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Protein Quality Evaluation of Cooked Hagfish (*Eptatretus burgeri*) Meats

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Abstract

The effect of cooking methods on *in vitro* and *in vivo* indices of the protein quality of hagfish meat were investigated. *In vitro* protein digestibilities of cooked meats (81.3~83.5%) were not significant different ($p<0.05$) from those of raw meat (82.9%), with the exception of steamed (110°C, 15 min) meat (86.3%). Convection oven cooking (220°C, 15 min) resulted in a higher trypsin indigestible substrate (TIS, 49.2 mg/g solid) compared with that of raw meat (38.9 mg/g solid). Free amino acid content of raw meat was decreased after boiling (100°C, 10 min). Both convection oven and microwave cooking (2,450 MHz, 3 min) decreased available lysine from 4.9 g /16 g N to 3.8~4.1 g/16 g N. *In vivo* apparent protein digestibilites (AD) of hagfish meat were similar for raw (92.4%) and cooked meats, but were somewhat lower than ANRC (Animal Nutrition Research Council) casein (94.3%). The PERs (3.7~4.1) and NPRs (3.7~4.9) of cooked meats were significantly higher ($p<0.05$) than those of raw meat (PER 3.3, NPR 3.6) and ANRC casein (PER 2.5, NPR 2.6), despite their lower *in vivo* protein digestibilities. These results demonstrate that cooking at optimal conditions resulted in remarkably positive effects on *in vitro* and *in vivo* protein qualities of hagfish meats. Therefore, steamed hagfish meat is an excellent source of high quality protein from seafood products.

Key words: hagfish meat, cooking method, *in vitro* and *in vivo* protein quality

INTRODUCTION

Hagfish (*Eptatretus burgeri*) is a readily available and widely consumed sea eel, commonly eaten as a proteinaceous side dish by Koreans. Hagfish meat is sometimes believed to be an aphrodisiac by laymen because of its snail-like look and copious amounts of jelly-like slime produced by glands in the skin. Hagfish is commonly prepared by broiling with soy sauce and hot pepper paste; several kinds of heating methods may be used, including the traditional method in the Pusan region of grilling over a straw fire. While some studies have investigated the food quality of other eels (silver conger eel, eel and conger eel) (1-4) and hagfish utilization (5,6), little information is available on the nutritional quality of hagfish protein.

The aim of this study was to investigate the effect of cooking methods on the protein quality of hagfish meats. Changes in the *in vitro* protein qualities were determined by amino acid analysis, available lysine retention, trypsin indigestible substrate (TIS) formation, and protein digestibility after processing. *In vivo* protein quality parameters (PER, NPR and AD) were obtained through experiments using growing rats.

MATERIALS AND METHODS

Preparation of sample

Live hagfish (*Eptatretus burgeri*) were purchased at a local fish market and transported to our laboratory in crushed ice. The whole fish, including guts, weighed 200 g on average. The fish were eviscerated, skinned, bones removed, and cut latitudinally into 6 cm pieces. Boiled samples were prepared in boiling tap water (1 : 3 w/w sample vs water) at 97±1°C for various durations (5,10,15 mins), and steamed samples steamed on a stainless steel screen in a steamer at 100°C for the same durations as boiled samples. Hagfish meat pieces were also cooked in a microwave oven (MH-713 SF) for 2, 3 and 4 minutes at 2,450 MHz, and grilled meats were prepared in convection oven at 220°C for 5, 10, and 15 minutes.

Proximate composition analysis

Moisture, lipid, protein (N×6.25) and ash were determined by the standard procedures of the AOAC (7). 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°C for 25 hours. Cysteine and cystine were determined using reduced glutathione as a standard according to the method of Felker and Waines (8). Tryptophan was released using the alkaline hydrolysis method (5 N NaOH) of Hugli and Moor (9).

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Free amino acid contents were determined in 5′-sulfosalicylic acid (SSA) deproteinized samples of 80% ethanol extracts using an amino acid analyzer (Biochrom 20, Pharmacia Biotech.) loaded with lithium buffer (pH 2.2).

Available lysine was measured by the method of Carpenter (10). Browning development in samples was checked according to the procedure of Chung and Toyomizu (11), and the results expressed as the values of O.D. × 100. Trypsin indigestible substrate content (TIS) was quantified using the method of Ryu and Lee (12) which is modified procedure of Rhinehart (13). The TIS results were expressed as purified soybean trypsin inhibitor equivalents.

