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High Temperature-Cooking Effects on Protein Quality of Fish Extracts

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Abstract

Fish extracts were processed at high temperature (136.7~140°C) for possible use as functional food ingredients. Raw fish meats and those hydrothermal extracts were compared with respect to *in vitro* and *in vivo* protein qualities. 95% of fat in raw meats was reduced in extracts but there were not remarkable changes in other macronutrients in freeze-dried extracts. Most of essential amino acids were decreased significantly but two times more proline and glycine were detected in extracts. High temperature cooking resulted 2.1~3.7 times of higher total free amino acid content in fish extracts compared with raw meat, and taurine and glutamic acid were increased especially. Severe protein damages were occurred when *in vitro* protein quality indices such as available lysine, hydrophilic browning, trypsin inhibitor formation and *in vitro* protein digestibility were measured on fish extracts. *In vivo* protein qualities were also strongly influenced by high temperature ; however rat-body-weight gain was nearly zero during PER assay, and rat PER or NPR of fish extracts were significantly lower ($p<0.001$) than those of control (ANRC casein) and original raw fish meats.

Key words: fish extracts, high temperature cooking, *in vitro* and *in vivo* protein qualities

INTRODUCTION

In combined high-pressure/heat treatments applied to fish for sterilization and manufacturing specially designed fish products (fish extracts), one of the most important limitations is the lack of nutritive data on thermal properties. For protein foods in general, severe heat treatment may lessen nutritive value (1,2) due to denaturation, aggregation or gelation and thermal hydrolysis. Such changes can be evaluated in different ways. *In vitro* methods including amino acid analysis, digestibility and available lysine level, and rat bioassays (PER and NPR) are recommended and frequently used as quality indicators.

Various studies have been published on the changes in protein quality of fish meat (3-9) following heat treatments. None of those results allows protein quality of extracts to be determined, so that their nutritional value is limited.

Several published reports concern the optimization of heat treatment for fish extraction (10) and their functional properties (11), and an angiotensin I-converting enzyme inhibitory activity (12). Ryu et al. (13) suggested that new equation model for *in vitro* protein digestibility prediction using fish extracts and their original meat samples.

Fish extracts which have been favored by Korean as a health food are conventionally processed at boiling temperature for over 10 hours. Most of fresh water fish could be used as materials for extracts. Crucian carp, snakehead fish and eel are most frequently consumed in the form of extracts and they have been served to pregnant women, old adults, patients and undernourished persons. Recently, health food manufacturers designed an autoclave type extractor to increase yields and to shorten the processing time. In spite of the risk of damage to proteins, those products has been consumed widely without considering nutritional value.

Therefore, our study was designed to ascertain the real food quality of fish extracts processed at high temperature using *in vitro* and *in vivo* protein quality assays.

MATERIALS AND METHODS

Materials

Live loach (*Misgurnus anguillicaudatus*), crucian carp (*Carassius carasius*), bastard halibut (*Paralychthys olivaseus*) and jacopever (*Sebastes schlegeli*) were obtained in the local fish market. The live specimens were eviscerated and scaled, and then cooked as described by Lee et al. (10). In case of loach, skins were scrubbed in 5% salt water to remove foreign bodies prior to processing.

Preparation of fish extracts

Fish meat blocks (3 cm×3 cm×2 cm) and whole rubbed-off loaches were cooked in an autoclave type extractor (Dong Kwang, NTC-730). Cooking temperature (°C), time (hour) and amount of added water (sample *vs* water ratio, w/w) for fish extracts were based on the previous experiment (10) designed to optimize cooking conditions which could reach 60% of thermal hydrolysis level using response surface methodology. Adopted cooking conditions are as follows: loach (140°C, 10.08 hour, 1 : 1<sample *vs* water, w/w>), crucian carp (136°C, 7.25 hour, 1 : 1.1<sample *vs* water, w/w>) and bastard halibut (140°C, 9.85 hour, 1 : 1<sample *vs* water, w/w>) and jacopever (140°C, 9.38 hour, 1 : 1<sample *vs* water, w/w>). Filtered extracts through

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cheese cloth were freeze dried for further experiments.

Proximate composition analyses

Moisture, lipid, protein (N×6.25) and ash were determined by the standard procedure of AOAC (14). All analyses were done in triplicate.

