Bull. Korean Fish. Soc. 19(5), 493~501, 1986 韓水誌 19(5), 493~501, 1986

Effect of Lipid on the Protein Denaturation in Cooking Fish Meat

Kang-Ho LEE, Byeong-Dae CHOI
Department of Food Science and Technology, National Fisheries University of Pusan
Nam-gu, Pusan 608, Korea
and
Hong-Soo RYU
Department of Nutrition and Food Science, National Fisheries University of Pusan
Nam-gu, Pusan 608, Korea
(Received June 15, 1986)

The present work aims to estimate the effect of heat treatment on the *in vitro* protein digestibility and formation of trypsin inhibitor or trypsin indigestible substrate(TIS) of raw and defatted flounder. It was also carried out to assess the formation of lipid-protein complexes under the conditions of different ratio of lipid addition.

The *in vitro* protein digestibility increased when steamed for 5 min showing 88.09% in raw and 90.56% in defatted samples, respectively. After 40 min steaming, the digestibility decreased by 2~4%. As for microwaving, heating for 1 min resulted in slight increase of digestibility, however, heating for 7 min did decrease of digestibility by 3~4% for both raw and defatted materials.

There was no difference in fatty acid composition found with heat treatment. The major fatty acids of flounder meat were $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, $C_{20:5}$, $C_{22:6}$ and the ratio of the unsaturated to saturated was 67.3:32.6.

Fat oxidation and nonenzymatic browning were enhanced by heat treatment and protein solubility decreased necessarily as the brown pigment formation increased.

On the other hand, the effects on the digestibility and TIS of the complexes formed from interaction of lipid and myofibrillar or meat protein of flounder were examined. The interaction of protein with lipid was considered to mostly contribute to the drop of digestibility of fish products. The digestibility of myofibrillar protein was 93.72% for flounder, and it generally decreased as the amount of lipid added to protein and reaction time increased. Also mixed and heated samples were more active in digestibility decline than those mixed after heating. The result probably indicated that lipid-protein interaction was involved in the drop of digestibility which coincided with protein denaturation.

Introduction

Lipid-protein interaction may lead to undesirable changes in nutritional and functional properties of food. It is so important in biological processes and in food systems, sometimes determining the odor, texture, color, *in vitro* enzyme digestion of food (Almquist, 1956; Karel, 1977). These interactions, which occur when lipids and proteins are present in the same system, are responsible for the formation of trapped complex(Karel, 1977; Greene, 1971; Shenouda and Piggott, 1975; Roubal, 1969).

Most fish fats contain more than 50% of unsaturated fatty acids, of which about half are polyunsaturated, lipid oxidation is always considered to be one of the major factors contributing to difficulties in

the preservation. The oxidation of lipids contaning unsaturated fatty acids causes chemical changes in proteins via nonenzymatic browning and formation of thermal cross-linkage during processing, storage and cooking.

The effects of lipid on proteins *in vitro* are as follows: loss of enzyme activity (Choi and Tappel, 1969), loss of solubility due to aggregation or complex formation (Pokorny and Janicek, 1968; Andrews *et al.*, 1965), chain scission (Zirlin and Karel, 1969), as well as loss of specific amino acids.

To gain more information about lipid-protein interaction at different ratio of lipid with protein, *in vitro* protein digestibility and trypsin indigestible substrate(TIS) were investigated. The objective of the present work was to determine the formation of lipid-protein complexes from the degree of heat denaturation influencing the drop of *in vitro* protein digestibility.

Materials and Method

1. Materials

The flounder(*Pleuronichthys cornutus*, weight 170 ±18 *g*, length 18±6 *cm*) was purchased from Jagalchi Fish Market of Pusan on July 15th, 1985 and transported on ice to low temperature room. Fresh flounder were skinned, eviscerated, ground with motar, and finally stored in a low temperature cabinet at —30°C during the experiment. After heat treatment and vacuum freeze-dried, samples were ground with mortar to 100 mesh.

2. Preparation defatted sample

The lipids of raw material were extracted with chloroform-methanol(2:1, v/v) in a blender by the procedure of Bligh and Dyer's(1959). The residue was used as defatted sample.