In vitro protein digestibility was measured by a modified pH-drop method (14) of AOAC (15). The new equation for calculating *in vitro* digestibility is as follows:

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

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-leucine equivalent by the OPDA method (16).

C-PER (computed protein efficiency ratio), DC-PER (discriminant computed protein efficiency ratio) and predicted digestibility were calculated by the corrected AOAC procedure (15). Protein digestibility via the new pH-drop method (14) and amino acid profiles were used in the calculation of the *in vitro* protein quality indices.

Rat bioassays

Male weanling (21～22 day old) Sprague-Dawley rats were used in the *in vivo* apparent protein digestibility (AD), protein efficiency ratio (PER), and net protein ratio (NPR) assays. Rats were individually housed in stainless steel cages in a room maintained at 22～24°C, 50～60% relative humidity, with alternating 12 hour periods of light and dark. Rats were placed on an adaptation diet for 4 days, weighed, and then randomly assigned to experimental groups (10 rats per group). Each group was fed an experimental diet containing 10% protein for 28 days. Diets were formulated using the procedure for PER (17) as outlined by AOAC (18). To reduce lipid oxidation, diets were stored at -20°C in airtight packs pre-weighed for daily consumption. Food and water were supplied *ad libitum*. The data were collected during routine PER tests (18). Food consumption was measured throughout the study; and feces were collected for eight days (days 18～26). An ANRC control diet group, using casein as the protein source, was included in each AD study as described by Dunlap et al. (19). A NPR assay, which has the advantage of compensating for protein maintenance requirements in addition to growth requirements, was run according to the procedure of Bender and Dowell (20).

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, Cary, NC USA) (21). Dunnett's T-test was used to compare the control (ANRC casein) with each individual cooked hagfish meat sample (22).

RESULTS AND DISCUSSION

Effect of cooking conditions on *in vitro* protein digestibility

In vitro protein digestibility and TIS of hagfish meats cooked by various heating methods are compared (Table 1). When attempting to estimate protein quality, digestibility (23) and TIS (12) must be evaluated because the TIS content of processed proteins is closely related to its *in vitro* protein digestibility. As shown in Table 1, steaming for 15 min resulted in the highest protein digestibility (86.3%), but there were no differences between different times for boiled meats. Microwaving time (2～4 min) did not affect protein digestibility, but hagfish meats cooked in a convection oven showed some decreased protein digestibility for 5 min and 15 min. A similar digestibility pattern could be observed in TIS content, which increased with longer cooking times. Boiled samples had lower TIS concentrations than steamed samples, but there was no differences by cooking time in boiled and steamed samples. Micro-

Table 1. Effect of cooking conditions on *in vitro* protein digestibility and trypsin indigestible substrate (TIS) content in cooked hagfish meats

Sample[1)]	Time	*In vitro* digestibility (%)	TIS[2)] (mg/g solid)
Raw		82.87±0.41$^{cd3)}$	38.95±0.97^{c}
Steamed	5 min	82.30±0.66^{def}	36.38±0.72^{e}
	10 min	83.08±1.05^{bcd}	30.93±0.10^{fg}
	15 min	86.29±0.41^{a}	27.17±1.18^{i}
Boiled	5 min	84.11±0.91^{bc}	32.10±0.97^{f}
	10 min	84.58±1.06^{b}	31.59±0.55^{fg}
	15 min	83.05±0.63^{bcd}	29.55±0.64^{h}
Cooked in			
Microwave	2 min	82.26±0.69^{def}	37.44±0.49^{de}
oven	3 min	82.62±0.51^{cde}	30.36±0.83^{gh}
	4 min	81.98±1.63^{def}	38.49±0.91^{cd}
Convection	5 min	81.28±1.09^{ef}	41.12±1.10^{b}
oven	10 min	84.58±0.66^{b}	31.59±0.30^{fg}
	15 min	80.93±0.21^{f}	49.23±0.53^{a}

[1)]Boiled at 100°C (sample : water, 1 : 3 w/w), steamed at 100°C, cooked in microwave oven at 2450 MHz and cooked in convection oven at 220°C.
[2)]Determined as equivalent of soybean trypsin inhibitor.
[3)]Means in the same row with different superscripts are significantly different at $p<0.05$.

waved samples had similar TIS contents to raw meat, and cooking meats in a convection oven resulted in significantly higher levels of TIS ($p<0.05$) compared to raw meat. The remarkable increase in TIS content observed in samples cooked by a convection oven appear to be related to browning products from amino-carbonyl reactions, protein-protein interaction, and the formation of insoluble complexes that are products of lipid-protein reactions (24,25).

