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Protein Quality Evaluation of Cooked Monkfish (*Lophiomus setigerus*) Meats

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To investigate the effect of cooking methods on protein quality of domestic fresh monkfish meat (FMM) and imported frozen monkfish meat (IMM), *in vitro* protein qualities were determined by amino acid anlysis, trypsin indigestible substrate (TIS) formation, and protein digestibility using the four-enzyme method. Crude protein contents of the boiled FMM and IMM were 90% of the dry base, which were higher than fresh FMM (82%) and IMM (84%). Profiles of total amino acid in FMM and IMM were not changed by cooking methods. Total free amino acid contents decreased to 29.0-33.6% for boiled (100℃, 10 min) and 24% for steamed (100℃, 10 min) samples. *In vitro* protein digestibilities of boiled and steamed FMM increased 86.6-86.8%, compared to raw IMM (82.9%), boiled and steamed IMM (85.1-85.5%) and raw IMM (83.6%). TIS of FMM (23.6 mg/g solid) and IMM (15.9 mg/g solid) showed no significant ($p<0.05$) difference in cooking methods. The C-PERs (computed protein efficiency ratio) of boiled FMM (2.63) and IMM (2.50) were significantly higher (<0.05) than raw (1.97) and steamed FMM(1.97) and IMM(1.94). These results demonstrate that boiling of FMM and IMM improves protein digestibility and C-PER when compared to steamed FMM and IMM. Therefore, boiling could be an excellent means to maintain high-protein quality of monkfish meat. Also, the cooking method may be applicable to the preparation of monkfish stew without any loss of free amino acids.

Key words: Monkfish, Cooking method, Protein quality, *Lophiomus setigerus*

Introduction

Monkfish lives in the muddy waters at the bottom of the semitropical or tropical seas. It's tail, which consists of lean and flavorful flesh, is popular for cooking. Therefore, the meat is comparable to lobster meat in western countries (D'Amico, 1996). The liver of monkfish is also used by people in Korea and Japan as a nutritious and caloric seafood dish, owing to its high level of fat and vitamin A. As well as cooked monkfish meat, its intestine, and slimy and limp skin is served as a popular seafood dish in Korea. With a high demand of monkfish meat in Korea, more than a half of her annual consumption were imported from China (NFPQIS, 2000). Although monkfish is known to be a palatable and unique tasteful seafood source, little is known about food qualities of fresh monkfish meat (FMM) comparing with the imported frozen monkfish meat (IMM) from China.

The present study compared the food qualities of the domestic fresh monkfish meat with those of the frozen imported products. Moreover, the changes in protein qualities during cooking was checked to provide fundamental data for preparing nutritious monkfish dishes.

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Materials and Methods

Preparation of sample

Chilled domestic whole monkfish (*Lophiomus setigerus*, 1,400-1,700 g of body weight, 40-50 cm in length) were purchased at a local fish market (Jagalchi, Busan) and transported to the laboratory kept in crushed ice. Same species of imported frozen monkfish from China, stored at -20±1℃ for 3 months, was obtained from the frozen fish company (Dongyoung-

Gold Plaza) in Busan. Chilled and frozen fish were thawed in running tap water for 1 hour. Meat blocks (6×4×3 cm) and skin pieces (6×3 cm) were boiled in tap water (sample vs water, 1:3 w/w) at 97±1℃ for 10 min. Steamed samples were cooked on a stainless steel screen in a steamer at 100℃ for 10 min using the same meat blocks and the skin pieces as boiled samples. All the cooked samples were freeze dried, and stored at -24℃ until experiments. Boiled water with meat blocks (6×4×3 cm) for various durations (5, 10, 15 min) was used for determining the released free amino acid contents.

Proximate composition analysis

Moisture, lipid, protein (N×6.25) and ash contents were determined by the standard procedures of the AOAC (1990). All analyses were done in triplicate.

