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In vitro and *In vivo* Protein Qualities of Boiled Fish Extracts with Spicy Vegetables

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Abstract

To evaluate the quality of fish extracts with spicy vegetables (garlic, onion and ginger) in suppressing fishy oder, fish extracts of crucian carp, loach, bastard halibut and jacopever were processed at 100°C for 6 hours, and their *in vitro* and *in vivo* protein qualities were determined. Protein and total lipid contents were closely related to the degree of discarding floated lipid on fish extracts and the kinds of added spicy vegetables. Boiling (100°C) appeared to improve *in vitro* protein qualities slightly more than hydrocooking (110°C), but those with mild processing tended to result in better protein qualities than high temperature cooking (136~140°C). Spicy vegetables did not have remarkable effects on improving *in vitro* protein quality parameters. Fish extracts with 10% ginger were generally higher in *in vitro* protein quality than with the other vegetables. In spite of higher *in vivo* protein digestibility of fish extracts containing spicy vegetables processed under mild conditions (100°C), PERs of those extracts were not higher than those of extracts processed at high temperature.

Key words: boiled fish extract, *in vitro* protein quality, *in vivo* protein quality, spicy vegetable

INTRODUCTION

Conventionally processed fish extracts have been favored by Koreans as a healthy and pharmaceutical food. Most freshwater fish like crucian carp, snakehead and eel can be used as materials for the extracts. On the other hand, most large scale cultured seawater fishes such as bastard halibut and jacopever have been consumed as raw fish dishes or traditional spicy soup dishes. But their food quality and availability are decreased severely by a long transporting and distribution time. Sometimes natural disasters such as red tide, also results in drastic decease in their production. However, more effective utilizing methods should be used due to their limitation in use and production. To find a way for utilizing mass produced cultured fish, we designed fish extracts containing Korean favored spicy vegetables (garlic, onion and ginger) to block the fishy and rancid odor. Because other researchers reported severe protein damages in fish extracts processed at high temperature (1,2), fish extracts were processed under mild conditions in order to diminish protein damage, and their protein qualities were evaluated using *in vitro* and *in vivo* assays.

MATERIALS AND METHODS

Materials

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

Preparation of fish extracts

Cubes of fish flesh (3 cm×3 cm×2 cm) and whole rubbed-off loaches were prepared and placed in 5 L round bottom flasks with reflux cooler. Spicy vegetables were mixed with fish samples at the ratio of 1 : 5 (w/w). Sesame oil added samples were prepared in the same way as that of spicy vegetables contained samples. Boiled fish extracts were processed in water at 100°C for 6 hrs.

Proximate composition analyses

Moisture and protein (N×6.25) were determined by the standard procedure of AOAC (3). 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 (4) using reduced glutathione standard. Tryptophan was determined using an alkaline hydrolysis (5 N NaOH) by Hugli and Moor method (5).

Extraction of free amino acid was done in 80% ethanol and then deproteinized with sulfsalicylic 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) (6).

Available lysine was measured by the method of Carpenter (7). Browning development in samples was checked according

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to the procedure of Chung and Toyomizu (8) 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 (9) which was modified from Rhinehart (10). Results of TI were expressed as purified soybean trypsin inhibitor equivalents.

In vitro protein digestibility was measured by the modified pH-drop method (11) of AOAC. The new equation of calculating *in vitro* digestibility is $Y = 151.9440 - 8.7855X_1 + 1.1389X_2$, 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 (12). Protein digestibility via the new pH-drop method (11) 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 were placed into individual stainless steel cages and 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 an experimental diet containing 10% protein for 28 days. Diets were formulated using the procedure for PER (13) as outlined by AOAC (14). To reduce the quality deterioration of diets from lipid oxidation, diets were stored in -20°C freezer in airtight individual packets for daily consumption throughout the experiment. Food and water was supplied *ad libitum*. The data were collected during routine protein efficiency ratio tests (14). 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. (15). 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 of Bender and Dowell (16). To estimate the maintenance requirements, a group of rats was fed 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.