Since steaming for 15 min., boiling for 10 min, microwaving for 3 min and cooking for 10 min in a convection resulted in the best protein digestibility and TIS content, those times were selected as conditions for sample treatment for further study on protein quality.

Proximate compositions

The results of the cooking methods on the macronutrient compositions of raw and / or cooked hagfish meats, determined by proximate analysis, are presented in Table 2. Macronutrient composition values of raw hagfish were different from those typically seen in other eel fish. Raw hagfish meat was slightly higher in protein and lipid than reported by Kim (5). These differences can probably be attributed to differences in size, species, seasonal variation, and habitat. Protein and lipid content in raw hagfish meat were lower than those in eel (*Anguilla japonica*) and conger eel (*Astroconger myriaster*), as reported by Choi et al. (2) and Park et al. (3). 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 meats cooked in a convection oven 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 cooked hagfish meats are shown in Tables 3 and 4. Raw and cooked hagfish meats were high in glutamic acid, aspartic acid, arginine and the essential amino acids, leucine and lysine. The sum of those amino acids accounted for more than 45% of the total amino acid content. The amino acid profile was similar to a previous report (26), with the exception of lower values of valine in this study. Essential amino acid losses can be reduced by steaming and boiling rather than microwave and convection oven cooking. The most abundant free amino acid was proline; followed by glycine, valine, taurine, threonine, and alanine, in that order (Table 4). The total free amino acid content was significantly affected by the cooking methods. This effect was greatest for boiled meat, which had only 27% of the free amino acid content of raw meat. It should be noted that the final free amino acid content in microwaved meat was about 99% of that of the raw meat. However, about 70% of taurine was lost during boiling and half in steamed and microwaved meats. Taurine is a

Table 3. Total amino acid profiles of cooked[1)] hagfish meats (g/16 g N)

Amino acid	Raw	Steamed	Boiled	Cooked in	
				Microwave oven	Convection oven
Trp	0.98	0.88	0.95	1.02	1.01
Asp	8.69	8.78	8.83	8.27	7.86
Thr	4.14	3.40	3.35	4.34	4.04
Ser	3.66	3.78	3.77	3.66	3.51
Glu	14.33	14.09	14.23	13.28	13.44
Pro	6.14	5.62	5.03	5.45	5.89
Gly	7.07	6.86	7.07	6.79	6.88
Ala	5.02	5.59	5.54	5.09	5.03
Cys	1.24	1.19	1.16	1.20	1.02
Val	5.42	4.91	4.88	4.81	4.40
Met	2.65	2.69	2.63	2.66	2.65
Ile	4.54	4.63	4.64	4.04	4.25
Leu	7.52	7.60	7.58	7.39	7.08
Tyr	3.29	3.42	3.20	3.26	2.97
Phe	4.41	4.43	4.40	4.25	4.32
His	1.82	1.85	1.77	1.74	1.67
Lys	8.82	8.65	8.69	8.16	7.93
Amm	1.04	1.23	1.26	1.26	1.40
Arg	7.40	6.72	7.36	6.37	7.51
Total	98.18	96.32	96.34	93.04	92.86

[1)]Cooking conditions are as same as in the footnotes in Table 2.

Table 2. Proximate composition of cooked hagfish meats (g/100 g sample)

Sample[1)]	Moisture	Total lipid	Crude protein	Crude ash
Raw	71.73 ± 1.04^{a}[2)]	7.03 ± 0.05^{c} (24.99)[3)]	19.62 ± 0.17^{d} (69.40)	1.13 ± 0.04^{b} (4.00)
Steamed	62.55 ± 0.40^{d}	10.26 ± 0.12^{a} (27.39)	24.80 ± 0.19^{a} (66.22)	0.83 ± 0.03^{c} (3.02)
Boiled	67.48 ± 0.76^{c}	7.80 ± 0.10^{b} (23.98)	20.53 ± 0.82^{c} (63.13)	1.67 ± 0.12^{a} (5.13)
Cooked in				
Microwave oven	69.24 ± 0.69^{b}	7.91 ± 0.40^{b} (25.71)	21.76 ± 0.22^{b} (70.74)	1.08 ± 0.02^{b} (3.51)
Convection oven	71.20 ± 0.86^{a}	6.05 ± 0.10^{d} (21.00)	19.99 ± 0.52^{c} (69.41)	1.53 ± 0.23^{a} (5.31)

[1)]Boiled at 100°C for 10 min (sample : water, 1 : 3 w/w), steamed at 100°C for 15 min cooked in microwave oven at 2450 MHz for 3 min and cooked in convetion oven at 220°C for 10 min.
[2)]Means in the same row with different letters differ significantly ($p<0.05$).
[3)]Data in parenthesis mean g/100 g solid.

