Studies on the Preparation and Utilization of Filefish Protein Concentrate (FPC)

II. The Effect of Processing Conditions on the Functional Properties

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말쥐치 濃縮蛋白質의 製造 및 利用에 関한 研究

제 2 보 : 製造方法에 의한 機能性의 変化

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Abstract

Isopropyl alcohol extracted filefish protein concentrate (FPC) and NaOH hydrolyzed filefish protein isolate (FPI) were used for the investigation on the effect of processing conditions on the molecular distribution and functional properties. The molecular distribution of FPC on polyacrylamide gel showed a resemblance to that of fish muscle, but that of alkaline hydrolyzed FPI showed the severe degradation of protein. The content of several amino acids in FPI were lower than those of FPC. The pepsin digestibility of the FPC dried at high temperature was relatively high. FPC didn't exhibit a significant difference in nitrogen solubility at the pH range of 3.0 – 9.0, while FPI showed a wide difference with the pH change. FPI was more suspensible and rehydrated in water than FPC. Although the aeration capacity of FPI was very low, foam viscosity was higher than that of FPC. In contrast with aeration capacity, FPI presented higher emulsion capacity and lower emulsion viscosity than FPC. The size of fat globule in the emulsion of FPC was larger than that of FPI. In general, most functional properties decreased with the increment in drying temperature, except water holding capacity.

Introduction

A multitude of low-cost protein sources could be utilized for protein ingradients. These include soybean, leaf, grains, fish, oilseed, legume, etc. Among these materials, fish is one of the most valuable protein sources containing abundant animal protein qualitified nutritively, and the production of fish protein concentrate (FPC) is one of the most acceptable methods of preservation. Most FPCs have several limitations of low functional properties and organoleptic properties which

are the most important natures of protein ingradient. New processes based on solubilization with enzyme or chemicals have been developed to improve functional properties of FPC. To produce a protein isolate, there are four essential steps, namely, (1) (1) solution of the protein in aqueous medium with proper pH and/or salt conditions, (2) removal of the undissolved residue (bones, grit, scales, etc.) from the protein solution, (3) recovery of the protein from the solution as a curd by proper pH adjustment or dilution, (4) purification and drying of protein fraction. Fish protein isolate (FPI) displays, to some extent, better functional properties than FPC, but the

formation of peptides or amino acids causes bitter tastes. Because of severe alkaline hydrolysis for the preparation of FPI, extensive racemization or decomposition of several amino acids take place. (2) Groot et al. (3) reported that the exposure of protein products to awueous alkaline resulted in the formation of the amino acid derivatives, lysinoalanine (LAL) poorely absorbed in biological metabolic stystem. Yanez et al. (4) suggested the losses of lysine and NPU of fish protein caused by drying.

As structural components, protein contributes specific functional properties such as gel formation, emulsification, aeration, dispersibility, etc.

Hermansson⁽⁵⁾ reported that the balance between forces underlying protein-protein and protein-solvent interactions by which most functional properties is determined, is affected by pH, concentration, temperature, nature of solvent, presence of other component, etc. Functional properties of protein, as well as other properties, are closely related to the denaturations caused by heating, drying, extreme dilution, raciation, chemical solutes, freezing, etc. According to Butler,⁽⁶⁾ as temperature increased, disorganization of structure can occur through the disruption of noncovalent forces which stabilize the etructure and shape of grotein molecule at low temperature.

Drying is an essential step as well as solvent extraction, in preparing FPC and FPI, and responsible for protein denaturation.

The objectives of this study are to investigate the molecular distributions of filefish muscle protein, FPC and FPI, and the differences in the functional properties of these proteins, at the various drying conditions.

Materials and Methods

Materials

Filefish (Navodon modestus) was obtained from a Fisheries Cooperative in Sam Chun Po on December, 1981, and preserved at -20 °C for the experiments.

Preparation of filefish protein concentrate

Filefish protein concentrate (FPC) was prepared by the methods of Son.⁽⁷⁾

Drying conditions were divided into three types, forced air-drying and vacuum-drying at the temperature of 30°C, 50°C and 70°C. Freeze-drying was performed after washing the lipid extracted residue once with hot

water (about 60°C) to increase freezing point.