Table 1. *In vitro* protein quality indices for boiled fish extracts containing spicy vegetables

Sample	Available lysine	Browning (O.D×100)		*In vitro* digestibility (%)	TI (mg/g solid)
		Lipophilic	Hydrophilic		
Crucian carp					
Raw	9.14±0.04	3.45	0.75	89.86	40.14
CCA	9.96±0.09	1.02	2.22	86.67	38.01
COA	8.64±0.02	1.32	2.40	86.25	42.45
CGA	7.98±0.10	1.42	2.42	85.42	33.75
CKA	9.12±0.11	1.24	2.32	86.44	37.14
CSA	7.96±0.08	2.25	3.26	85.35	35.22
Bastard halibut					
Raw	7.86±0.02	1.55	0.55	89.99	53.86
BHA	7.84±0.13	1.35	2.8	85.60	42.51
BOA	6.48±0.15	1.8	2.5	86.21	40.83
BGA	7.68±0.07	1.55	4.2	84.62	38.29
BKA	7.12±0.03	1.66	3.42	85.31	41.61
BSA	7.96±0.08	2.31	3.66	85.22	44.27
Loach					
Raw	7.50±0.05	4.75	0.35	89.64	46.12
LHA	6.98±0.10	0.45	1.62	85.83	41.80
LOA	6.78±0.06	0.90	2.60	85.38	41.02
LGA	7.10±0.13	0.50	1.50	85.20	42.69
LKA	7.46±0.05	1.00	3.20	85.53	39.06
LSA	7.46±0.08	0.95	3.40	85.69	42.10
Jacopever					
Raw	9.84±0.03	2.45	0.65	90.25	45.92
JHA	8.16±0.05	0.45	4.60	86.16	39.27
JOA	5.50±0.13	0.50	6.65	85.76	43.43
JGA	6.70±0.22	0.60	5.20	85.58	41.00
JKA	7.28±0.11	0.70	2.40	87.26	39.37
JSA	7.90±0.14	1.20	10.65	86.88	40.08

Cooked at 100°C for 6 hrs and then filtered
CCA: Crucian carp extracts
COA: (Crucian carp+20% Onion) extracts
CGA: (Crucian carp+20% Garlic) extracts
CKA: (Crucian carp+20% Ginger) extracts
CSA: (Crucian carp+20% Sesame oil) extracts
BHA: Bastard halibut extracts
BOA: (Bastard halibut+20% Onion) extracts
BGA: (Bastard halibut+20% Garlic) extracts
BKA: (Bastard halibut+20% Ginger) extracts
BSA: (Bastard halibut+20% Sesame oil) extracts
LHA: Loach extracts
JHA: Jacopever extracts
LOA: (Loach+20% Onion) extracts
JOA: (Jacopever+20% Onion) extracts
LGA: (Loach+20% Garlic) extracts
JGA: (Jacopever+20% Garlic) extracts
LKA: (Loach+20% Ginger) extracts
JKA: (Jacopever+20% Ginger) extracts
LSA: (Loach+20% Sesame oil) extracts
JSA: (Jacopever+20% Sesame oil) extracts

RESULTS AND DISCUSSION

In vitro protein qualities

Various *in vitro* protein quality indices for boiled fish extracts are presented in Table 1. The amount of available lysine of crucian carp was almost the same as fish meat extracts processed at 110°C, and that of bastard halibut was higher than those previously reported (2). Loach and jacopever had more than 60% of available lysine compared with that in same fishes cooked at high temperature (1). These results indicated that temperature dependence on lysine availability is apparent. Development of liphophilic brown pigment in every fish extracts containing spicy vegetables were much lower than those of raw fish except for the sesame oil added extracts, but severe

hydrophilic browning occurred in all fish extracts. Sesame oil added fish extracts both showed remarkable hydrophilic and lipophilic browning. Even though the hydrophilic browning in boiled fish extracts were higher than those of raw meats, these values were no more than 25~50% of the browning at high temperature (110~140°C) (1). Improvement of browning discoloration in boiled fish extracts was not made any better by adding spicy vegetables. Although a measurable difference was not found within extracts containing spicy vegetables, ginger had some inhibitory effect against hydrophilic browning.