3. Heat treatment

Twenty grams of raw and defatted flounder were heated in the microwave oven (Model ER-5000, Gold Star, CO.) for 1, 3, 5 and 7 minutes. Twenty grams of meat dispersed in a petri dish (*ϕ* 9 *cm*) was steamed on a water bath for the periods of time 5 to 40 minutes.

4. Chemical analyses

(1) *In vitro* protein digestibility

The *in vitro* digestibility of the flounder was determined by multienzyme method of Hsu *et al.* (1977) as improved by Satterlee *et al.* (1979) using ANRC sodium casein as the reference protein.

(2) Trypsin indigestible substrate(TIS)

The content of TIS of all samples was determind using the procedure of Rhinehart method(1975). The results were represented in trypsin inhibitor equivalents, which equals to the *mg* of purified soybean trypsin inhibitor (Sigma, 10,000 BAEE units/*mg* protein) per gram sample(dry basis). The standard curve used in measuring TIS content was shown in Fig. 1. For the reference, method by Hamerstrand *et al.* (1981) was employed to determine the TIS content quantitavely.

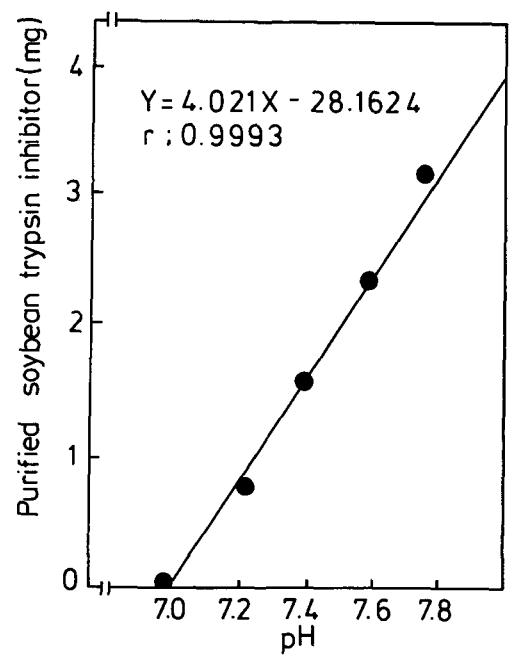

Fig. 1. Relationship of pH at 10 minuaes to purified soybean trypsin inhibitor concentration.

(3) Nonenzymatic browning index

The extents of nonenzymatic browning in each

samples was determined by the method of Chung and Toyomizu(1976).

(4) Determination of peroxide value(POV) and carbonyl value(COV)

The peroxide value was determined by the procedure of AOAC(1980) and carbonyl value was performed according to the procedure of Henick(1954).

(5) Protein solubility

The methods of Morr *et al.* (1985) and Montecavalo *et al.* (1984) were used to determine the protein solubility of vacuum freeze-dried flounders.

(6) Fatty acid composition

The lipids of heat treated samples were extracted by the procedure of Bligh and Dyer's(1959). Methyl ester of each fatty acid was prepared from the lipids by a procedure described Fujino (1978) and determined using GLC.

Operation conditions of GLC

Instrument	Pye-Unicam series 304
Column	1.5 $m \times 4.0$ mm i.d., stainless steel
Packing material	15% DEGS on 60-80 mesh chromosorb W
Carrier gas	1.25 kg/cm^2, Nitrogen
Column temp.	195°C
Detector temp.	FID at 250°C
Injector temp.	250°C

(7) Preparation of myofibrillar protein

Extraction method for myofibrillar protein was using the procedure of Groninger(1973) and Groninger and Miller(1975).

(8) Interaction of lipid to protein

The raw, defatted samples and isolated myofibrillar protein were mixed with lipid extracted from raw(10:0.5, 10:1, 10:2 and 10:4) for 2 and 4 hrs at room temperature(24±1°C) and 50°C. Except for the buffering capacity of protein itself, no attempt was made to control the pH of the lipid-protein mixtures. After mixing the protein and lipids, all samples were freeze-dried (FTS System's Freeze Dryer, Model TD 3-561 FD 8-84, 2-3 Torr, plate temp. 20°C).

