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Improvement of Rheological and Functional Properties of Salmon FPC by Enzymatic Partial Hydrolysis

2. Rheological and functional properties of salmon FPC hydrolysates

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To enhance application of FPC in food industry, salmon FPC hydrolysates were produced and their functional properties were investigated. Hydrolysates of salmon FPC showed high solubilities ranged from approximately 80~95%. Emulsifying activity index (EAI) values of hydrolysates were higher than that of egg white, however sugar ester showed the highest EAI value of 32.5. Though sugar ester had the best emulsifying stability value (ΔEAI) of 9.65, hydrolysates showed better ΔEAI value than that of egg white. Foam Activity of hydrolysates was predominantly better than those of controls and also foam stability value showed similiar trend. Osmolality of hydrolysates was increased with the increase of degree of hydrolysis (DH) and concentration of protein, but it showed very lower osmolality than that of NaCl. Flow property of hydrolysates showed different upward-downward curves, and hysteresis loop increased with the increase of DH. ACE inhibitory activity showed similiar value ranged from 21.1~24% at all DH values.

Key words : salmon FPC, hydrolysate, functional and rheological properties

Introduction

Fish muscle protein is widely recognized as a good nutritious protein source, because it is cheap and easy to prepare, but has limited use in food industry due to its relatively poor protein functional properties such as solubility, emulsifying property and foam activity, etc. Therefore, chemical or enzymatic modification of fish protein has been proposed to improve its functional properties so that it can be used in food industry (Brekke and Eisele, 1981).

Enzymatic hydrolysis has been found effective in improving the functional properties of many proteins, because it does not harm the nutritive value of a protein, is nontoxic and specific, and because the use of enzymes in food is accepted by public. As well as decreases in the size of the protein, enzymatic hydrolysis may alter all of properties of the original molecule such as solubilities, intramolecular bond arrangements and properties of charged groups (Montecalvo JR. et al., 1984).

Many studies have shown the advantage of enzymatic hydrolysis with various protein sources. Fish protein hydrolysates from eviscerated mullet (*Mugil cephalus*) showed slightly lower nutritional value than casein, but it can be used in food industry because it had better solubility and almost same nutritional values (Rebeca et al., 1991). Chobert et al. (1988) reported that the emulsifying capacity of casein hydrolysates increased at the isoelectric point while emulsifying capacity of whey protein hydrolysates increased at alkaline pH, and the emulsifying activity of the hydrolysates was higher than that of casein and whey proteins. Muilangi et al. (1996) reported that hydrolysates of heat-denatured whey protein isolate, which was generated by trypsin, had better solubility, emulsifying properties and foaming properties at all hydrolysis level than that of heat-denatured whey protein.

In this paper, we report the changes in solubilities, emulsifying properties, foaming activities, and other functional properties such as osmolality, angiotensin I-coverting enzyme inhibitory acticity, and rheological properties of salmon FPC hydrolysates during its hydrolysis.

Materials and Methods

1. Preparation of salmon FPC hydrolysates

Salmon FPC was prepared from frozen salmon meat (*Onchorhynchus keta*) by the method of Lee et al. (1978).

After 10 g of salmon FPC was dispersed in distilled water, trypsin (Sigma Chemical Co.) was added to the salmon FPC and the mixture was incubated at 40℃ and at pH 8.0 for 2, 4, 6, 8 hours. The ratio of enzyme : substrate[E/S] was 1 : 100 and pH of solution was adjusted with 0.1 M NaOH before and during hydrolysis. After desired hydrolysis time, the pH of the mixture was adjusted to pH 7.0 with 0.1 M citric acid. The solution was heated 90℃ for 5 min to inactivate enzyme, and then it was centrifuged at 1,200 g for 10 min. The supernatant was filtered and freeze-dried (Freezer dryer Model : SFDSM12, Samwon Co.).