In vitro digestibility of boiled fish extracts was not severely altered. This small decrease in *in vitro* digestibility was higher by 7~8% than extracts cooked at high temperature (130~140°C) (1) and by 1~2% than those of hydrocooked (110°C) except in bastard halibut as in previous reports (2). On the other hand, boiled bastard halibut extracts resulted in almost the same *in vitro* digestibility with those processed at higher temperature. This result may be associated with a higher sensitivity of weak muscle structure to heat denaturation than the other fish specimens used. Changes in the content of trypsin indigestible substrates (TI) showed a similar trend in *in vitro* digestibility. About 50% of TI in high temperature cooked (130~140°C) samples were formed in boiled extracts, and those levels was slightly lower than extracts processed at 110°C (1,2).

Changes in free amino acid composition

Total free amino acid content and amino acid profiles of boiled fish extracts were compared to those of original raw fish meats (Table 2 and 3). Total amount of free amino acids was measured two more times in boiled fish extracts by OPDA method than those in raw meat, but bastard halibut showed poor free amino acid content compared with other fishes. Generally, content of released free amino acid from fish meat by heat treatment is dependent on the tenderness of fish meat related to sensitivity against protein denaturation and heating condition (time, pressure and temperature) (1,17). So, boiled fish extracts contained about 1,000 mg/100 g solid less of total free amino acid than high temperature treated extracts. Spicy vegetables do not have a negligible effect on the content of nitrogenous compounds. Therefore, they would not affect total free amino acid content in fish extracts. When free amino acid content of fish extracts with spicy vegetables were compared each other, they showed similar total free amino acid content. Likewise, in total free amino acids determined through amino acid analyzer there was a similar trend of variation in content according to type of fish and spicy vegetables (Table 3). The major boiled fish extracts free amino acids were taurine, histidine, glycine and glutamic acid except for bastard halibut. There appeared to be a unique result in bastard halibut showing extremely low contents of glycine, glutamic acid and histidine which were present in large amounts in the other extracts. 3~4 times more taurine in extract (2 times in crucian carp) than their original meats were presumably due to the taurine from the head and other parts of fish (1).

Table 2. Free amino acid content of boiled fish extracts[1)] containing spicy vegetables determined by OPDA method (% dry base)

Sample	DL-Leucine	DL-Lysine
Crucian carp		
Raw	2.24±0.05	2.73±0.04
CCA	4.63±0.07	3.98±0.06
COA	4.61±0.04	3.96±0.03
CGA	4.57±0.08	3.92±0.07
CKA	4.60±0.11	3.95±0.10
CSA	4.54±0.09	3.90±0.08
Bastard halibut		
Raw	2.02±0.09	1.74±0.08
BHA	4.10±0.09	3.51±0.07
BOA	3.87±0.07	3.33±0.06
BGA	3.96±0.13	3.40±0.11
BKA	4.43±0.02	3.80±0.02
BSA	3.67±0.12	3.15±0.10
Loach		
Raw	1.65±0.01	1.78±0.01
LHA	4.44±0.00	3.81±0.00
LOA	4.45±0.06	3.82±0.05
LGA	4.07±0.03	3.50±0.02
LKA	4.47±0.14	3.84±0.12
LSA	4.33±0.00	3.71±0.00
Jacopever		
Raw	1.75±0.03	1.94±0.02
JHA	3.84±0.07	3.30±0.06
JOA	4.04±0.03	3.47±0.03
JGA	3.58±0.09	3.08±0.08
JKA	3.88±0.11	3.34±0.10
JSA	3.52±0.06	3.02±0.05

[1)]Same samples as presented in Table 1

Changes in total amino acid profiles

To ascertain the protein quality of fish extracts, total amino acid profile was presented in Table 4. It has generally been reported that changes in total amino acid profiles of fish meats sterilized at 115~124°C (F_0 8~21) were not detectable (18), but significant changes in essential amino acid profiles could result by heating at 136°C for 6 hrs (1).