Preparation of filefish protein isolate

The preparation of filefish protein isolate (FPI) was performed by the methods of Son.

Drying conditions for the preparation of FPI were same as used for that of FPC.

Analysis of chemical composition

The content of chemical component was determined in accordance with the procedure of A.O.A.C.⁽⁸⁾

Analysis of amino acid composition

The amino acid compositions of filefish muscle, FPC and FPI were measured with automic amino acid analyzer (Hitachi Model 835, Japan)

Determination of trimethylamine content

The trimethylamine (TMA) content was determined by the procedure of A.O.A.C.

Comparison of electrophoretic pattern

SDS-polyacrylamide gel (PAG) electrophoresis was performed by the methods of Weber and Osborn. (9)

Each 50 mg of FPC and FPI, or 0.1~g of fish muscle was added to 5~ml of 0.01M sodium phosphate buffer solution (pH 7.0) containing 6M urea, 1% sodium dodecyl sulfate (SDS), 1% 8-mercaptoethanol and 0.015% bromphenolblue. The mixture was boiled for 5~min, and then centrifuged at $2,800 \times G$ for 30~min. Fifty microliters of supernatant were applied to a gel.

Soluble protein was prepared by homogenizing 2g of fish muscle or each 1g of FPC and FPI with 30ml of 0.01M sodium phosphate buffer (pH 7.0), and then centrifuged at $2,800 \times G$ for 30min. The supernatant was mixed with 6M urea, 1% SDS, 0.5% 8-mercaptoethanol serveral drops of glycerine and 0.015% bromphenolblue, and then incubated at 37% for 2hrs. One hundred microliters of this solution were layered on the top of gel. Gel buffer was 0.2M sodium phosphate buffer solution (pH 7.0) containing 0.2% SDS.

The acrylamide concentration of gel was 7.5%, and all gels contained 2M urea. For the determination of high molecular weights, 5.0% gel was used also. Electrophoresis was performed at constant current of 6 mA per gel for 3 hrs. Staining was done at 60 - 65 °C for 2 hrs, and destaining at room temperature.

Molecular weight of protein unit separated in SDS-PAG was determined by comparing the mobility with

Bovine Albumin Cross-linked (Sigma Co., U.S.A.) in 5.0% gel and Bovine Hemoglobin Cross-linked (Sigma)

in 7.5% gel.

Disc-polyacrylamide gel electrophoresis was performed according to the methods of Davis. (10)

Determination of pepsin digestibility

Pepsin digestibility was determined by the procedure of A.O.A.C.

The pepsin used for digestion was manufactured by Sigma Co. The activity of enzyme was 1;10,000.

Determination of functional properties

1. Nitrogen solubility

Nitrogen solubility was determined by modifying the methods of Butler. (6) Half gram of FPC or FPI was dispersed in 20ml of deionized water with a magnetic mixer (Yamato Type MH-61, Japan). The pH was adjusted from 3.0 to 9.0 with 0.1N HC1 or 0.1N NaOH solution. After adding 20ml again, the mixture was stirred for 20min, and then centrifuged at $3,800 \times G$ for 10min. at room temperature. Five milliliters of filtrate passed through Whatman #1 paper were taken for nitrogen analysis by microkjeldahl method (A.O.A.C.).

2. Water holding capacity

Eastimate of water holding capacity was based on the methods of Cobb et $al.^{(11)}$ Duplicated samples, 0.5g of each was rehydrated with 20ml of water in centrifuge tube, standing for 6hrs at room temperature, and then centrifuged at 4,000 \times G for 20 min. at room temperature. The volume of supernatant passed through the damped whatman #1 paper was meas

Water holding capacity (%) =

3. Dispersibility

Dispersibility was determined by modifying the methods of Dubrow. (12) Sample (0.5g) was added to 20ml of water and stirred for 20min. The pH was adjusted to 7.0 with 0.1 N NaOH or 0.1 N HCl solution. After adding 20ml of water again, the dispersion was stirred for 2 hrs, and poured into 50ml graduated cylinder. It was allowed particles to settle, standing for 90min. The 5ml of supernatant was carefully transfered into a tared bottle and dried completely for about 20hrs at 103 °C. Dispersibility was calculated by the following equation.