Results and Discussion

1. Proximate composition of raw flounder

The results of the proximate analyses of the raw flounder was shown in Table 1. An 82.6% (dry basis) of crude protein and 10.3% (dry basis) of crude fat was contained in sample. The contents of crude protein (N×6.25) and crude fat was smilar to earlier report (Jeong, 1983).

Table 1. Proximate composition of the raw flounder

(%, dry basis)

Crude protein	Crude fat	Ash
82.58	10.30	5.53

2. Effect of heat treatment on nitrogen solubility

The effects of steaming and microwaving on nitrogen solubility are shown in Table 2. The total nitrogen of raw (12.63%) and defatted (13.74%) flounder seemed to be affected by heat treatment. The solubility of raw and defatted flounder heated was decreased with increase in cooking time. Steaming for 20 and 40 minutes caused an exteme reduction of nitrogen solubility of samples showing 33.6% and 29.6% for the raw and 34.6% and 31.9% for the defatted, respectively. Microwaving for 5 and 7 minutes drastically reduced the solubility to 21.2% and 19.2% of raw, and 29.4% and 27.6% of defatted, respectively.

3. Development of nonenzymatic browning during the heat treatment

For both raw and defatted flounder, browning gradually developed by steaming for 40 minutes (Table 3). Probably brown pigments did not increase during the steaming because of the low lipid contents and carbonyl compounds (Table 3). Brown pigments of raw flounder were slightly higher than that of defatted sample. The same tendency was shown in defatted flounder meat heated with microwaving. However, the browning development

Table 2. Effect of steaming and microwaving on the nitrogen solubility of flounder

Heat treatment (min)	Raw			Defatted		
	Total nitrogen(%)	Soluble nitrogen(%)	Solubility* (%)	Total nitrogen(%)	Soluble nitrogen(%)	Solubility* (%)
Steaming						
0	12.63	10.42	82.53	13.74	12.38	90.10
5	12.63	5.10	40.37	13.74	5.78	42.10
10	12.63	4.47	35.38	13.74	5.15	37.46
20	12.63	4.24	33.56	13.74	4.75	34.57
40	12.63	3.74	29.61	13.74	4.38	31.89
Mirowaving						
0	12.63	10.42	85.53	13.74	12.38	90.10
1	12.63	3.75	29.73	13.74	5.25	38.27
3	12.63	3.08	24.37	13.74	4.60	33.51
5	12.63	2.63	21.23	13.74	4.03	29.36
7	12.63	2.42	19.19	13.74	3.79	27.55

*The ratio of flounder: deionized H_2O was 1:100 (w/v).

Table 3. Changes in the peroxide value(POV), carbonyl value(COV) and browning pigment of flounder when heat treated

Heat treatment (min)	Browning pigment (O.D./g solid)		POV (*meq/kg*)	COV (*meq/kg*)
	raw	defatted	raw	raw
Steaming				
0	0.05	0.04	11.48	28.19
5	0.07	0.06	18.04	31.41
10	0.09	0.08	22.35	42.27
20	0.13	0.09	30.07	63.60
40	0.16	0.12	43.79	94.62
Microwaving				
0	0.05	0.04	11.48	28.19
1	0.08	0.06	15.48	45.18
3	0.09	0.06	23.94	91.07
5	0.19	0.08	47.71	159.13
7	0.27	0.09	58.01	187.82

revealed much higher values in the raw materials heated by microwaving for 5 minutes or so than the defatted as indicated in Table 3. In case of the raw sample, the browning pigments were excessively increased probably because of abundance of carbonyl compounds formed by lipid oxidation (You and Lee, 1982).

4. Changes of fatty acid composition by heat treatment

The fatty acid composition of steamed and microwaved flounder meat is given in Table 4 and 5, respectively. The major fatty acids, found in the heat treated were palmitic acid, palmitoleic acid, oleic acid, eicosapentaenoic acid and docosahexaenoic

Table 4. Effect of steaming on the fatty acid composition of raw flounder

(*g*/100 *g* solid)