It could be confirmed that there was a slight decreasing tendency in most of the essential amino acids, particularly tryptophan and cysteine which decreased by approximately 60% in crucian carp extracts. Representative heat labile amino acid lysine is used as an index of protein quality, which was maintained to more than 80~90% in all fish extracts (17,19). On the other hand, boiled fish extracts (crucian carp and bastard halibut) had only 35~50% of tyrosine of the original fish meat. These results were comparable to the results of hydrocooked (2) fish extracts showing 70~80% tyrosine loss. For the fish extracts with 20% ginger, the ginger protected against the decrease of total amino acids with some exceptions. From the base of results above, change of protein quality was prevented to some degree by adding spicy vegetables and moderate heating conditions because total amino acid would be expected to affect overall protein quality. The tendency of the decrease in most essential amino acid was not evitable as in the previous report (1). It was well known that representative water soluble

Table 3. Free amino acid composition of boiled fish extracts[1)] containing spicy vegetables (mg/100 g solid)

Amino acid	Raw CC	CCA	CKA	Raw BH	BHA	BKA	Raw LO	LHA	LKA	Raw JA	JHA	JKA
Phophoserine	2.24	0	0	3.00	0	0	2.62	0	0	2.32	58.40	56.20
Taurine	342.19	514.64	598.63	386.99	1,240.47	1,248.87	215.58	818.74	798.89	391.07	1,155.59	1,122.77
Urea	50.21	97.99	80.86	43.05	141.75	96.46	32.57	96.14	102.10	52.12	39.22	51.92
Phosphoethanol-amine	4.64	0	0	3.85	0	0	13.62	0	0	9.21	15.36	12.21
Aspartic acid	30.54	28.33	23.65	21.06	28.50	28.86	14.32	23.11	72.62	35.22	24.32	32.19
Hydroxyproline	2.52	11.11	3.45	3.17	41.51	35.81	13.62	40.46	50.21	16.76	38.42	24.21
Threonine	51.42	98.77	101.17	20.83	114.11	100.75	45.86	263.88	403.70	25.52	125.35	144.80
Serine	35.38	66.47	71.24	21.05	88.95	66.65	28.34	142.70	226.97	46.78	126.59	134.10
Asparagine	54.87	57.91	191.30	28.94	140.24	145.90	43.48	123.29	164.37	34.70	156.34	231.36
Glutamic acid	49.15	142.37	132.56	14.86	72.11	48.90	31.11	216.46	212.82	41.14	157.60	165.40
