chemicals Private Ltd., Bangalore, India) and by the spiking or addition of standards.

5. Statistical Analyses

Data were analyzed by analysis of variance using the SPSS statistics program (version 12, 2000; SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was used to identify the difference between treatments at a 5% significance level ($p < 0.05$).

III. RESULTS AND DISCUSSION

1. Proximate Composition and Glycogen Content

Table 2 shows the proximate composition and glycogen content of raw abalone and commercial processed abalone and analogue products. The moisture content ranged from 68.5% to 78.1%, protein from 10.5% to 17.5%, crude fat from 0.1% to 0.3%, ash from 1.0% to 3.0%, carbohydrates from 3.5% to 17.9% and glycogen content from undetected to 3.6% (Table 2). The proximate compositions of raw abalone (C1) and commercial processed abalone (P1) were not different except that the ash content of P1 (2.87%) was higher than that of C1 (1.93%) (Table 2) due to the seasonings and other additives in the processed abalone. On a dry-weight basis, protein (58.10%) was predominant in abalone analogue products, followed by carbohydrates (23.32%) because starch was added to improve the texture and reduce the cost. Abalone contained more glycogen than abalone analogue products (Table 2). Abalone analogue products S1, S2 and S3 were manufactured by the same company but are different types of product. The proximate compositions of S1 and S2 were similar except that S2 had a higher moisture content, also with respect to S3 (Table 2). Abalone analogue product S3 contained lower moisture and protein contents but

higher ash and carbohydrate contents than all the other analogue products S1, S2 and S4, indicative of the starch and seasonings that were added to this product. Abalone analogue product S4 contained the lowest ash content (Table 2), indicative of less added salt and monosodium glutamate (MSG). Its nutrition label showed a sodium concentration less than half that of all the other samples (data not shown). Glycogen was only detected in abalone analogue product S4, at a level lower than in abalone samples C1 and P1 (1.79% vs 2.63% and 1.98%, respectively) (Table 2). The glycogen was due to abalone extract being added to the fish surimi during processing, as noted in the ingredient list (Table 1). Marine shellfish and freshwater clams are in general rich in glycogen, compared to fish muscle; glycogen plays an important role in metabolism and supplies energy for gametogenesis in many bivalves (Wu and Shiau, 2002). Glycogen also improves the characteristic flavor of shellfish although it itself is tasteless (Konosu, 1973).

Table 3. pH values and ammonia levels (mg/100 g) of raw and commercial prepared abalone and abalone analogues.

Sample	Product type	pH	NH_3
C1	Raw abalone	$6.75 \pm 0.04^{b,1,2}$	5.06 ± 0.70^{d}
P1	Processed abalone	6.79 ± 0.01^{b}	12.57 ± 1.05^{c}
S1	Abalone analogue	6.72 ± 0.10^{b}	23.66 ± 1.28^{a}
S2	Abalone analogue	6.77 ± 0.06^{b}	17.58 ± 1.40^{b}
S3	Abalone analogue	6.94 ± 0.07^{a}	18.95 ± 2.30^{b}
S4	Abalone analogue	6.45 ± 0.02^{d}	6.41 ± 1.08^{d}

[1] Values are expressed as mean ± standard deviation, $n = 3$.

[2] Values with different superscripts within the same column are significantly different ($p < 0.05$).

2. pH and Ammonia Level of Abalone and Abalone Analogue Products

The pH of raw abalone (C1 = 6.75) did not change significantly after processing (P1 = 6.79) as shown in Table 3. It was, however, slightly higher than the pH of 6.6 measured in processed abalones (*Haliotis asinia*) by Siripatrawan et al. (2009). The pH of abalone analogue products S1 to S4 ranged from 6.45 to 6.94. Product S4 (pH 6.45), made from fish surimi, was significantly lower in pH than raw abalone (6.75), processed abalone (6.79) and abalone analogues S1, S2 and S3 (6.72, 6.77 and 6.94, respectively), which were made from squid, but pH can not be used for monitoring quality changes in fish products (Morkore and Lilleholt, 2007). A significantly lower ammonia level (6.41 mg/100 g) was found in abalone analogue S4 than in abalone analogues S1, S2 and S3. Cephalopod surimi has previously been reported to contain higher amount of ammonia than fish surimi (Vecchione and Galbraith, 2001). The optimum pH for fish surimi gelation lies within the range of pH 6.5-7.5 (Park et al., 2013), but the pH of abalone analogue S4 was a little lower than this.