Dispersibility (%) =

4. Fat absorption

Half gram of sample was dispersed in 10ml of refined soybean oil by standing for 2 hrs at room temperature, and centrifuged at $2,800 \times G$ for 20 min. The volume of supernatant was measured.

Fat absorption (%) =

Aeration property

Aeration capacity was measured by the methods of Gronigner. Gronigner. Gronigner. Gronigner. Gronigner Gr

Foam stability was observed by comparing the changes in foam volume at the time interval. Foam viscosity was measured by modifying the methods of Rasekh. (14) A 3g of sample in protein weight was dispersed in 100ml of 0.2M citrate phosphate buffer solution (pH 7.0), and then whipped a homogenizer (International Co. Korea) at 6,000 rpm for 6 min. The viscosity was determined with Brookfield viscometer Model LVT (U.S.A.) at the time intervals of 5, 30 and 60 min.

6. Emulsion property

Emulsion capacity was determined according to the methods of Webb. (15) One gram of sample and 150ml of 3% NaCl solution were blended with Waring blender (Model EP-1, U.S.A.) at 6,000 rpm for 2 min. for a complthe dispersion. Thirty millilters of refined soybean oil were added to the dispersion and emusified at 6,000 rpm for 3 min. Emulsification was continued at 12,000 rpm adding oil at the rate of 0.7 ml/sec. until electric resistance of emulsion increased abruptly. Emulsion capacity was calculated by the following equation.

Emulsion capacity =

Volume of oil emulsified (ml)
Weight of protein in sample (g)

Emulsion viscosity was determined as follows. One gram of sample in protein weight was dispersed in 55ml of 0.1 M sodium phosphate buffer solution (pH 7.0) containing 3% NaCl by a homogenizer Seiki Co. Japan) at 9,000 rpm for 2 min. The dispersion was emulsified with 45ml of soybean oil at 13,000 rpm for 5 min. The emulsion was allowed to stand at 25 °C in water bath for 5 min., and then the viscosity was measured by Brookfield

viscometer LVT (U.S.A.). As soon as emulsion was prepared, light microscopy of fat globule in emulsion was carried out. The breakdown of emulsion was observed during storage at room temperature.

Results and Discussion

Proximate composition

As shown in Table 1, the protein content of FPC is about 76-78%, and that of lipid is 0.4 - 0.5%. FPI contains greater amount of protein than FPC.

Amino acid composition

As discribed in Table 2, the contents of both leucine and lysine, kinds of essential amino acids in fish muscle are slightly higher than those of other amino acids. FPC and FPI contain the larger proportions of alanine, methionine, leucine, tyrosine, phenylalanine and histidine than fish muscle. The contents of serveral amino acids in FPI, such as glycine, alanine, lysine and arginine are lower than those in FPC. Because of severe alkaline hydrolysis, the racemization of some amino acids, formation of lysinoalanine (LAL) and other changes in amino acids maybe occured during the process.

Trimethylamine content

Trimethylamine (TMA) should be removed for acceptability of FPC and FPI. Table 3 shows the TMA contents FPC and FPI. There was no significant dif-

Table 2. Amino acid composition of filefish muscle, FPC and FPI

. Essential amino acid

	Filefish muscle	FPC	FPI (%)
Aspartic acid	10.04	10.78	11.00
Threonine*	3.16	3.13	3.20
Serine	2.12	2.18	1.81
Glutamic acid	14.10	15.42	14.95
Glycine	5.41	6.54	4.65
Alanine	4.80	8.68	8.26
Valine*	8.54	5.42	5.39
Methionine*	1.86	2.53	2.57
Isoleucine*	4.06	3.82	4.42
Leucine*	7.12	7.78	8.49
Tyrosine	1.48	2.65	2.61
Phenylalanine*	3.69	3.95	4.15
Lysine *	8.19	7.77	7.36
Histidne	2.82	3.01	3.24
Arginine	6.10	4.24	4.34
Proline	3.05	3.64	2.77
Tatal	86.54	91.54	89.21

Conditions

Flow rate: Ninhydrin 0.3 ml/min. Buffer solution 0.225 ml/min.