Fatty acid	Steaming time (min)				
	0	5	10	20	40
Saturate					
12:0	0.60	0.78	0.39	0.39	0.87
14:0	4.20	3.90	3.68	3.85	4.33
15:0	1.09	0.96	0.90	0.93	1.18
16:0	18.39	18.85	18.93	19.05	18.83
17:0	2.37	2.34	2.01	2.04	2.36
18:0	5.94	6.49	6.43	6.22	6.19
Total	32.59	33.32	32.53	32.58	33.76
Monene					
14:1	0.60	0.48	0.43	0.48	0.78
16:1	10.94	10.51	10.19	10.81	10.75
17:1	1.88	2.10	1.90	1.92	2.02
18:1	15.62	15.61	15.81	15.92	15.70
20:1	5.26	4.38	5.01	4.42	4.56
22:1	1.93	1.74	1.71	1.72	1.69
Total	36.23	34.82	35.05	35.29	35.45
Polyene					
18:2	1.21	1.44	1.32	1.14	1.35
18:3	0.89	0.84	0.58	0.39	0.45
20:2	0.77	0.30	0.72	0.33	0.34
20:4	2.99	3.30	3.68	3.55	3.21
20:5	10.86	10.38	10.61	10.78	10.35
22:4	1.69	1.56	1.78	1.71	1.75
22:5	4.76	4.74	4.72	4.90	4.73
22:6	8.01	9.30	9.20	9.43	8.61
Total	31.18	31.86	32.61	32.23	30.64

Table 5. Effect of microwaving on the fatty acid compositoon of raw flounder

(*g*/100 *g* solid)

Fatty acid	Microwave heating time (min)				
	0	1	3	5	7
Saturate					
12:0	0.60	0.67	0.32	0.34	0.34
14:0	4.20	3.97	3.82	3.84	4.03
15:0	1.09	0.94	0.92	0.97	0.93
16:0	18.39	18.76	18.45	18.93	17.65
17:0	2.37	2.14	2.13	2.31	2.20
18:0	5.94	6.21	5.98	6.18	5.49
Total	32.59	32.69	31.62	32.57	30.64
Monoene					
14:1	0.60	0.49	0.48	0.50	0.48
16:1	10.94	10.72	10.71	10.56	11.34
17:1	1.88	1.79	1.82	1.97	1.80
18:1	15.62	15.99	15.79	15.71	16.41
20:1	5.26	4.56	5.05	5.03	6.02
22:1	1.93	1.97	1.97	1.94	2.57
Total	36.23	35.52	35.82	35.71	38.52
Polyene					
18:2	1.21	1.16	1.21	1.22	1.27
18:3	0.89	0.40	0.58	0.53	0.66
20:2	0.77	0.31	0.71	0.78	1.01
20:4	2.99	3.48	3.26	3.31	2.75
20:5	10.86	10.85	10.85	10.59	11.07
22:4	1.69	1.79	1.71	1.58	1.57
22:5	4.79	4.91	5.00	4.68	4.90
22:6	8.01	8.89	9.24	9.03	7.43
Total	31.18	31.79	32.56	31.72	30.84

acid. Especially, palmitic acid was the most predominans. Among the monoenic fatty acids, palmitoleic acid and oleic acid were found to be the major constituents comprising about 73% of the total monoenes. The principal acids in the polyunsaturated were eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid. The level of eicosapentaenoic acid was fairly high in polyunsaturated group. There was no great changes found in fatty acid composition during heat treatment because the level of carbonyl compounds were not greatly changed(Table 3).

5. Changes in the *in vitro* protein bigestibility and trypsin indigestible substrate content of flounder during heat treatment

Changes in the *in vitro* protein digestibility of raw and defatted flounder processed under various conditions of steaming and microwaving are presented in Table 6. The *in vitro* protein digestibility increased at the early stages of heating by steaming and microwaving and afterward it decreased. Especially, in the defatted samples it was rapidly decreased by steaming for 40 minutes.

These results agreed with the fact that proper heat treatment increases the digestibility of squid, oyster and pollock protein (Ryu, 1983). The proper condition of heat treatment which might result in a desirable denaturation, could get the fish meat more suscepitble to enzyme hydrolysis.