***In vitro* protein quality assay**

Total amino acid profiles were determined with an amino acid analyzer (Biochrom 20, Pharmacia Biotech), using samples hydrolyzed with 6 N HCl *in vacuo* at 110℃ for 25 hr. Cysteine and cystine were determined using reduced glutathione as a standard according to the method of Felker and Waines (1978). Tryptophan was released using the alkaline hydrolysis method (5 N NaOH) of Hugli and Moor (1972).

Free amino acid contents were determined in 5'-sulfosalicylic acid (SSA) deproteinized samples of 80% ethanol extracts using an amino acid analyzer (Biochrom Ltd., England) loaded with lithium buffer (pH 2.2).

Browning development in samples was checked according to the procedure of Chung and Toyomizu (1976), and the results were expressed as the values of O.D.×100. Trypsin indigestible substrate content (TIS) was quantified using the method of Ryu and Lee (1985) which is modified procedure of Rhinehart (1975). The TIS results were expressed as purified soybean trypsin inhibitor equivalents.

In vitro protein digestibility was measured by a modified pH-drop method (Ryu et al., 1998) of AOAC (1990). The new equation for calculating *in vitro* digestibility is as follows:

$$Y=151.944015-8.78545\cdot X_1-1.138901\cdot X_2$$

where Y=% *in vitro* digestibility, X_1=terminal pH after 20 min of digestion by the pH-drop method, and X_2=free amino acid content expressed as D.L.-leucine equivalent by the OPDA method (Church et al., 1983).

C-PER (computed protein efficiency ratio), DC-PER (discriminant computed protein efficiency ratio) and predicted digestibility were calculated by the corrected AOAC procedure (1982). Protein digestibility via the new pH-drop method (Ryu et al., 1998) and amino acid profiles were used in the calculation of the *in vitro* protein quality indices.

Statistical analysis

Data from proximate compositions, available lysine content, browning and *in vitro* protein qualities were evaluated by analysis of variance (ANOVA) using SAS Version 6.12 (SAS, 1997).

Results and Discussion

Proximate compositions

The results of cooking methods on the macronutrient compositions of fresh and/or frozen monkfish meats, determined by proximate analysis, are presented in Table 1. Macronutrient composition values of fresh raw monkfish were not different from those seen in the frozen raw monkfish. Raw monkfish meat was slightly higher in lipid and lower in protein than reported by others (NFRDA, 1995; Chung and Toyomizu, 1998). These differences can probably be attributed to differences in size, species, seasonal variation, and habitat. Moisture content in raw monkfish meat and skin were over 86%, and those were some higher than those in other fish meats, generally. Steamed meats lost more of their moisture content than the meats cooked by other methods. The loss of moisture affected the other proximate components, especially the lipid content. The major differences in lipid loss in the steamed meats may be the result of lipid dripping during cooking. The crude protein content was significantly decreased by boiling, which probably accounts for the water soluble proteins found in the water.

Amino acids composition

Total amino acid profiles and free amino acid compositions of raw and cooked monkfish meats are given in Table 2 and 3. The results showed that glutamic acid was the major amino acid in all samples (14.09-15.82 g/16 g N). Aspartic acid, lysine and arginine were other major amino acids. The sum of those amino acid was more than 40% of total amino acid content. Profile of amino acid was similar to the other reports (NFRDA, 1995). Following cooking, steaming and boiling could not reduce the cooking