(): Dry base

6.6

75.7

Temperature: Colume 53 °C Reaction vessel 90 °C

Table1. Proximate composition of FPC, FPI and whole filefish

Protein (%) Lipids (%) Ash (%) The volatile (%) **FPC** F-30:7ª 8.2 76.8(83.7) 0.4(0.4)14.9(16.2) F-50:3 8.5 78.4(85.7) 0.4(0.4)12.8(14.0) 7.6 F-70:1.5 78.2(84.6) 0.5(0.5)13.0(15.0) V-30:8b 76.1(82.9) 0.5(0.5)14.5(16.0) 8.2 V-50:4 8.5 76.4(83.5) 0.5(0.5)14.8(16.2) V-70:2 75.7(82.7) 0.5(0.5)16.0(18.5) 8.5 FR¢ 7.6 77.0(83.3) 0.5(0.5)15.2(16.5) FPI IF-30:9d 7.0 (7.6) 7.5 85.0(91.9) 0.2(0.2)IV-30:11e 6.3 (6.8) 7.5 85.5(92.4) 0.3(0.3)

0.4(0.4)

5.0(20.6)

a: Forced air-dried FPC at 30°C for 7 hrs

86.4(92.4)

16.3(67.1)

- b: Vacuum-dried FPC at 30 °C for 8 hrs
- c: Freeze-dried FPC

IFR^f

Whole filefish

3.0(12.3)d: Forced air-dried FPI at 30 °C for 9 hrs

6.5 (6.9)

- e: Vacuum-dried FPI at 30 °C for 11 hrs
- f: Freeze-dried FPI

ference in TMA content of FPC and FPI varing with the processing condition. TMA in whole filefish was mostly removed by IPA and alkaline, and it is supposed that drying contributes to removal of the remnant TMA.

Table 3. Trimethylamine content of whole filefish, FPC and FPI

	(): Dry base		
	Trimethylamine (% × 1000)		
Whole filefish	0.17(0.70)		
FPC			
F-30;7	0.15(0.16)		
F-50;3	0.14(0.15)		
F-70;1.5	0.14(0.15)		
V-30;8	0.16(0.17		
V-50;4	0.14(0.15)		
V-70;2	0.15(0.16)		
FR	0.16(0.17)		
FPI			
IF-30;9	0.11(0.12)		
IV-30;11	0.11(0.12)		
IFR	0.12(0.13)		

Electrophoretic pattern

1. SDS-PAG electrophoresis

As indicated in Fig. 1, the molecular distribution of FPC was very similar to that of muscle protein.

There was not notivible difference in the pattern among FPCs prepared differently in drying condition. However, it was found electrophoretically that FPI was severely degraded. The soluble protein of FPC is composed of 3 or 4 units which molecular weights are relatively small, as shown in Fig.2. Table 4 presents the molecular weight of protein unit, marked as alphabet, in filefish muscle, and the color intensity of protein unit on gel. Two units, namely A and B, which may be myosin heavy chains, were not found in the soluble protein of filefish muscle. The soluble protein of FPC or FPI mainly consists of two units which molecular weights are about 44,000 and 15,000 dalton, supposed to be tropomyosin and myoglobin.