Heat processing to a mild extent can improve the digestibility of protein by destroying protease inhibitors as well as opening of the protein structure through denaturation. As the protein was extremely heated, the polymerization in a drop in protein digestibility possibly because of physical changes in protein structure which render the protein unavail-

Table 6. Differences in the *in vitro* protein digestibility and indigestible substrate(TIS) content of flounder following the various steaming and microwaving time

	Steaming (min)					Microwaving (min)				
	0	5	10	20	40	0	1	3	5	7
Dig. (%)										
Raw	85.83	88.09	86.85	86.74	85.95	85.83	87.41	86.74	84.48	83.58
Defatted	90.23	90.56	88.99	87.86	86.17	90.23	91.47	90.80	90.35	90.12
TIS(*mg/g*)										
Raw	14.81^{R}	13.26	12.79	12.09	11.78	14.81^{R}	14.01	13.04	11.78	11.15
	0.42^{H}	0.44	0.39	0.34	0.30	0.42^{H}	0.37	0.32	0.29	0.28
Defatted	11.01^{R}	10.59	10.14	10.02	9.60	11.01^{R}	10.22	9.69	9.45	9.27
	0.49^{H}	0.46	0.46	0.41	0.37	0.49^{H}	0.43	0.37	0.34	0.33

R: Rhinehart method (1975), H: Hamerstrand method (1981)

able. Table 6 illustrates the differences in the *in vitro* protein digestibility and TIS content of raw and defatted flounder with heating times. The *in vitro* protein digestibility was decreased until the steaming time reached 40 minutes, but TIS content did not change significantly (about 1~2%). The variation of TIS content in raw flounder was superior to defatted sample. As shown in Table 6, TIS content was rather low in microwaved raw flounder and dropped from 14.81 to 11.15 *mg/g*.

Lee *et al.* (1984) reported that trypsin indigestible substrates in several fishery products increased during the storage because of the lipid oxidation. The content of trypsin indigestible substrate were reduced by the heat treatment. During the heat treatment, the destruction of trypsin inhibitor activity in flounder seemed to be higher than formation of trypsin indigestible substrates.

6. Lipid-protein interaction

In general, oxidized unsaturated lipids in seafoods tend to bind proteins and form insoluble lipid-protein complexes (Rouble and Tappel, 1966 ; Roubal, 1969; Castell, 1971 ; Khayat and Schwall, 1983). The

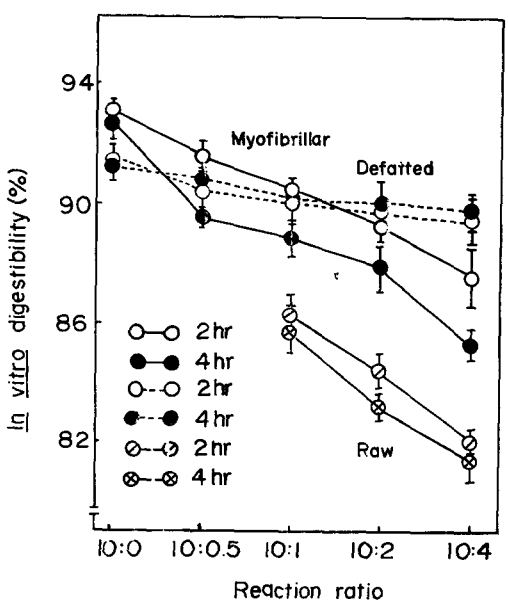

Fig. 2. *In vitro* protein digestibisity of protein-lipid complexes by different mixing ratios at room temperature (24±1°C).

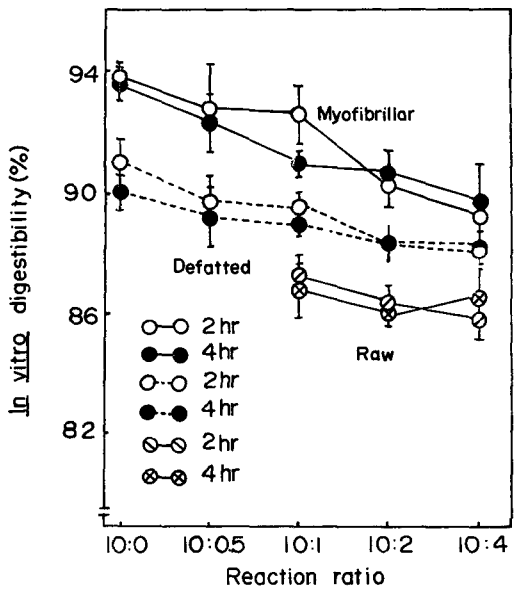

Fig. 3. *In vitro* protein digestibility of protein-lipid complexes by different mixing ratios at 50°C.