Table 1. Proximate composition (%) of the raw and cooked monkfish

Sample			Moisture	Crude Fat	Crude Protein (N×6.25)	Crude Ash
Meat	Fresh	Raw	87.30±1.1[a]	1.65±0.3[a]	10.52±0.6[c]	0.79±0.2[a]
		Boiled[1]	75.97±0.8[b]	0.22±0.1[c]	21.66±0.8[b]	1.06±0.2[a]
		Steamed[2]	75.83±1.4[b]	0.47±0.1[c]	21.27±1.0[b]	1.08±0.3[a]
	Frozen[3]	Raw	85.74±1.4[a]	1.47±0.2[a]	11.92±1.1[c]	0.79±0.3[a]
		Boiled	76.51±1.3[b]	0.80±0.2[b]	21.47±0.7[b]	1.07±0.2[a]
		Steamed	74.39±0.9[b]	0.16±0.2[c]	23.99±1.0[a]	1.17±0.2[a]
Skin	Fresh	Raw	86.35±1.2[a]	2.93±0.5[a]	12.88±0.8[d]	0.31±0.1[bc]
		Boiled	83.20±1.0[b]	0.23±0.2[b]	15.99±0.6[c]	0.32±0.1[bc]
		Steamed	81.40±1.2[bc]	0.61±0.1[b]	17.61±0.8[ab]	0.60±0.1[a]
	Frozen	Raw	86.35±0.9[a]	2.45±0.3[a]	12.42±1.2[d]	0.28±0.1[bc]
		Boiled	81.70±1.2[bc]	0.40±0.1[b]	16.50±0.9[bc]	0.20±0.1[c]
		Steamed	79.86±1.0[c]	0.28±0.3[b]	18.34±0.5[a]	0.44±0.2[ab]

[1]Boiled at 97±1℃ for 10 min (water/meat=3:1, v/w).
[2]Steamed at 100±1℃ for 10 min (water/meat=2:1, v/w).
[3]Frozen products were thawed in running water for 1 hour and then experimented.
[a-d]Means in the same row with different subscripts are significantly different at $p<0.05$.

Table 2. Total amino acid profiles (g/16 g N) of various monkfish meats[1]

Amino acid	Fresh			Frozen		
	Raw	Boiled	Steamed	Raw	Boiled	Steamed
Trp	0.97	1.01	0.92	0.91	1.08	1.07
Asp	9.58	10.02	8.96	9.90	10.46	9.98
Thr	4.34	4.48	4.42	4.37	4.75	4.47
Ser	4.34	4.43	4.24	4.20	4.61	4.53
Glu	15.34	15.29	14.09	15.21	15.82	15.54
Pro	3.47	4.08	3.09	3.95	2.36	3.66
Gly	5.91	5.71	4.57	4.65	5.13	5.09
Ala	5.20	5.30	5.27	4.67	5.26	5.07
Cys	0.89	1.31	1.59	0.90	0.91	0.88
Val	4.50	4.46	4.40	5.11	5.45	4.64
Met	3.08	2.74	3.35	3.08	2.81	3.16
Ile	4.69	3.58	4.98	5.70	4.65	4.92
Leu	6.51	8.09	8.34	9.38	7.74	7.63
Tyr	3.49	3.96	3.57	3.73	3.68	3.98
Phe	3.57	4.06	4.08	2.87	4.38	3.83
His	3.61	3.43	3.94	3.25	3.65	3.70
Lys	8.40	8.61	8.71	8.85	8.49	8.74
Amm	2.99	1.51	1.68	1.54	1.98	2.50
Arg	8.12	6.91	8.78	6.70	8.17	7.34
Total	98.99	99.00	98.98	98.97	101.39	100.73

[1]Same cooked meats were used as shown in Table 1.

loss of essential amino acids except lysine in frozen samples. The most abundant free amino acids was taurine, sweet taste amino acids (alanine and lysine) and glutamic acid in order (Table 3). Those free amino acids accounted for about 54% of total free amino acid and contributed in the unique taste of monk fish meat. Total free amino acid in both fresh and frozen samples was markedly decreased after boiling (Tables 3, 4). Only 71% and 66% of total free amino acid were remained after boiling in the fresh meat and the frozen meat, respectively. Drastic decrease (52-73%) in free amino acid by boiling was found in monkfish skin when determined by OPDA method (Church et al., 1983, Table 4). The decrease in total free amino acid content of the boiled samples may be explained by the release of water soluble amino acids from meat and skin during boiling. But in case of steamed samples, lower decrease in total free amino acid were occurred than in the boiled sample. The final free amino acid content in cooked meat and skin of frozen meat was lower than those in fresh samples owing to additional release of free amino acid during thawing in running tap water.