2. Disc-PAG electrophoresis

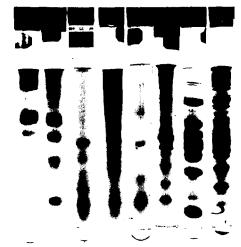
Figure 3 shows the disc-PAG electrophoretic pattern of soluble protein in 0.2 M Tris-HCl buffer (pH 7.0). Their units were found in the gel of FPC, which pattern was similar in all soluble proteins of FPCs,



H.G. IFr IV30 IF30 FR V70 V50 V30 F70 F50 F30 Raw

Fig. 1. SDS-PAG electrophoretic pattern of filefish muscle, FPC and FPI, in 7.5% gel

Raw; filefish muscle, F30; forced air-dried FPC at 30 °C, F50; forced air-dried FPC at 50 °C, F70; forced air-dried FPC at 70 °C, V30; vacuum-dried FPC at 30 °C, V50; vacuum dried FPC at 50 °C, V70; vacuum dried FPC at 70 °C, FR; freeze-dried dried FPC, IF 30; forced air-dried FPI at 30 °C, IFr; freeze-dried FPI, H.G.; Bovine Hemoglobin Cross-linked.



B.A. H.G. SIF IF30 SF F30 SRaw Raw

Fig. 2. Comparison of the SDS-PAG electrophoretic pattern of filefish muscle (Raw), FPC (F30) and FPI (IF30) with the pattern of their soluble proteins (SRaw, SF, SIF)

H.G.: Bovine Hemoglobin Cross-linked B.A.: Bovine Albumin Cross-linked

Table 4. The proximate molecular weight and color intensity of protein unit in filerfish muscle, FPC, FPI and their soluble proteins, determined in 7.5% gel.

		nit Mwt	Color intensity					
	Unit		Rawa	sRaw ^b	F-30 ^c	sF-30 ^d	IFe	sIF ^f
	A	290,000	+		++			
	— в	198,000	+ +		++			
THE PARTY OF THE P	— c	160,000	+ +	+	+			
	— D	135,000	+ +	+				
	_ E	96,000	+	+ +	+			
	_ F	90,000	+++	+++	++			
	G	72,000	+	+	+			
	Н	67,000	+	+				
	I	61,000	+ +	+++	+ +			
)]	57,000	+	+ +	+			
	к	50,000	+++	+ + +	+ +			
	L	44,000	+++	+ + +	+++	+++	++	+ +
	М	41,000	+ +	+ + +	+			
	N	36,000	+++	+++	+ +		+ +	+
	` 0	32,000	+ +	+ +	+			
	P	26,000	+ +	+ + +	+ +	+	+	
	Q	20,000	+ +	+	+ +	++		+
	\sim R	15,000	+	+++	++	+ +	+	+ +

- a: Muscle protein of raw filefish
- b: Soluble protein of muscle protei
- c: Forced air-dried FPC at 30°C
- d: Soluble protein of F-30
- e: Forced air-dried FPI at 30 °C
- f: Soluble protein of IF

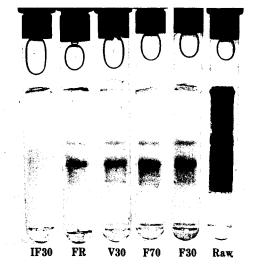


Fig. 3. Disc-PAG electrophoretic pattern of soluble protein of filefish muscle (Raw), FPC (F30, F70, V30, FR) and FPI (IF30) at pH 7.0

whereas FPI left only light trace. Probably, it is due to the degradation by alkaline hydrolysis.

Pepsin digestibility

Filefish protein concentrate dried at high temperature exhibits the higher digestibility than that dried at low temperature, as shown in Table 5. The drying at high temperature caused the reformation of protein structure to be easily digestible by proteolytic enzyme.

Functional properties

1. Nitrogen solubility

Figure 4 shows the nitrogen solubilities of FPC and FPI in the pH range of 3.0-9.0. FPC was not widely different at the change of pH, however, FPI exhibited the marked contrast of solubility. Freeze-dried FPC and FPI presented relatively low solubility. In the pH range from 3.0 to 6.0, the solubility of FPI was lower than that of FPC, but at the pH over 6.0, suddenly increased. As exhibited in Table 6, drying at high temperature has a

Table 5. Pepsin digestibility of FPC and FPI

	Pepsin digestibility (%)		
FPC			
F-30;7	93.4		
F-50;3	95.5		
F-70;1.5	96.0		
V-30;8	93.1		
V-50;4	97.7		
V-70;2	98.5		
ER	93.5		
FPI			
IF-30;9	96.3		
IV-30;11	95.2		
IFR	99.2		

harmful influence on the solubility at pH 7.0, and the FPI dried under vacuum or forced air condition was more soluble than FPC.