in vitro protein digestibility of lipid-protein complex by the different mixing ratios at room temperature (24±1°C) and 50°C was presented in Figure 2 and 3. The digestibility of the mixture was rapidly decreased with increase in reaction time and ratio. There were 5.53% and 7.44% drop of digestibility in lipid-isolated myofibrillar protein mixture, and 4.13% and 4.36% drop in lipid-raw meat mixture after 2 hours and 4 hours reaction at room temperature, respectively. But the digestibility of lipid-defatted meat mixture was not influenced by the reaction time and ratio at room temperature. There was little variations in the *in vitro* digestibility of lipid-defatted meat complexes caused by the increase in the reaction time and ratio at room temperature.

Figure 3 illustrates the results that the lipid interacted with protein at 50°C. The *in vitro* digestibility was decreased in all cases of test, but the digestibility at 50°C showed higher values than that of room temperature. At higher temperature like this, protein structures were unfolded and tended to be easily hydrolyzed by enzyme (Ryu, 1983). The defatted protein-lipid mixture showed

Table 7. Changes in the trypsin indigestible substrate (TIS)* contents of lipid-protein interaction following the different mixing ratio

Interaction ratio(w/w)	Room temp. (24±1°C) 2 hr	Room temp. (24±1°C) 4 hr	Interaction ratio(w/w)	50°C 2 hr	50°C 4 hr
Myofibrillar : Lipid			Myofibrillar : Lipid		
10 : 0.5	1.31	1.60	10 : 0.5	1.47	2.21
10 : 1	2.85	2.44	10 : 1	2.12	2.65
10 : 2	3.45	3.98	10 : 2	3.87	3.63
10 : 4	3.87	4.81	10 : 4	4.03	4.05
Defatted : Lipid			Defatted : Lipid		
10 : 0.5	12.23	12.97	10 : 0.5	11.42	12.62
10 : 1	12.94	13.08	10 : 1	12.63	13.92
10 : 2	13.83	13.14	10 : 2	13.05	14.62
10 : 4	13.95	14.55	10 : 4	13.70	14.36
Raw : Lipid			Raw : Lipid		
10 : 2	17.85	16.40	10 : 2	15.66	14.81
10 : 4	18.90	19.54	10 : 4	17.69	16.98

*Rhinehart method (1975)

Table 8. Changes in the *in vitro* protein digestibility and trypsin indigestible substrate(TIS)* contents of lipid-protein interaction heating after mixing and heating before mixing

Heat treatment (min)		Heating after mixing** Raw	Heating after mixing** Defatted	Heating before mixing** Raw	Heating before mixing** Defatted
Steaming					
5	Dig. (%)	83.01	87.14	87.75	89.33
	TIS (*mg/g*)	21.37	14.15	17.85	14.06
40	Dig. (%)	81.66	85.72	86.07	87.52
	TIS (*mg/g*)	22.04	15.80	18.39	15.24
Microwaving					
1	Dig. (%)	83.91	86.52	83.92	84.25
	TIS (*mg/g*)	21.81	15.79	20.57	14.38
7	Dig. (%)	81.44	84.37	82.79	81.77
	TIS (*mg/g*)	22.28	16.04	21.55	15.93

*Rhinehart method(1975).

**Interaction protein with lipid is 10:4(w/w). The mixtures were reacted at room temperature for 4 hours

88~90% digestibility. Based on the data in Table 7, TIS increased as the fat level of lipid-protein complexes increased. The relationship between *in vitro* protein digestibility and TIS could be explained by the fact that the digestibility was decreased because increase in the oxidized fats formed during the reaction reduced the relative amount of substrate which the enzyme could act. The comparative values for the *in vitro* protein digestibility and TIS content of the complexes formed by the interaction of lipid and protein are shown in Table 8. In order to obtain any informations concerning to the differences in the digestibility and TIS according to the sequences of heat treatment and mixing, both raw and defatted flounder meat were treated in either of two ways: that is, the meat was heated after mixing(HAM) or before mixing(HBM) with lipid.