Effect of cooking conditions on *in vitro* protein digestibility

In vitro protein digestibility and TIS of monkfish meats by various cooking methods were compared (Table 5). In estimating protein quality, protein digestibility (Gauthier et al., 1982) and TIS (Ryu and Lee, 1985) must be evaluated because TIS content of processed protein source is closely related to its *in vitro* protein digestibility. As shown in Table 5, cooking resulted some higher protein digestibility (2-4%) of monkfish meat than that of the fresh meat, but there was no differences for cooked monkfish skin samples. Cooking condition had little effect on protein digestibility of frozen monkfish samples as

Table 3. Free amino acid composition (g/100 g solid) of various monkfish meats[1]

Amino acid	Fresh			Frozen		
	Raw	Boiled	Steamed	Raw	Boiled	Steamed
Phosposerine	0.06	0.03	0.00	0.00	0.00	0.00
Taurine	1.51	1.42	1.38	1.49	1.41	1.39
Aspartic acid	0.08	0.01	0.02	0.22	0.08	0.08
Threonine	0.44	0.24	0.27	0.43	0.21	0.28
Serine	0.25	0.19	0.25	0.35	0.18	0.27
Glutaminc acid	0.88	0.56	0.65	0.72	0.46	0.58
α-amino adipic acid	0.09	0.05	0.05	0.16	0.06	0.05
Proline	0.78	0.37	0.31	0.55	0.27	0.34
Glycine	0.64	0.70	0.74	0.63	0.60	0.69
Alanine	1.27	0.94	0.86	1.13	0.60	0.91
Valine	0.18	0.11	0.16	0.29	0.17	0.18
Methionine	0.12	0.08	0.11	0.20	0.12	0.12
Isoleucine	0.17	0.09	0.14	0.20	0.14	0.14
Leucine	0.29	0.15	0.26	0.31	0.23	0.24
Tyrosine	0.16	0.08	0.12	0.20	0.12	0.13
Phenylalanine	0.16	0.09	0.16	0.22	0.13	0.14
Ammonina	0.03	0.02	0.02	0.01	0.01	0.01
Ornithine	0.02	0.01	0.01	0.02	0.02	0.00
Lysine	0.93	0.51	0.50	0.62	0.47	0.44
Histidine	0.30	0.18	0.21	0.32	0.14	0.19
Arginine	0.14	0.18	0.24	0.34	0.16	0.20
Total	8.50	6.03	6.46	8.40	5.58	6.38

[1]Same cooked meats were used as shown in Table 1.

Table 4. Free amino acid contents (g/100 g solid) of raw and cooked monkfish meats

Sample			D.L-leucine[1]	D.L-lysine
Meat	Fresh	Raw	3.35±0.02^{a}	2.88±0.02^{a}
		Boiled	2.04±0.02^{d}	1.76±0.02^{d}
		Steamed	2.18±0.01^{c}	1.88±0.01^{c}
	Frozen	Raw	3.24±0.03^{b}	2.78±0.03^{b}
		Boiled	1.43±0.01^{f}	1.23±0.01^{f}
		Steamed	1.84±0.05^{e}	1.59±0.04^{e}
Skin	Fresh	Raw	2.59±0.03^{a}	2.23±0.04^{a}
		Boiled	1.23±0.04^{d}	1.06±0.05^{d}
		Steamed	2.05±0.01^{c}	1.76±0.01^{c}
	Frozen	Raw	2.36±0.01^{b}	2.04±0.02^{b}
		Boiled	0.65±0.02^{f}	0.56±0.02^{f}
		Steamed	1.16±0.04^{e}	1.00±0.04^{e}

[1]Determined by OPDA method as equivalent of D.L-leucine and D.L-lysine.
[a-f]Means in the same row with different subscripts are significantly different at $p<0.05$.