2. Water holding capacity

Drying at high temperature increased the water holding capacity of FPC. It is supposed the formation of network produced in protein by drying at high temperature. The degraded FPI exhibits higher capacity than FPC. The water holding capacity of freeze-dried FPI was the greatest of all.

3. Dispersibility

At pH 7.0, both FPC and FPI dried by vacuum showed a superior dispersibility to those dried by forced air at same temperature. FPI was more suspensible in water than FPC.

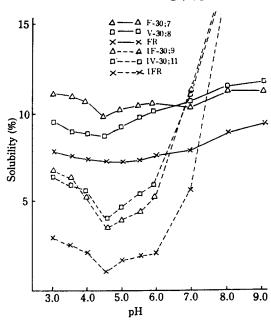


Fig. 4. Nitrogen solubility of FPC and FPI in the pH range of 3.0 - 9.0

4. Fat absorption

Generally, fish protein has lipophilic property rather than hydrophilic one. FPI absorbed more oil than FPC, as exhibited in Table 6. FPC dried at 50 °C presented higher absorption than others.

5. Aeration property

Comparatively, the aeration capacity of vacuumdried FPC at 30 °C for hrs was the greatest of all, and FPI showed the poor capacity. Freeze drying and drying at high temperature have a negative effect on the aera-

Table 6. Functional properties of FPC and FPI

FPC	Nitrogen solubility (%)	Water holding capacity (%)	Dispersibility (%)	Fat absorption (%)	Emulsion capacity (ml/g)
F-30:7	10.1	420	20.5	462	110
F-50:3	8.0	432	432 17.2 480		103
F-70:1.5	6.5	440	440 13.5 450		98
V-30	10.3	414	22.6	420	112
V-50:4	9.7	425	19.4	445	108
V-70:2	8.4	430	18.5	390	104
FR FPI	7.3	420	23.8	396	102
IF-30:9	10.9	800 52.6 660		660	160
IV-30:11	10.7	820	49.7	663	145
IFR	5.8	850	38.4	520	96

tion capacity of FPC and FPI. As indicated in Fig. 5, After 5 min., the foam volume was not changed greatly, and continued stable state.

Foam viscosity at pH 7.0 is presented in Fig. 6. Foam of freeze-dried FPI was still strong after 60min. Freeze-dried FPC has higher viscosity and more resistant than forced air or vacum-dried FPC.

6. Emulsion property

The emulsion capacity at pH 7.0 of vacuum-dried FPC was somewhat higher than that of forced air-dried FPC at the same temperature. Emulsion capacity showed a close relationship to the solubility. As exhibited in Fig. 7, the fat globule in FPC emulsion was bigger than that of FPI emulsion, and the FPC emulsion was holding

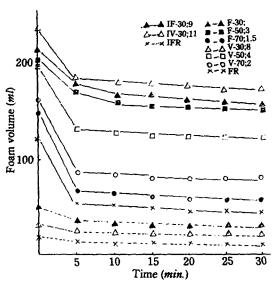
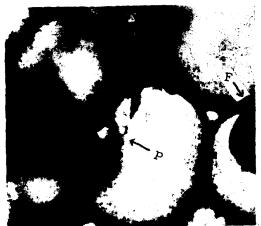
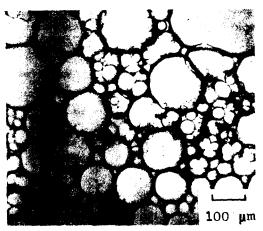


Fig. 5. Volume of foam prepared with FPC and FPI, and the change at the time interval



(Fat grobules in FPC emulsion)



(Fat gloubules in FPI emulsion)

Fig. 7. Photomicroscoph of the fat globule in the emision prepared with FPC and FPI