Table 4. Free amino acid composition of cooked[1)] hagfish meats (mg/100 g solid)

Amino acid	Raw	Steamed	Boiled	Cooked in	
				Convection oven	Microwave oven
Taurine	143.40	61.33	37.85	108.46	73.91
Aspartic acid	15.90	4.55	3.80	5.35	5.72
Hydroxyproline	2.33	1.77	1.52	1.88	1.62
Threonine	160.32	86.01	52.99	136.20	157.24
Serine	82.25	41.37	22.29	48.73	91.80
Glutamic acid	130.87	48.34	36.85	88.68	102.53
Sarcosine	6.73	1.69	2.08	3.43	10.37
Proline	804.25	910.55	210.26	778.06	994.06
Glycine	253.88	63.97	48.89	245.06	180.85
Alanine	198.34	83.68	52.69	147.50	83.81
Citrulline	2.31	1.02	1.36	1.23	2.65
α-aminobutyric acid	12.94	8.23	4.76	15.44	10.23
Valine	148.29	102.44	46.83	151.03	197.51
Cystine	42.35	30.73	14.05	45.12	57.22
Methionine	114.97	49.23	17.37	113.10	60.35
Cystathione	97.54	60.12	24.58	95.95	173.67
Ethanolamine	79.80	38.56	16.92	73.85	73.66
Anserine	159.30	44.96	43.22	157.49	176.26
Isoleucine	49.13	91.99	10.70	44.80	73.31
Leucine	25.88	29.42	12.08	24.07	28.22
Tyrosine	17.55	15.46	12.03	18.64	17.87
Phenylalanine	4.23	3.54	2.23	4.65	3.46
γ-aminobutyric acid	36.79	18.82	10.80	38.54	23.30
Ornithine	64.41	27.13	23.01	64.57	58.59
Lysine	53.00	30.60	20.78	44.90	47.50
Histidine	10.40	7.70	7.72	18.36	12.04
Arginine	8.32	3.55	2.32	7.87	4.66
Total	2,725.48	1,866.76	739.98	2,482.96	2,722.41

[1)]Cooking conditions are as same as in the footnotes in Table 2.

non-proteinous amino acid involved in the regulation of osmotic pressure and the metabolism of opines.

In vitro and *in vivo* protein qualities

To evaluate the protein quality of cooked hagfish meat, the content of available lysine was determined in cooked meats and the effect of the degree of browning on lysine availability was evaluated (Table 5). Boiled samples retained more available lysine than those prepared by other cooking methods ($p<0.05$). Cooking in a convection oven reduced available lysine by about 20%; which was expected since a severe lipophillic browning developed during convection oven cooking, and this protein-lipid interaction type of browning is known to reduce lysine availability (6,27). Similar effects of high temperatures on lysine availability have been reported in tuna meat cooked at 100°C for 3 hours, flame sterilized, and high temperature sterilized (115°C) (28). Hydrophilic browning was similar within cooked samples, though some slight differences were found in boiled samples.

Table 5. Effects of cooking methods on available lysine and brown color development in hagfish meats

Sample[1)]	Available lysine[2)] (g/16 g N)	Browning (O.D × 100)	
		Lipophilic	Hydrophilic
Raw	4.86±0.03[a3)]	12.2±0.02[b]	3.0±0.02[a]
Steamed	4.24±1.17[c]	2.7±0.05[e]	2.0±0.03[b]
Boiled	4.61±0.09[b]	2.9±0.08[d]	1.0±0.03[c]
Cooked in			
Microwave oven	4.14±0.03[c]	10.6±0.10[c]	3.0±0.10[a]
Convection oven	3.82±0.08[d]	17.0±0.10[a]	2.0±0.03[b]

[1)]Refer to the footnotes in Table 2.
[2)]Determined as FDNB reactive lysine.
[3)]Means in the same row with different letters differ significantly ($p<0.05$).