3. Free Amino Acids (FAAs) and Taurine

Total FAA of raw abalone was 1581 mg/100g, and in the five commercial processed abalone and abalone analogue products it ranged from 336 to 1283 mg/100g (Table 4). Raw and processed abalone (C1 and P1) and abalone analogue S4 characteristically possessed a much higher amount of FAA. The predominant FAA in raw abalone and commercial processed abalone was taurine, which accounted for 61% and 29% of total FAA, respectively. Taurine, proline, glycine, alanine and arginine are the most prevalent free amino acids in invertebrates (Konosu and Yamaguchi, 1982). Taurine in mammalian systems plays a role in bile salt formation, and is also important in osmoregulation, membrane stabilization, growth modulation, glycolysis, stimulation of glycogenesis, antioxidation and calcium homeostasis (Sturman, 1993; Stapleton et al., 1997; Redmond et al., 1998; Aly and Khafagy, 2014; Zhu et al., 2016). Taurine is also an important component in chicken essence, which has been marketed as a health food with the above-mentioned bioactivities (Wu and Shiau, 2002). Although taurine is the main FAA in cephalopods (D'Aniello et al., 1995; George-Zamora et al., 2011), abalone analogue products S1, S2 and S3 made from squid contained taurine at less than 2.5%, possibly due to leaching and heating during processing. Fresh and cooked jumbo squid mantle muscle is rich in arginine, histidine, taurine, alanine and methionine, but the concentration of taurine decreases after cooking (Rosas-Romero et al., 2010). A low concentration of taurine was also found by us in processed abalone (Table 4).

Significantly larger amounts ($p < 0.05$) of taurine, ornithine, serine and threonine were detected in raw abalone and commercial processed abalone product P1 compared to abalone analogue products S1-S4 (Table 4). Accumulated taurine in animals functions as the main cellular osmo-effector (Konosu and Yamaguchi, 1982; Amarowicz and Shahidi, 1997). Freshwater clams grown in low salinity may need to lower their taurine level for osmoregulation (Wu and Shiau, 2002). Abalone is a marine shellfish, so it needs more FAA such as taurine for osmo-regulation.

Total FAA in raw abalone and in commercial processed abalone was 4.7 and 3.6 times higher than that in commercial abalone analogue product S3 (Table 4). In addition, the raw and processed abalone samples contained higher taurine (61% and 29%, respectively), but lower alanine (1.3% and 4.0%, respectively) than the analogue products. Raw abalone and commercial processed abalone product can be regarded as rich sources of taurine, beneficial to human health. Taurine was also the most abundant FAA in small abalone (*Haliotis diversicolor*), followed

by arginine and glycine (Chiou et al., 2001). The total FAA level and the amino acid profile in the muscle of abalone in the present study were similar to those of small abalone (Hatae et al., 1995).

Table 4. Free amino acids (mg/100 g) of abalone and abalone analogue products.

Amino acid	C1	P1	S1	S2	S3	S4
Phosphoserine	3.4[b,1,2]	11.6[a]	1.8[cd]	1.2[cd]	1.1[d]	2.2[c]
Taurine	971.1[a]	361.2[b]	7.8[c]	6.3[c]	8.3[c]	4.1[c]
Aspartic acid	26.3[b]	61.7[a]	9.4[c]	8.5[c]	11.5[c]	11.0[c]
Threonine	14.9[b]	31.4[a]	6.7[c]	6.1[c]	6.4[c]	7.5[c]
Serine	13.7[b]	40.8[a]	8.4[c]	7.8[c]	6.7[c]	9.8[c]
Glutamic acid	22.4[d]	154.9[c]	591.0[a]	533.3[a]	35.8[d]	328.1[b]
Sarcosine	-[*]	1.0	-	-	-	-
Glycine	120.1[c]	52.1[b]	53.8[b]	48.1[b]	25.1[c]	260.0[a]
Alanine	0.8[e]	48.4[c]	146.6[a]	127.7[ab]	23.9[d]	119.1[b]
Citrulline	9.1	4.9	-	-	-	-
α-Aminobutyric acid	-	2.2[a]	1.1[b]	1.2[b]	1.8[a]	0.9[b]
Valine	16.6[b]	37.6[a]	1.9[d]	7.8[c]	11.8[c]	18.5[b]
Cystine	-	-	17.4[a]	5.1[c]	10.1[b]	-
Methionine	5.5	13.1	12.7	8.7	17.4	10.2
Isoleucine	8.5[b]	33.4[a]	13.5[b]	9.6[b]	12.6[b]	16.3[b]
Leucine	8.9[b]	49.7[a]	8.9[b]	6.6[b]	8.7[b]	9.2[b]
Tyrosine	16.3	16.0	-	-	-	-
Phenylalanine[b]	14.7	33.4[a]	11.7[c]	10.7[c]	12.6[c]	16.4[b]
β-Alanine	11.7	7.1	-	-	5.0	9.6
β-Amino-isobutyric acid	7.1	7.0	-	-	-	2.3
γ-Aminobutyric acid	0.6	6.6	-	-	-	1.5
Tryptophan	1.4	5.3	-	-	-	-
Ornithine	5.7[b]	10.2[a]	1.0[cd]	1.2[cd]	2.0[c]	1.0[d]
Lysine	10.4[b]	37.3[a]	7.0[b]	7.2[b]	9.8[b]	9.4[b]
Histidine	6.8[b]	9.4[a]	3.2[cd]	3.5[c]	3.8[c]	2.5[d]
Arginine	241.5[a]	126.7[b]	63.5[c]	70.0[c]	70.5[c]	8.7[d]
Proline	9.8[d]	43.4[c]	97.9[b]	93.9[b]	47.7[c]	431.7[a]
Total	1581.4[a]	1207.0[bc]	1066.9[cd]	972.5[d]	335.9[e]	1283.7[c]