In vitro protein-quality assay

Total amino acid composition of the sample was determined by the amino acid analyzer (Biochrom 20, Pharmacia Biotech). Samples were hydrolyzed with 6 N HCl *in vacuo* at 110°C for 25 hours. Cysteine and cystine were determined by the modified procedure of Felker and Waines (15) using reduced glutathione standard. Tryptophan was released using an alkaline hydrolysis (5 N NaOH) by Hugli and Moor method (16).

Extraction of free amino acid was done in 80% ethanol and then was deproteinized with sulfosalicylic acid. The free amino acid profiles of deproteinized samples were examined with lithium column on amino acid analyzer. Total free amino acid content was determined on 95% ethanol deproteinized samples of 75°C water extracts from freeze dried (70 mesh) samples using *o*-phthaldialdehyde spectrophotometric assay (OPDA method) (17).

Available lysine was measured by the method of Carpenter (18). Browning development in samples was checked according to the procedure of Chung and Toyomizu (19) and the results were expressed as the values of O.D×100. Trypsin-indigestible substrate content (TI) was quantified using the procedure of Ryu and Lee (20) which is modified from Rhinehart (21). Results of TI were expressed as purified soybean trypsin inhibitor equivalents.

The *in vitro* protein digestibility was measured by the modified pH-drop method (13) of AOAC (22). The new equation of calculating *in vitro* digestibility is $Y=151.944015-8.78545X_1-1.138901X_2$, where Y=% *in vitro* digestibility, X_1 =terminal pH at 20 min digestion of pH-drop method and X_2=free amino acid content expressed as D-leu. equivalent by OPDA method. C-PER (computed protein efficiency ratio), DC-PER (discriminant computed protein efficiency ratio) and predicted digestibility were calculated by the corrected procedure of AOAC (22). Protein digestibility via new pH-drop method (13) and amino acid profiles were used in the calculation of those *in vitro* protein-quality indices.

Rat bioassays

The 21~22 days old male weanling albino rats (Sprague-Dawley) were used in the *in vivo* apparent protein digestibility, PER and NPR assays. The rats placed into individual stainless steel cages were housed in a room maintained at 22~24°C, 50~60% RH with alternating 12 hour periods of light and dark. Rats were placed on an adaptation diet for 4 days, weighed at the end of the adaptation period, and then randomly distributed to experimental groups (10 rats per group). Each group was fed with an experimental diet containing 10% protein for 28 days. Diets were formulated using the procedure for PER (23) as outlined by AOAC (24). To reduce the quality deterioration of diets from lipid oxidation, diets were stored in -20°C refrigerator as the airtight individual small packs for daily consumption throughout experiments. Food and water were supplied *ad libitum*. The data were collected during routine protein-efficiency ratio tests (24). Food consumption was measured through the study, and feces were collected for eight days (day 18~26). A control diet of ANRC casein was included in each study of apparent digestibility assay of Dunlap et al. (25). A net protein ratio (NPR) assay, which has the advantage of considering protein maintenance requirements in addition to growth requirements, was run according to the procedure Bender and Dowell (26). To estimate the maintenance requirements, a group of rats was fed with a nonprotein diet for 10 days. The weight loss of this group was added to the weight gain of the test groups, thereby taking into account the maintenance as well as the growth requirement of the rat. The following equations were used to calculate the NPR and PER values.

% *in vivo* apparent protein digestibility

$$= \frac{\text{N. in diet (g)} - \text{N. in feces (g)}}{\text{N. in diet (g)}}$$

Dunlap et al. (25)

NPR

$$= \frac{\text{weight gain (g)} + \text{weight loss (g) of nonprotein group}}{\text{Total protein consumed (g)}}$$

Bender and Doell (26)

$$\text{PER} = \frac{\text{Gain in body weight (g)}}{\text{Protein intake (g)}}$$

Osborne et al. (23)

RESULTS AND DISCUSSION

Compositional analysis

The approximate compositional data for the extracts and their freeze-dried samples (Table 1) are compared with the original fish meats. Those values of fresh fish meats were generally consistent with the previous reports (27,28) but there were some differences in loach samples. Differences of our results from the others were likely due to the sources of samples such as eviscerated whole fish for ours and edible meats used in previous experiments. The moisture contents of fish extracts, processed at the conditions mentioned in "Preparation of fish extracts", were ranged from 94.82 to 97.21% and those freeze-dried products had from 3.30 to 5.22%. Total lipid contents in fish extracts (0.02~0.07%) and those freeze-dried samples (0.75~1.40%) were very low when compared to those in the original fresh meats which ranged from 1.60 to 4.22%. These lower contents would probably be removed lipids on extracts prior to filtering. Most