Sarcosine	15.71	33.64	31.49	15.58	22.06	35.18	21.56	14.00	11.97	32.43	56.60	49.72
α-Aminoapidic acid	12.12	50.17	32.32	7.01	13.09	14.68	9.76	5.66	15.66	17.31	10.72	9.00
Proline	50.23	66.33	62.80	26.26	74.76	63.22	59.17	118.23	171.76	68.72	152.80	177.56
Glycine	459.39	494.48	580.82	183.62	79.42	76.13	393.69	177.00	255.18	332.32	270.10	145.79
Alanine	90.47	168.08	199.32	83.21	220.52	198.12	73.08	310.31	336.93	90.54	153.56	158.83
Citrulline	16.90	0	0	6.86	0	0	13.98	0	0	15.21	20.12	18.42
α-Aminobutyric acid	12.71	5.18	5.11	11.34	6.07	6.22	11.45	8.52	11.72	11.32	26.90	16.18
Valine	40.39	100.68	100.24	29.46	80.73	68.78	42.24	80.87	182.08	40.65	49.87	45.52
Cystine	33.70	12.42	11.21	22.53	70.11	53.92	29.81	58.21	8.94	39.96	46.80	34.27
Methionine	33.70	53.33	53.04	22.51	45.01	67.00	39.14	43.79	87.89	23.11	54.68	46.48
Isoleucine	30.99	89.31	86.50	16.93	135.82	39.05	39.15	32.00	115.24	24.85	80.29	51.25
Leucine	51.57	159.12	151.04	32.12	73.74	116.09	49.86	52.33	111.45	59.35	96.87	95.50
Tyrosine	33.98	50.58	57.30	17.32	83.83	67.28	26.98	39.95	26.22	29.70	72.56	63.66
β-Alanine	16.43			15.23			15.32			15.36	9.11	4.28
Phenylalanine	37.25	84.25	85.35	21.18	10.57	67.76	31.80	48.82	62.82	36.85	69.00	47.70
β-Aminoisobutyric acid	27.49	0	0	6.92	0	0	17.97	0	0	6.53	0	0
γ-Aminobutyric acid	24.84	7.04	15.65	2.92	39.82	9.60	16.21	15.98	39.64	5.34	29.73	39.63
Ammonia	24.13	17.71	18.11	14.90	34.49	35.59	25.68	67.67	64.55	64.58	139.14	91.44
Ornithine	11.07	10.45	15.46	17.56	113.35	30.10	15.31	24.20	124.34	1051	13.71	18.82
Lysine	39.30	66.93	64.66	34.92	3.61	27.95	67.73	216.03	204.85	42.05	111.84	103.64
Histidine	234.82	642.13	623.70	21.29	20.79	13.81	46.89	233.14	253.02	36.30	210.28	150.71
3-Methylhistidine	14.44	0	0	5.65	20.26	18.73	14.49	12.59	15.74	13.56	10.22	14.69
Anserine	85.08	41.96	53.05	68.75	187.95	157.74	90.32	46.17	54.61	72.06	63.67	48.65
Carnosine	20.61	0	0	18.69	18.60	38.64	23.23	0	0	20.12	8.30	42.48
Arginine	28.00	25.88	29.16	19.21	85.57	69.32	20.49	54.73	48.60	37.74	187.14	40.32
Total	2,068.21	3,197.26	3,479.24	1,278.77	3,415.99	3,076.24	1,608.49	3,384.98	4,199.26	1,710.45	3,841.20	3,489.80