[*]Not detected.

C1: raw abalone, P1: processed abalone, S1-S4: abalone analogue products

[1] Values are expressed as means, $n = 3$.

[2] Different superscript letters in the same row are significantly different ($p < 0.05$).

Glycine (120.1 mg/100 g) and arginine (241.5 mg/100 g) were also abundant in raw abalone (C1), constituting 7% and 15% of the total FAA, respectively. In commercial processed abalone, glutamic acid, arginine and aspartic acid accounted for 12%, 10% and 5% of the total FAA, respectively. Glycine and glutamic acid were the most important amino acids among taste components related to the palatability of small abalone (Chiou et al., 2001). Arginine is typically rich in cephalopod and gastropod molluscs (Konosu and Yamaguchi, 1982). Its concentration (8.7 mg/100 g) was the lowest in abalone analogue product S4 among all the samples tested in the present study (Table 4). S4 was made from fish surimi and contained high amounts of proline (431.7 mg/100 g) and glycine (260.0 mg/100 g) (Table 4), together accounting for 58.6% of the FAA in the sample.

The FAA profile of abalone analogue products made from squid was different from that of the analogue made from fish surimi. Glycine, alanine, and especially proline are the most prevalent free amino acids in dried squid (Tsai et al., 1989) and proline is sometime used as a nutritional additive in preparing analogue product S4. The ingredients label of product S4 showed that abalone extract was added, possibly increasing the glycine content and sweetness of the product. The taurine, glycine and arginine concentrations of processed abalone were significantly lower than those of raw abalone (Table 4), possibly due to losses in cooking and blanching.

Monosodium glutamate (MSG), aspartic acid and glutamic acid are often used in processed foods to enhance umami taste (Fuke, 1994). Glutamic acid is the second most abundant FAA in cephalopod species (D'Aniello et al., 1995; George-Zamora

et al., 2011), but it is possible it is lost in the abalone analogue production process. Nonetheless, it was the dominant FAA in

Table 5. Nucleotide-related compounds (μmol/g) of raw abalone, prepared abalone and abalone analogue products.

	C1 Raw abalone	P1 Processed abalone	S1 Abalone analogue (made from squid)	S2 Abalone analogue (made from squid)	S3 Abalone analogue (made from squid)	S4 Abalone analogue (made from fish surimi)
ATP	$0.11 \pm 0.02^{b,1}$	0.25 ± 0.04^{a}	-*	-	-	-
ADP	0.21 ± 0.05^{a}	0.18 ± 0.05^{b}	0.02 ± 0.02^{c}	0.07 ± 0.01^{bc}	0.08 ± 0.04^{bc}	0.07 ± 0.01^{bc}
AMP	1.83 ± 0.16^{b}	0.16 ± 0.10^{b}	0.28 ± 0.06^{a}	0.38 ± 0.04^{a}	0.14 ± 0.06^{bc}	0.04 ± 0.02^{c}
IMP	0.12 ± 0.04^{d}	1.54 ± 0.05^{c}	1.42 ± 0.31^{c}	1.54 ± 0.33^{c}	3.19 ± 0.33^{a}	2.37 ± 0.50^{b}
Inosine	0.01 ± 0.01^{a}	0.22 ± 0.13^{a}	-	-	-	0.16 ± 0.05^{a}
Hypoxanthine	0.24 ± 0.10^{d}	1.34 ± 0.15^{c}	1.83 ± 0.20^{b}	1.86 ± 0.13^{b}	2.42 ± 0.11^{a}	0.41 ± 0.09^{d}
Total	2.52 ± 0.47^{c}	3.69 ± 0.32^{bc}	3.58 ± 0.25^{bc}	3.85 ± 0.25^{b}	5.84 ± 0.39^{a}	3.04 ± 0.58^{c}

* Not detected.

[1] Values are expressed as mean ± standard deviation, $n = 3$; within the same row values with different superscript letters are significantly different ($p < 0.05$).

products P1, S1, S2, S3 and S4, accounting for 12%, 55%, 54%, 10% and 25% of the total FAA, respectively. These values were much higher than that of raw abalone (C1) (1.4% glutamic acid in total FAA). The high concentration of glutamic acid in the processed products evidently comes from food additives.