Table 1. Proximate composition of fish meats and those extracts (%)

Sample	Moisture	Total lipid	Protein (N×6.25)	Crude ash
Raw fish meats				
Bastard halibut	76.83	1.60	20.37	1.19
Jacopever	80.44	2.10	15.62	0.86
Crucian carp	77.43	2.51	17.70	0.89
Loach	74.78	4.22	15.95	3.43
Fish extracts				
Bastard halibut	97.01	0.03	2.69	0.19
Jacopever	96.83	0.04	2.91	0.23
Crucian carp	97.54	0.02	2.34	0.12
Loach	94.82	0.07	4.68	0.42
Feeze dried fish extracts				
Bastard halibut	3.77	1.10	86.18	6.18
Jacopever	3.61	1.30	87.66	5.47
Crucian carp	3.30	0.75	90.40	4.79
Loach	5.22	1.40	85.31	7.67

extracts comprised solubilized proteins in water. Protein and ash contents of loach extracts were different from those of other fish extracts. Loach extracts exhibited two times higher protein and ash contents than the other extracts. Much meats and soften bones of loach were passed through filtering cheese cloth and those might be resulted in more protein and ash content.

For the fish extracts, the processing decreased several amino acids significantly including the essential amino acids tyrosine, cystine, leucine, isoleucine, lysine, methionine and threonine (Table 2). Although slight differences were noted within fish samples, heating fish for 7.25~10.08 hour at 136~140°C caused a loss of tyrosine of averaged 54%. The other amino acids such as cystine, methionine, isoleucine and leucine decreased by approximately 30%. Some decrease in lysine ranging from 22 to 27% was noted in fish extracts. It has already been reported that the degradation of amino acids in fish meats by heating at 115°C or 124°C was negligible (8). Because their samples were processed for short period (Fo value 8 or 21), those conclusion could be possible. But the different results were reported a severe decrease in essential amino acids of fish samples heated at 116°C for 27 hour (29) and at 82°C for 3 hour (30). Shim et al. (31) also reported that most of essential amino acids decreased by 25~49% when fish meat (brown sole) were processed at 100°C for 10 minutes. However, the decrease in amino acids of fish extracts presumably could arise as a result of degradation due to high temperature cooking for long period. In contrast to the results of decreased amino acids, severe increases were observed for high water soluble amino acids, proline and glycine, as expected.

The free amino acid contents of fish extracts were compared with those original fish meats (Table 3 and 4). 35 kinds of free amino acids were checked, and taurine and glycine were major free amino acids in both sea fish and freshwater fish meats. In case of taurine which is known as a representative non-proteinous amino acid, 3 times more level of taurine was determined in all extracts when compared with their original fish meat. Those results was presumably through large amount of taurine present in fish tissues like brain and viscera included with meats in fish extracts preparation. Histidine was also observed as major free amino acid in crucian carp meat. But in case of fish extracts various free amino acids like threonine, aspartic acid, glutamic acid and ammonia could be included as major free amino acids from thermolysis during long time cooking at high temperature. The total content of free amino acids

Table 2. Total amino acid profiles of various fishes and those extracts (g/16 g N)

Amino acid	Bastard halibut	Jacopever	Crucian carp	Loach	HEH	JEH	CEH	LEH
Trp	1.44	1.54	0.95	0.90	1.45	0.94	0.88	0.96
Asp	9.79	10.78	11.03	9.46	8.63	8.43	9.07	8.31
Thr	4.55	4.93	5.21	4.71	4.12	3.75	3.99	4.03
Ser	4.09	4.38	4.76	4.44	4.40	4.39	4.20	4.38
Glu	13.86	13.94	15.60	15.12	15.19	14.63	15.22	14.93
Pro	2.37	3.81	3.12	3.60	6.36	8.02	6.98	7.22
Gly	4.12	4.00	4.64	4.68	10.68	10.78	11.13	9.54
Ala	5.58	5.87	6.18	5.96	7.94	7.58	7.74	7.16
Cys	0.69	0.68	0.92	0.88	0.59	0.55	0.46	0.59
Val	5.54	4.68	5.08	5.03	5.25	4.06	3.74	4.02
Met	1.92	2.76	2.92	4.64	1.96	1.80	1.98	2.35
Ile	4.30	4.51	4.79	5.27	3.24	3.31	3.15	3.26
Leu	8.01	8.53	8.90	9.10	6.87	6.62	6.76	6.61
Tyr	3.68	3.42	3.44	2.85	0.58	1.58	0.93	1.35
Phe	5.50	4.76	5.61	5.16	4.50	4.75	4.63	4.39
His	1.91	1.47	2.21	1.85	1.64	1.29	1.80	1.86
Lys	9.16	9.49	9.92	9.20	7.16	7.08	7.30	7.10
Amm	1.79	1.10	1.39	1.28	1.77	1.70	1.73	1.83
Arg	5.35	5.74	6.03	7.89	6.48	6.23	5.96	5.61
Total	93.65	96.39	102.7	102.02	98.81	97.49	97.65	95.50