In general, HAM showed lower values of the digestibility and therefore high TIS contents than HBM. In case of HAM there was not any differences found in the digestibility for both the raw and the defatted between the heating methods of steaming and microwaving. Nevertheless, the defatted samples showed higher digestibility than the raw materials in the both cases of heating. However, in the case of HBM the samples heated

by steaming revealed higher digestibilities than those by microwaving. The result probably indicated that lipid-protein interaction was involved in the drop of digestibility which concurred with protein denaturation.

References

Almquist, H.L. 1956. Changes in fat extractibility and protein digestibility in fish meal during storage. J. Agric. Food Chem. 4, 638—639.

Andrews, F., J. Bjorksten, F.B. Trenk, A.S. Henick and R.B. Koch. 1965. The reaction of an autoxidized lipid with proteins. J. Am. Oil Chem. Soc. 42, 779—781.

AOAC 1980: "Official Methods of Analysis," 13th ed., Association of Official Analytical Chemists, Washington, DC.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37, 911—915.

Castell, C.M. 1971: Metal-catalyzed lipid oxidation and changes of protein in fish. J. Am. Oil Chem. Soc. 48, 645—649.

Chen, B.H. and C.V. Morr. 1985. Solubility and foaming properties of phytate-reduced soy protein isolate. J. Food Sci. 50, 1139—1142.

Choi, K.S. and A.L. Tappel. 1969. Inactivation of ribonuclease and other enzymes by peroxidizing lipids and by malonaldehyde. Biochem. 8, 2827—2830. Cited from Kanner, J. and M. Karrel 1976.

Chung, C.H. and M. Toyomizu. 1976. Studies on the browning of dehydrated foods as a function of water activity— I . Effect of A_w on browning in amino acid-lipid systems. Bull. Jap. Soc. Fish. 42(6), 697—702.

Greene, B.E. 1971. Interrelations between myoglobin, lipids, flavour and color of meat. Proceedings of the Meat Industry Research Conference. p. 27.

Groninger, H.S. Jr. 1973. Preparation and properties of succinylated fish myofibrillar protein. J. Agr. Food Chem., 21(6), 978—981.

Groninger, H.S. Jr. and R. Miller. 1975. Preparation and aeration properties of an enzyme-modified succinylated fish protein. J. Food Sci. 40, 327—330.

Hamerstrand, G.E., L.T. Black and J.D. Glover. 1981. Trypsin inhibtors in soy products; Modification of the standard analytical procedure. Cereal Chem., 58, 42—45.

Henick, A.S., M.F. Benca and J.H. Mitchell Jr. 1954. Estimating carbonyl compounds in rancid fats and foods. J. Am. Oil Chem. 51, 928—930.

Hsu, H.W., D.L. Vavak, L.D. Satterlee and G.A. Miller. 1977. A multienzyme technique for estimation protein digestibility. J. Food Sci. 42, 1269—1273.

Jeong, B.Y., D.S. Byun and J.H. Pyeun. 1978. Nutritional evaluation of muscle protein of flounder, *Limanda herzensteini*, dried by different methods. J. Korean Soc. Food and Nutr. 7, 1—6.

Karel, M. 1977. Interaction of food proteins with water and with lipids, and some effects of these interactions on functional properties, in "Biotechnological Applications of proteins and Enzymes," Z. Bohak and N. Sharon edited, Academic Press, New York, p. 317.

Khayat, A. and D. Schwall. 1983. Lipid oxidation in seafood. Food Tech., 37(7), 130—140.

Kim, S.A. 1984. Influence of lipids on the *in vitro* protein digestibility of dried fish. Ph. D. thesis of Nat. Fish. Univ. of Pusan.

Kimoto, W.I. and A.M. Gaddis. 1971. Catalytic decomposition of autoxidized unsaturated fatty acids. J. Am. Oil Chem. Soc., 48, 331—334.

Lee, K.H., J.H. Jo and H.S. Ryu 1984: Distribution of trypsin indigestible substrates (TIS) in seafoods and its changes during processing. 2. Changes in TI and *in vitro* apparent digestibility of boiled and dried anchovy during processing and storage. Bull. Korean Fish. Soc. 17(2), 101—108.

Montecavalo, J. Jr., S.M. Constantinides and C.S. Yang. 1984. Optimization of processing para-

meters for the preparation of flou flounder frame protein products. J. Food Sci. 49, 172—176.