Table 5. *In vitro* protein digestibility and trypsin indigestible substarte (TIS) of raw and cooked monkfish meats

Sample			*In vitro* digestibility (%)	TIS (mg/g solid)[1]
Meat	Fresh	Raw	82.90±0.09^{f}	15.90±0.09^{f}
		Boiled	86.85±0.10^{a}	17.99±0.08^{f}
		Steamed	86.62±0.04^{b}	16.55±0.06^{e}
	Frozen	Raw	83.58±0.10^{e}	23.55±0.05^{c}
		Boiled	85.49±0.10^{c}	24.56±0.05^{a}
		Steamed	85.05±0.08^{d}	24.06±0.03^{b}
Skin	Fresh	Raw	85.61±0.06^{a}	13.89±0.05^{c}
		Boiled	85.38±0.06^{b}	15.16±0.04^{d}
		Steamed	84.82±0.08^{d}	14.31±0.03^{e}
	Frozen	Raw	83.13±0.08^{f}	16.15±0.05^{c}
		Boiled	85.16±0.06^{c}	19.50±0.04^{b}
		Steamed	84.14±0.04^{e}	21.72±0.06^{a}

[1]Determined as equivalent of soybean trypsin inhibitor.
[a-f]Means in the same row with different subscripts are significantly different at $p<0.05$.

compared to fresh samples. Raw and cooked monkfish, representative low fat fish, possessed lower TIS than the other fish samples (Lee et al., 1984a, b; Lee and Ryu, 1987). Matsushita (1975) has reported that oxidized fat resulted in TIS through the inactivation of enzyme activity and enzyme indigestible complex by lipid and protein interaction. Meat samples

Table 6. Browning pigments in raw and cooked monkfish meats

Sample		Browning pigments (O.D×100)	
		Lipophilic	Hydrophilic
Fresh	Raw	3.0±0.1[b]	0.6±0.2[d]
	Steamed	3.5±0.1[a]	1.0±0.1[c]
	Boiled	3.4±0.1[a]	0.9±0.1[c]
Frozen	Raw	3.3±0.2[a]	0.9±0.1[c]
	Steamed	1.7±0.2[d]	1.9±0.2[b]
	Boiled	2.2±0.2[c]	2.3±0.2[a]

[a-d]Means in the same row with different subscripts are significantly different at $p<0.05$.

had higher TIS content than skin samples, and fresh samples retained a significant lower level of TIS ($p<0.05$) compared with that in frozen samples. Although remarkable increase in TIS content could not observed during cooking, changes in protein digestibility seemed to be characterized by a notable hydrophilic browning products from amino-carbonyl reaction as shown in Table 6. Those browning induced lower digestibility can be explained by the fact that protein structure became more resistant to enzyme hydrolysis (Nesheim and Carpenter, 1967; Lee and Ryu, 1987).

In vitro protein qualities

To ascertain the overall protein qualities of various cooked monkfish meats, *in vitro* protein quality assays were performed and their results were presented in Table 7. Steamed and boiled fresh meat had about 4% higher *in vitro* protein digestibility than raw fresh meat while the digestibility of frozen samples was not markedly increased after cooking. In case of the predicted digestibility, cooking had a significant effect on digestibility of fresh and frozen monkfish meats ($p>0.05$). Predicted digestibility of cooked samples was about 9-10% higher than those of fresh and frozen meats. When fresh or frozen monkfish meat were boiled in water, C-PER values increased from 1.97 to 2.63 and 2.50, respectively. Steaming did not affect PER value, and those result was not similar to the other results of cooked fish meats (Moon et al., 1999; Hwang et al., 2002). It also revealed that boiled monkfish meat had a significant difference in DC-PER as compared with their raw fresh meat, but the difference was not noticeable in the cooked meats of frozen monkfish. Previous studies have demonstrated that C-PER is a reliable estimate of the quality of a food protein (Satterlee et al., 1979; Seet et al., 1983; Ryu and Lee, 1985), and excellent correlations were obtained between the values derived from C-PER with those from the bioassay techniques when raw fish meats were used as the protein sources. However, with the cooked monkfish meat there was a great discrepancy between C-PER and DC-PER, C-PER is better in evaluating protein quality of monkfish meat because of its low lipid levels.