CEH: crucian carp extracts processed at 136.7°C for 7.25 hours, HEH: halibut extracts processed at 140°C for 9.85 hours
LEH: loach extracts processed at 140°C for 10.08 hours, JEH: jacopever extracts processed at 140°C for 9.38 hours

Table 3. Free amino acid composition in various fish meats and those extracts (mg/100 g solid)

Amino acid	Bastard halibut	Jacopever	Crucian carp	Loach	HEH	JEH	CEH	LEH
Phosphoserine	3.00	2.32	2.24	2.62	42.98	56.08	30.00	26.86
Taurine	386.99	391.07	342.19	215.58	1,495.89	1,150.02	777.16	750.12
Urea	43.05	52.12	50.21	32.57	192.91	192.91	151.94	199.23
Phosphoethanolamine	3.85	9.21	4.64	13.62	16.32	14.02	12.02	12.02
Aspartic acid	21.06	35.22	30.54	14.32	82.78	96.16	86.86	131.04
Hydroxyproline	3.17	16.76	2.52	13.62	32.76	36.46	20.44	37.52
Threonine	20.83	25.52	51.42	45.86	172.54	146.96	163.77	277.64
Serine	21.05	46.78	35.38	28.34	101.22	127.78	126.47	140.24
Asparagine	28.94	34.70	54.87	43.48	198.23	145.16	103.34	86.70
Glutamic acid	14.86	41.14	49.15	31.11	197.09	178.88	140.43	142.40
Sarcosine	15.58	32.43	15.71	21.56	52.77	46.60	36.36	55.42
α-Aminoapidic acid	7.01	17.31	12.12	9.76	15.99	14.72	8.27	6.68
Proline	26.26	68.72	50.23	59.17	110.43	174.80	191.84	133.73
Glycine	183.62	332.32	459.39	393.69	197.23	325.64	494.48	398.98
Alanine	83.21	90.54	90.47	73.08	250.63	250.56	209.92	230.77
Citrulline	6.86	15.21	16.90	13.98	23.12	20.12	16.14	15.72
α-Aminobutyric acid	11.34	11.32	12.71	11.45	17.88	21.12	22.66	23.26
Valine	29.46	40.65	40.39	42.24	103.98	83.48	100.68	102.48
Cystine	22.53	39.96	33.70	29.81	70.11	56.92	55.32	57.40
Methionine	22.51	23.11	33.70	39.14	55.89	51.40	58.85	87.24
Isoleucine	16.93	24.85	30.99	39.15	135.99	75.99	89.30	66.10
Leucine	32.12	59.35	51.57	49.86	83.74	91.12	160.99	165.90
Tyrosine	17.32	29.70	33.98	26.98	97.63	75.34	89.10	68.08
β-Alanine	15.23	15.36	16.43	15.32	12.11	9.11	14.44	13.44
Phenylalanine	21.18	36.85	37.25	31.80	76.32	87.58	73.02	95.18
β-Aminoisobutyric acid	6.92	6.53	27.49	17.97	4.22	3.22	3.88	5.34
γ-Aminobutyric acid	2.92	5.34	24.84	16.21	39.82	27.70	27.04	27.96
Ammonia	14.90	64.58	124.13	25.68	145.02	142.00	114.08	187.77
Ornithine	17.56	10.51	11.07	15.31	55.06	28.12	39.90	30.96
Lysine	34.92	42.05	39.30	67.73	87.32	110.84	100.22	134.54
Histidine	21.29	36.30	234.82	46.89	140.60	134.56	600.13	233.14
3-Methylhistidine	5.65	13.56	14.44	14.49	20.26	29.84	16.58	19.64
Anserine	68.75	72.06	85.08	90.32	187.95	80.745	60.22	87.14
Carnosine	18.69	20.12	20.61	23.23	18.60	16.60	24.90	23.90
Arginine	19.21	37.74	28.00	20.49	85.92	157.14	80.99	75.82
Total	1,278.77	1,710.45	2,068.21	1,608.49	4,621.31	4,259.69	4,301.74	4,150.36