[1)]Same samples as presented in Table 1

Table 4. Total amino acid profiles of boiled fish extracts[1)] containing spicy vegetables (g/16 g N)

Amino acid	Raw CC	CCA	CKA	Raw BH	BHA	BKA	Raw LO	LHA	LKA	Raw JA	JHA	JKA
Trp	0.95	0.35	0.30	1.44	0.99	0.89	0.90	0.96	0.96	1.54	1.25	1.10
Asp	11.03	8.76	9.56	9.82	7.94	8.12	9.46	7.20	7.04	10.78	7.90	8.02
Thr	5.21	2.88	2.96	4.56	2.88	2.93	4.71	2.92	2.75	4.93	2.69	2.58
Ser	4.76	2.62	2.55	4.10	2.80	3.10	4.44	3.17	2.73	4.38	3.38	3.28
Glu	15.60	13.59	13.89	13.90	13.97	12.71	15.12	12.48	11.83	13.94	12.86	11.63
Pro	3.12	7.31	6.80	2.38	11.99	13.03	3.60	6.60	7.98	3.81	6.52	7.84
Gly	4.64	14.97	13.54	4.13	12.23	12.91	4.68	14.43	14.26	4.00	17.11	18.37
Ala	6.18	8.74	8.72	5.60	8.04	8.09	5.96	8.29	7.96	5.87	8.75	9.21
Cys	0.92	0.23	0.22	0.69	1.14	1.07	0.88	0.61	0.72	0.68	0.70	0.58
Val	5.08	6.11	6.92	4.55	5.66	5.62	5.03	5.32	6.33	4.68	5.70	6.14
Met	2.92	1.98	1.96	1.93	0.74	0.81	4.64	2.76	3.07	2.76	0.69	0.65
Ile	4.79	2.94	3.01	4.31	2.70	2.16	5.27	2.61	2.35	4.51	2.07	2.00
Leu	8.90	6.04	6.28	8.03	5.79	4.74	9.10	5.90	4.61	8.53	3.48	4.24
Tyr	3.44	1.20	1.24	3.69	0.83	0.83	2.85	2.12	3.18	3.42	2.25	3.41
Phe	5.61	4.53	4.80	5.52	4.24	5.11	5.16	3.69	4.64	4.76	4.08	3.27
His	2.21	1.77	1.73	1.92	1.56	1.22	1.85	2.19	1.95	1.47	1.11	1.19
Lys	9.92	8.79	9.16	9.19	7.22	6.74	9.20	6.64	6.41	9.49	6.35	6.19
Amm	1.39	0.59	1.42	1.80	2.88	1.12	1.28	2.12	4.77	1.10	2.69	2.10
Arg	6.03	5.54	5.47	5.37	6.07	5.11	7.89	5.42	5.37	5.74	5.98	6.18
Total	102.7	98.94	100.76	93.11	99.67	96.31	102.02	95.43	98.91	96.39	95.56	97.98

[1)]Same samples as presented in Table 1

Table 5. *In vitro* and *in vivo* protein qualities of various boiled fish extracts[1)] containing 20% ginger

Sample	C-PER	DC-PER	Rat-PER	Predicted digestibility (%)	*In vitro* digestibility (%)	*In vivo* digestibility (%)
ANRC casein	2.5	2.5	2.28	87.74	90.3	86.86
CCA	2.66	2.49	0.30	105.22	86.67	84.91
CKA	2.71	2.59	0.26	102.78	86.44	86.90
BHA	2.34	2.29	0.18	89.74	85.60	86.13
BKA	2.53	2.34	0.25	106.43	85.31	87.69
LHA	2.20	2.07	-	103.77	85.83	90.50
LKA	2.35	2.51	0.27	71.81	85.53	83.04
JHA	2.67	2.62	-	96.30	86.16	85.41
JKA	2.73	2.65	0.10	99.20	87.26	85.26

[1)]Same extracts as presented in Table 1

amino acid in fish meat such as proline, glycine and alanine were increased by hydrocooking. Extremely high glycine (280~320%) and proline (crucian carp 200~235%, bastard halibut 280~310%) content were also achieved as in the previous report (1).

Overall *in vivo* protein qualities

Table 5 showed the results of *in vivo* protein qualities of fish extracts using the albino rat. Rat-PERs of boiled fish extracts ranged from 0.25 to 0.30 (0.10 for jacopever+ginger) and these were not superior to the other kinds of fish extracts (1,2). But in cases of apparent *in vivo* protein digestibility, fish extracts showed a similar or higher digestibility of ANRC casein. 8~10% more than *in vivo* digestibility compared to identical fish samples processed at severe condition (1,2) means that boiling would improve protein quality of fish extracts in respect of digestibility. *In vitro* digestibility was consistent with tendency of *in vivo* digestibility, but showed slightly higher digestibility than *in vivo* digestibility. From our observation, rat weight gain was not observed during experimental periods and these results were similar to previous reports (1). But we could not observe that feces of rats fed with fish extracts diets contained considerable amounts of brown semiliquid material and some alopecic rats. These results could be attributed to improvement in acceptability of diet by adding spicy vegetable and reduction increase of the digestibility and essential amino acid. In spite of some improvement in protein quality by higher *in vivo* digestibility of boiled fish extracts, low PERs of those extracts indicated that most free amino acids may be used in maintenance of body tissue and physical movement as reported for poor PERs of high level free amino acid foods (2,19).

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