Table 6 shows the *in vitro* protein digestibility and PER values of cooked hagfish meats. Steamed meat had about 4% higher *in vitro* protein digestibility than raw and oven cooked meat, but C-PER values were not different. The predicted digestibility was the same for all cooked meats ($p<0.05$) with the exception of convection oven cooked samples. In each case, predicted digestibility was about 10% higher than actual values. However, those digestibility values may not be a reliable indicator of actual protein quality (12). Similar tendencies were also seen in DC-PER values of cooked hagfish meats.

To ascertain the overall *in vivo* protein qualities of various cooked hagfish meats, rat bioassays were performed and their results were presented in Table 7. All cooked samples had about 2% lower *in vivo* apparent digestibilities (AD) than that of the standard protein, ANRC casein (94.34%). Cooking hagfish meats had no effect on AD. The higher AD than *in vitro* digestibility, combined with

Table 6. *In vitro* protein quality of cooked hagfish meats

Sample[1)]	*In vitro* digestibility (%)	Predicted digestibility (%)	C-PER	DC-PER
ANRC casein	90.30±0.03[a2)]	87.20±0.10[c]	2.50±0.05[b]	2.50±0.06[c]
Raw	82.87±0.25[d]	94.60±0.76[b]	2.67±0.07[a]	2.61±0.04[b]
Steamed	86.29±0.13[b]	94.78±0.70[b]	2.67±0.07[a]	2.72±0.04[a]
Boiled	84.58±1.57[c]	95.04±0.15[b]	2.72±0.07[a]	2.71±0.06[a]
Cooked				
Microwave oven	82.62±0.64[d]	95.37±0.41[b]	1.97±0.01[c]	2.67±0.04[ab]
Convection oven	83.08±1.01[d]	97.74±0.76[a]	1.97±0.04[c]	2.67±0.04[ab]

[1)]Refer to the footnotes in Table 2. [2)]Means in the same row with different letters differ significantly ($p<0.05$).

Table 7. *In vivo* protein qualities of cooked hagfish meats

Group	AD (%)	NPR	Uncorrected PER	Corrected PER
ANRC Casein	94.34	2.63±0.43	2.20±0.34	2.50
Raw	92.36	3.65±0.71*	2.90±0.25*	3.29
Steamed	92.59	3.79±0.24*	3.64±0.21**	4.14
Boiled	92.09	4.12±0.32**	3.23±0.17**	3.67
Cooked in				
Microwave oven	92.41	4.89±0.23**	3.26±0.30*	3.70
Convection oven	92.33	3.83±0.42*	2.94±0.17*	3.34

Values are given as Means±SEM (n = 10).
*$p<0.05$, **$p<0.01$ compared with ANRC casein group.

the lack of difference in digestibility between samples, suggests that cooking does not cause severe protein damage. When hagfish meat was cooked in a microwave oven or boiled in water, NPR values were increased from 3.65 to 4.89 and 4.12, respectively. Steaming resulted in the highest PER value of 4.14, while microwaved and boiled samples had PER values of 3.70 and 3.67, respectively. Although the lowest PER, 3.34, was in meat cooked in a convection oven, PERs of all cooked samples were significantly higher than the casein reference. Previous studies have demonstrated that C-PER is a reliable estimate of the quality of a food protein (12,28,29). Excellent correlations were obtained between the values derived from C-PER with those of the bioassay techniques when raw fish meats were used as the protein sources. However, with cooked hagfish meat there was a great discrepancy between rat PER and C-PER or DC-PER, and similar discrepancies between *in vivo* digestibility and predicted digestibility calculated using only amino acid profiles. Even though it has some advantages in estimating protein quality, C-PER is not a good tool for evaluating protein quality of hagfish meat because of its high lipid levels.

CONCLUSION

In vitro digestibility was about 84% in hagfish meats cooked by several heating methods. The cooking method which resulted in the highest digestibility (86.29%) was steaming. The amount of essential amino acids, such as lysine and leucine, were high in all hagfish meats regardless of cooking method. The major free amino acids in all hagfish meats were proline, glycine, valine, taurine, threonine, alanine. Values of C-PER and DC-PER in hagfish meats cooked by steaming and boiling were higher than that of ANRC casein. PERs and NPRs cooked hagfish meats were superior to ANRC casein in rats. *In vivo* apparent digestibilities of hagfish meats prepared by all methods were greater than 90%.

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