CEH: crucian carp extracts processed at 136.7℃ for 7.25 hours, HEH: halibut extracts processed at 140℃ for 9.85 hours
LEH: loach extracts processed at 140℃ for 10.08 hours, JEH: jacopever extracts processed at 140℃ for 9.38 hours

ranged from 1,608 to 2,068 mg/100 g solid for raw fish meat but those extracts had from 4,150 to 4,620 mg/100 g solid of total free amino acid content using amino acid analyzer. There appeared to be similar total free amino acid contents in same fish samples using OPDA method (Table 4). Even though some variations were found within fish samples due to meat composition and structure, about 2.7 times of total free amino acids were determined in fish extracts as compared with their fish meats. A severe increase in nonproteinous nitrogen compounds as taurine and ammonia, and nonessential amino acids hydroxyproline, asparagine, glutamic acid and arginine accounted for much of these increase in free amino acid contents. Unlike those nitrogenous compounds essential amino acids contents, which are susceptible to nonenzymatic browning, were not increased remarkably. Perhaps those amino acids could not contribute to increase total free amino acid contents in fish extracts.

Table 4. Free amino acid content of various fish meats and those extracts determined by OPDA[1] method (g/100 g solid)

Sample	DL-Leucine	DL-Lysine
Raw fish meats		
Bastard halibut	1.23±0.03	1.40±0.02
Jacopever	1.75±0.03	1.94±0.02
Crucian carp	2.24±0.05	2.73±0.04
Loach	1.65±0.01	1.78±0.01
Fish extracts		
Bastard halibut	4.83±0.01	4.15±0.01
Jacopever	4.78±0.01	4.10±0.01
Crucian carp	4.74±0.03	4.07±0.03
Loach	4.76±0.02	4.09±0.01

[1]Determined as equivalent of D.L.-leucine and D.L.-lysine

In vitro protein qualities

The data in Table 5 indicate the *in vitro* protein qualities of freeze-dried fish extracts. High temperature cooking for long period resulted in 55% more of available lysine loss in raw meats. Many researchers have reported that the fish meats sterilized for short period retained 85% more of total available lysine (6-8). The findings in available lysine loss of our study indicated that the cooking time and tem-

perature were not desirable for fish extracts in order to ensure protein qualities. Excessively high temperature during processing caused some destruction of "total" lysine content and available lysine loss. Those damage was progressed drastically when protein sources were exposed to moisture heat (50~250 g/kg) for long time (32). Similar pattern of available lysine loss due to browning could be confirmed by external color test and hydrophilic brown pigment development. Although it has been known (33) that the available lysine was retained 80% over even after sterilization of mackerel meat (at 110°C for 104 min and at 120°C for 46 min) and non-enzymatic browning reaction was still at the initial stage, it was assumed that non-enzymatic browning reaction taken place during fish extracts processing was already at final stage. Reaction could also occur within protein themselves between the free amino groups of lysine and arginine and the free acid groups of aspartic acid and glutamic acid, or amide groups of asparagine and glutamine (6).

Protein sources processed for long time often lose their nutritional value through formation of proteolytic enzyme indigestible substrate (20) and those results is closely related to *in vitro* protein digestibility. All fish extracts had a double trypsin indigestible substrate (TI) content, expressed as soybean trypsin inhibitor equivalents, compared to the raw fish samples (Table 5). TI increase seems to be generally characterized by browning products from amino-carbonyl reaction, or protein-protein interaction as described above. Similar findings have also been reported that there was a direct relationship between brown pigment development and TI formation (34). *In vitro* digestibilities of fish extracts were markedly affected by high temperature cooking for long time and their values were lowered by 9% compared to raw fish meats. Those lowered *in vitro* digestibility with larger amount of TI, browning development and poor available lysine retention would be expected to affect overall protein quality of fish extracts.