Table 7. *In vitro* protein quality of raw and cooked monkfish meats[1]

Sample		*In vitro* digestibility (%)	Predicted digestibility (%)	C-PER	DC-PER
ANRC casein		90.30	87.20	2.50	2.50
Fresh	Raw meat	82.90[a]	83.41[a]	1.97[a]	2.07[a]
	Boiled meat	86.85[d]	93.52[e]	2.63[c]	2.70[d]
	Steamed meat	86.62[d]	93.25[e]	1.97[a]	2.50[b]
Frozen	Raw meat	83.58[a]	88.30[a]	1.97[a]	2.50[a]
	Boiled meat	85.49[b]	96.69[d]	2.50[b]	2.68[b]
	Steamed meat	85.05[b]	98.46[e]	1.94[a]	2.67[b]

[1]Same cooked meats were used as shown in Table 1.

[a-d]Means in the same row with different subscripts are significantly different at $p<0.05$.

Released total nitrogen and free amino acid in boiling water

When the fresh or frozen monkfish meat was subjected to preparing monkfish soup, called as *agutang* in Korean, various nitrogenous compound including free amino acid are released from heated meat into boiling water. Figs. 1 and 2 show the amount of the released crude protein and total free amino acid expressed as D.L-lysine from monkfish meat into boiling water during 20 minute boiling. As shown in Fig. 1, the released crude protein content of fresh monkfish meat increased up to 10 min boiling, with no significant change from 10 min to 15 min. Progressive releasing took place by continuous boiling after 15 min. On the other hand, the frozen monkfish meat did not release the crude protein throughout the boiling period. It should be noted that crude protein and free amino acid (Fig. 2) in the frozen meat might not be extracted into boiling water within 10 min. This can be explained by the fact that some of nitrogenous compounds in frozen samples already had been released during thawing by drip. Prolonged boiling resulted in concentration of boiling water through vaporization and led the higher level of crude protein in boiling water after 10 min. Tendency of

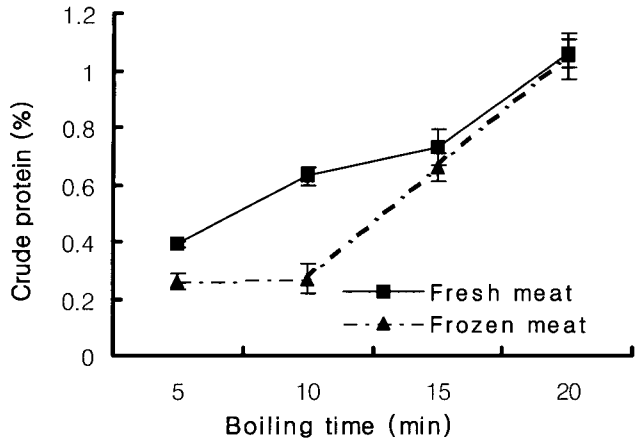

Fig. 1. Changes in the released crude protein from boiled monkfish meats. 100 mg of monkfish meats were boiled in 30 mL of distilled water and the released crude protein contents were expressed as crude protein g/100 g of boiled water.

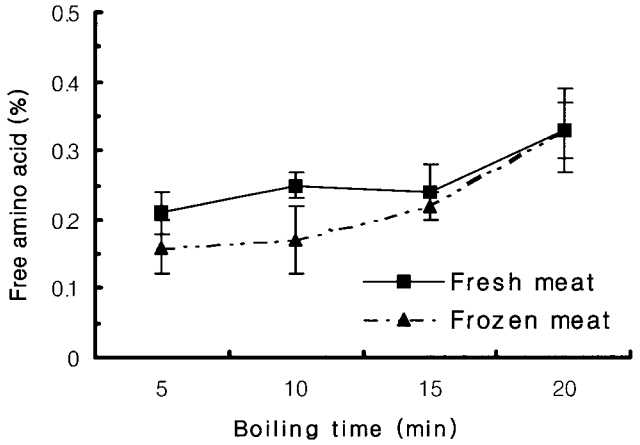

Fig. 2. Changes in the released free amino acid from boiled monkfish meats. Cooking condition was as same as indicated in Fig. 1, and free amino acid content were expressed as the D.L-lysine equivalents (g/100 g boiling water).

changes in free amino acid content during boiling was similar to crude protein as shown in Fig. 2.

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