Pokorny, J., B.A. El-Zeany and G. Janicek. 1974 b. Browning reactions of oxidized fish lipid with proteins, In Prox. of 1st Int'l. Congress Food Sci. Tech. 1, 217—219. Cited from Khayat, A. and D. Schwall 1983.

Rhinehart, D. 1975. A nutritional characterization of the distiller's grain protein concentrates. MS thesis of Univ. of Nebraska-Lincoln, p. 29.

Roubal, W.T. and A.L. Tappel. 1966. Damage to proteins, enzyme and amino acids by peroxidizing lipids. Arch. Biochem. Biophy. 113, 5—8.

Roubal, W.T. 1939. Trapped radicals in dry lipid-protein systems undergoing oxidation. J. Am. Oil Chem. Soc. 47, 141—144.

Roy, R.B. and M. Karel. 1973. Reaction products of histidine with autoxidized methyl-linoleate. J. Food Sci. 38, 893—897.

Ryu, H.S. 1983: Nutritional evaluation of protein quality in some seafoods. Ph. D. thesis of Nat. Fish. Univ. of Pusan.

Satterlee. L.D., J.G. Kendrick and G.A. Miller. 1979: Rapid *in vitro* assays for estimating protein quality. Food Tech. 31, 78—81.

Shenouda, S.Y.K. and G.M. Pigott. 1975. Lipid-protein interaction during aquous extraction of fish protein; Actin-lipid interaction. J. Food Sci. 40, 523—532.

Zirlin, A. and M. Karel. 1969. Oxidation effects in a freeze-dried gelation-methyl linoleate system. J. Food Sci. 34. 160—164.

魚肉 調理時 脂質이 蛋白質 變性에 미치는 影響

李 康 鎬 · 崔 炳 大 · 柳 洪 秀*
釜山水産大學 食品工學科 *釜山水産大學 食品營養學科
(1986년 6월 15일 수리)

要 約

蛋白質의 어떠한 加熱變性 程度에서 消化率 低下에 影響을 미치는 lipid-protein complex가 가장 많이 形成되는 가를 究明하기 위하여 도다리(*Pleuronichthys cornutus*) 肉蛋白質을 加熱變性시키고 four-enzyme system을 利用하여 *in vitro* digestibility를 測定하였으며, 여기에 도다리 肉에서 抽出한 脂質을 添加하여 lipid-protein complex가 形成되는 現象을 調査한 結果는 다음과 같다.

In vitro digestibility는 5分間 煮熟하였을 때 生試料가 88.09%, 脫脂試料가 90.56%이었으며, 40分 後에는 各各 85.95%, 86.17%를 나타내었다. Microwaving의 경우 1分 加熱 後의 消化率은 生試料가 87.41%, 脫脂試料는 91.47%이었고, 7分 加熱 後에는 3~4% 程度가 減少하였다.

lipid-protein complex 生成에 起因된 것으로 생각되는 trypsin indigestible substrates(TIS)는 加熱時間, 添加되는 脂質의 量, 相互作用時間에 따라 增加하는 傾向을 나타내었다.

도다리 肉에서 抽出한 脂質의 主要 脂肪酸은 $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, $C_{20:5}$, $C_{22:6}$ 이었고, 加熱時間에 따른 脂肪酸 組成의 變化는 거의 없었으며. 不飽化脂肪酸의 含量이 67% 程度를 차지하였다. POV, COV의 增加와 함께 褐變物質의 生成이 增加하였고, 蛋白質 溶解度는 microwaving하였을 때 가장 낮았다(19.19%).

lipid-protein 相互作用에서 脂質의 含量이나 酸化程度가 酵素的 消化에 가장 큰 沮害因子로 作用하였으며, 生試料 및 脫脂試料에 脂質을 섞어 加熱處理하면서 反應시킨 것과 蛋白質을 加熱變性시킨 후 脂質을 添加하여 反應시킨 것을 比較하면 lipid-protein 相互作用으로 因하여 前者가 後者보다 낮은 消化率을 나타내어 蛋白質과 脂質의 相互作用은 蛋白質 變性現象과 동시에 일어날 可能性이 높음을 알 수 있었다.