Table 5. *In vitro* protein-quality indices for fish meats and those extracts

Sample	Available lysine[1] (g/16 g N)	Browning (O.D×100)		TI[2] (mg/g solid)	*In vitro* dig. (%)
		Lipophilic	Hydrophilic		
Raw fish meats					
Bastard halibut	3.93	1.55	0.55	53.86	89.99
Jacopever	4.92	2.45	0.65	45.92	90.25
Crucian carp	4.57	3.45	0.75	40.14	89.86
Loach	3.75	4.75	0.35	46.12	89.64
Fish extracts					
Bastard halibut	1.76	1.58	10.51	87.54	81.17
Jacopever	2.22	2.48	17.95	85.10	81.40
Crucian carp	1.82	4.10	6.20	86.17	80.74
Loach	2.28	5.00	6.10	88.93	81.77

[1]Determined as FDNB reactive lysine
[2]Determined as equivalent of soybean trypsin inhibitor

Overall *in vivo* protein qualities

To ascertain the overall *in vivo* protein qualities of fish extracts, rat bioassays were performed and their results were presented in Table 6. In experiment of apparent *in vivo* protein digestibility, all fish meat showed better digestibilities than standard protein ANRC casein (89.99%). On the other hand, fish extracts had a significant lower (10.5~15.9%) digestibility of their original fish meats, indicating high temperature cooking seems to progress severe protein damage. Similar results have been presented that the apparent digestibility of heat-damaged cod flour (116°C for 27 hour) was only 68% by chicks bioassay (35). Surprisingly, our observation is that the recorded rat PERs of fish extracts were 0.5 below while those PERs of raw fish meats were 2.8 over except loach (2.1). Inspection of the results for PER assay indicates that severe heat treatment for fish extracts reduced weight gain to almost zero. It is known that feed consumption and weight gain during PER assay generally depend on the feed acceptability and digestibility. We could observe that feces of rats given fish extracts diets contained a considerable amount of brown semi-liquid material and some alopecic rats on same diets. Like as reduced feed intake, the feed consumption behavior of fish-extracts-diet-fed rats were quietly different from those of raw fish meats or casein-diet-fed rats. The explanation of those extremely lower PER and NPR from fish extracts may be reduced feed intake, poor essential amino acids profile with available lysine retention and remarkably lower

Table 6. *In vitro* and *in vivo* protein qualities of fish meats and those extracts

	C-PER	DC-PER	Rat PER	NPR	Predicted digestibility	*In vitro* digestibility	*In vivo* digestibility
ANRC casein	2.50	2.50	2.50	3.18	87.74	90.3	89.99
Raw fish meats							
Bastard halibut	2.74	2.81	2.91	4.00^{**}	95.61	89.99	92.59^{*}
Jacopever	2.59	2.50	3.40^{**}	5.25^{***}	91.07	90.25	91.12
Crucian carp	2.59	2.67	2.83	4.24^{**}	90.88	89.86	93.90^{**}
Loach	2.59	2.50	2.09	3.68^{*}	89.04	89.64	89.86
Fish extracts							
Bastard halibut	2.24	2.99	0.26^{***}	1.16^{***}	100.27	81.17^{***}	85.25^{**}
Jacopever	2.28	3.11	0.37^{***}	1.14^{***}	100.88	81.40^{***}	75.26^{***}
Crucian carp	2.27	3.08	0.23^{***}	1.02^{***}	100.27	80.74^{***}	75.26^{***}
Loach	2.23	2.90	0.50^{***}	1.09^{***}	101.45	81.77^{***}	79.33^{***}

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

digestibility due to browning development and TI formation resulted those protein qualities. It was also assumed that the deficiency of some essential amino acids as tyrosine and histidine and limited an unknown growth factors (36) associated with those lower nutritional index. Additional work will be needed to check and compare the effects of processing on rat's health. Whether the health problems would occur under the present processing condition was not investigated.

Previous studies (6,37,38) have proven that C-PER procedures can reliable estimate the quality of a food protein. Excellent correlation (r=0.8450) was obtained between the values derived by the C-PER procedure with that of the bioassay techniques when the raw fish meats were used as protein sources. However, in case of fish extracts, there was a great discrepancy between rat PER and C PER or DC PER. Similar tendency also noted in the *in vivo* digestibility as compared with predicted digestibility calculated using amino acid profile solely. Even if there would be some advantages in estimating protein quality, C-PER could not be a good tool for evaluating protein quality of severe damaged proteins.

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