P: Insoluble protein particle

F: Foam

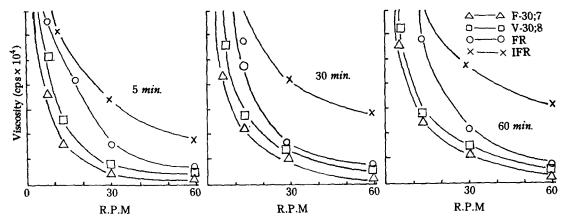


Fig. 6. Foam viscosity of FPC and FPI after 5,30 and 60 min.

a lot of insoluble protein particles on the surface of fat globule. After 1 hr, the emulsion prepared with FPC was mostly separated from water phase, whereas that with FPI was not, but from insoluble particles. Standing for 3 hrs, the FPI emulsion was faintly separated from water phase, demonstrating the slight breakdown, in contrast with the quick one of FPC emulsion. After 24 hrs, oil phase appeared on the top of two emulsions by coalescence of fat globules, and water phase was clearly seperated from FPI emulsion. The viscosity of emulsion prepared is presented in Fig. 8. The emulsion of freezedried FPC exhibits the highest viscosity, and the FPI emulsion presented relatively low viscosity.

The forced air-dried FPC shows higher emulsion viscosity than vacuum-dried FPC. The FPI emulsion was disrupted with appearance of a sudden increase of viscosity at the high rpm of viscometer (over 3.0 rpm).

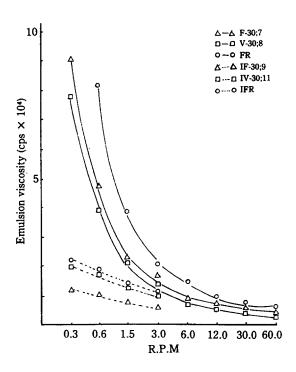


Fig. 8. Viscosity of the emulsion prepared with FPC and FPI.

요 약

매년 200,000톤 이상이 어획되고 있으나 주로 단순한 어포로서만 이용되고 있는 말쥐치를 원료로 하여, isopropyl alcohol에의해 지방이 제거된 filefish pro-

tein conceutrate(FPC)과, NaOH용액에 의한 가수 분해에 이어 lactic acid용액으로 단백질을 침전시켜 얻은 filefish profein isolate(FPI)를 식품에 참가이 용하기 위하여, 제조공정 및 건조조건에 따른 기능성 의 변화를 비교 검토하였다.

영양적인 면에서 보면, FPI의 pepsin소화율은 FPC의 경우보다 높았으나, lysine, leucine, alanine 등의 몇몇 아미노산 합량의 손실이 있었다.

FPC의 단백질 분자조성은 어욱의 경우와 비슷하였으나 FPI의 단백질은 대부분 degradation되었다.

FPC의 수용액상에서의 질소 용해도는 용액의 pH 변화에 따라 큰 차이가 없었으나 FPI의 경우는 크게 변하였으며 pH7.0에서는 FPI가 FPC보다 높은 용해 도를 나타냈다. 냉동건조된 FPC와 FPI는 진공 또 는 강풍건조로 제조한 것보다 용해도가 낮았다.

용해도, 보수력, 분산력, 유화 형성 능력, 보유력(保油力) 등의 기능성을 비교해 본 결과, FPI가 FPC보다 대부분의 기능성이 우수하게 나타났으나 기포 형성능력과 emulsion의 점도는 FPC가 우수하였다.

FPC와 FPI의 Emulsion의 형태는 커다란 차이를 보였으며, FPC emulsion의 지방구는 FPI emulsion 의 경우보다 훨씬 크고, 그 표면에 녹지 않은 단백질 입자를 포집하고 급속히, 유화에 관여하지 않은 수용 액과 분리되는 현상을 나타냈다.

제조시 건조온도를 증가시킬수록, 변성에 의해 대부분의 기능성이 낮아지고 있으나, 보수력은 단백질 구조상의 변화에 의해 높아지는 경향을 보였다.

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