The aspartic acid concentration of processed abalone was also higher than that of raw abalone (Table 4). The high quantity of glycine in Product S4 might also be artificial, coming from added glycine. Glycine contributes to the sweet taste of seafood (Konosu and Yamaguchi, 1982; Fuke, 1994) and the same may be true for this abalone analogue product. Altogether, our findings provide confirmation that Product S4 was not made from authentic processed abalone.

Raw abalone had a higher concentration of total FAA than the processed abalone and the commercial abalone analogue products, and Product S3 had the lowest concentration. The FAA profiles of products S1, S2 and S3 were different from those of raw abalone C1 and processed abalone product P1. Glutamic acid, glycine, alanine, arginine and proline were the major FAA of Product S3, together accounting for 59.7% of the total FAA, while taurine accounted for only about 2.5%. The same amino acids were dominant in Products S1 and S2, the free amino acid profiles of which were very similar except for valine and cystine (Table 4), and which were manufactured by the same company.

Alanine was the predominant FAA in abalone analogue products S1 to S4 (7.1% to 13.7%). Alanine has been recognized as a taste-active compound for shellfish (Konosu and Yamaguchi, 1982; Fuke, 1994), and it is often added together with MSG to improve food taste. Product S3 had less alanine and MSG added than S1, S2 and S4 (Table 4). Alanine, arginine, glutamic acid and glycine are recognized as taste-active components of various foods (Konosu and Yamaguchi, 1982; Fuke, 1994). The high concentrations of these FAAs in raw abalone, processed abalone and commercial abalone analogue products contributed to the taste of these products.

4. ATP-Related Compounds

Total concentrations of ATP derivatives in processed abalone (P1) and abalone analogue products (S1 to S4) were higher than that of raw abalone (Table 5). Abalone analogue product S3 (5.84 μmol/g) contained the highest content of total ATP derivatives. ATP is rapidly degraded to IMP after fish death (Siripatrawan et al., 2009). Our results were in good agreement with those of Siripatrawan et al. (2009), who found that raw abalone accumulated mainly AMP instead of IMP or adenosine. Very low activities of AMP-deaminase and adenosin-deaminase activities in abalone result in AMP accumulation (Watanabe et al., 1992), and accumulation of AMP was also observed in this study. AMP imparts an umami taste by synergism with glutamic acid, specifically the sensation of savoriness (Hatae et al., 1995). Chiou et al. (2001) proposed that AMP may be the most important taste component related to the palatability of small abalone. In the present study, processed abalone contained high levels of both IMP (1.54 μmol/g) and hypoxanthine (Hx) (1.34 μmol/g) (Table 5). Both concentrations were significantly higher than for those compounds in raw abalone. Howgate (2005) reported that the loss of flavor and freshness in several fish species was correlated with decreasing IMP concentrations. Li et al. (2011) suggested that Hx accumulation in fish meat reflects the initial phase of autolytic deterioration and bacterial spoilage. A high Hx level was found in processed abalone (P1) and abalone analogue products S1, S2 and S3 (Table 5).

The ATP-related compounds of abalone analogue products S1, S2 and S3 all had similar profiles, possibly due to the inclusion of squid meat ingredients that were high in IMP as a flavor enhancer (Konosu and Yamaguchi, 1982; Fuke, 1994). Abalone analogue product S4 made from fish surimi contained less Hx than the others (Table 5). The ATP-related compounds were different between raw and processed abalone, and their profiles were also different between commercial processed abalone and abalone analogue products because different flavor additives were used, and these flavor enhancers may have been

further degraded during heat processing. The ratio of ATP-related compounds is, therefore, not suitable for use as a quality index of processed abalone and abalone analogue products.

IV. CONCLUSION

The proximate composition and glycogen content of abalone analogues were different from those of raw and processed abalone on account of the ingredients and flavor enhancers used. The proximate composition, pH and ammonia concentrations of raw hybrid abalone varied only slightly among the samples tested, but half of the taurine and glycogen was lost after processing. As a result, the taurine and arginine levels of commercial processed abalone as well as abalone analogue products were less than those of raw abalone. Free glutamic acid, glycine and proline were higher in commercial processed abalone and abalone analogue products than those of raw abalone. The extra free amino acids might have come from the ingredient sources and flavor enhancers. The results of this study should allow the distinction of authentic processed abalone from analogues prepared from different ingredients. Finally, although some adenosine 5'-triphosphate (ATP) related compounds were added to products S1, S2 and S4 as flavor additives, those compounds might have degraded during processing. Thus, their concentration is not suitable for use as an index of freshness for commercial processed abalone and abalone analogue products.

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