2. Measurement of solubility

Solubility of salmon FPC hydrolysates was determined according to Franzen and Kinsella (1976) with slight modification. Hydrolysate (0.1 g) was dispersed in 15 mℓ of 0.2 M NaCl, and pH was adjusted to 2, 4, 6, 8, 10 with 0.2 M NaOH or 0.2 M HCl. The solution was stirred for 45 min at room temperature and centrifuged at 1,200 rpm for 30 min. The amount of nitrogen of supernatant was measured by micro kjeldhal method. Solubility was calculated as follows.

$$\text{Solubility}(\%) = \frac{\text{Soluble}-\text{N}}{\text{Total}-\text{N}} \times 100$$

3. Emulsifying properties

Emulsifying activity: Emulsifying activity of samples and controls (egg white and sugar ester, Myeong Shin Chem.) was evaluated turbidimetrically according to the method of Pearce and Kinsella (1978) with slight modification. Emulsion of each sample was prepared with 10 mℓ of 1% (w/v) protein and 3.3 mℓ corn oil and then tripricate emulsions were homogenized (Ace-Homogenizer, AM-7) for 30 sec at room temperature. A 0.2 mℓ aliquot of emulsion was diluted (1/250 final dilution) with 49.8 mℓ of 0.1% SDS solution containing 0.1 M NaCl (pH 7.0). Absorbance of the diluted emulsion was measured at 500 nm with Milton Roy spectrophotometer using 0.1% SDS solution containing 0.1 M NaCl (pH 7.0) as a blank. The emulsifying activity was expressed as its emulsifying activity index (EAI) and the EAI was calculated in units of m^2/g as follows.

$$\text{EAI}\,(m^2/g) = \frac{2.303 \times 2 \times A_{500} \times \text{dilution}}{c \times \Phi \times 10{,}000}$$

where the dilution factor is 250, c is the weight of protein or protein equivalent /unit volume of aqueous phase before emulsion formation (g/mℓ=0.01), and Φ is the oil volume fraction (0.25) of the emulsion, and 10,000 is the conversion parameter.

Emulsifying stability: Emulsifying stability was measured accroding to Chobert et al. (1988). The stock emulsions prepared above were held at 20℃ for 24 hrs and then were heated at 80℃ for 30 min. After aliqouts were cooled to room temperature and stirred, turbidity was measured as described above and expressed as EAI (80℃). The emulsion stability was calculated as follows.

$$\Delta\text{EAI}\,(\%) \frac{\text{EAI}\,(\text{max}) - \text{EAI}\,(80℃)}{\text{EAI}\,(\text{max})} \times 100$$

where EAI (max) is the maximum value obtained before storing of emulsion. The smaller the value of ΔEAI (%), the better the stability.

4. Foaming properties

Foaming activity: Foaming activity of hydrolysates and egg white (control) was measured by the method of Sathe and Salunkhe (1981), with slight modification. Foam was formed by whipping the 1% protein solution in homogenizer at 10,000 rpm, for 5 min, at room temperature. The overrun was calculated by the following equation.

$$\%\ \text{overrun} = \frac{\text{Volume of foam} - \text{Volume of protein solution}}{\text{Volume of protein solution}} \times 100$$

Foam stability: was measured by monitering drainage after foam was held for 30 min at 25℃. Foam stability was calculated as follows

$$\text{Foam stability} = \frac{\text{Volume of foam} - \text{Volume of drainage}}{\text{Volume of foam}}$$

5. Osmolality

Osmolality was measured using osmometer (model 5002, Fisher Co.). 2 mℓ of sample solution was pipetted into the sample tube and placed in the cooling chamber

of the instrument, and osmolality was automatically recorded after samples was supercooled and seeded.

6. Rheological properties

Rheological properties of hydrolysates were evaluated using corn plate viscometer (Brookfield DV−II+ C/P, sample volume 2 mℓ). After equilibration at 25℃, samples were sheared at a programmed rate increasing from 0 to 100 s^{-1} (upward flow curve) and subsequently decreasing from 100 to 0 s^{-1} (downward flow curve).

7. Angiotensin I-coverting enzyme (ACE) inhibitory activity

ACE inhibitory activities were evaluated by the method of Cushman and Cheung (1970). For each assay, 100 μℓ of ACE inhibitor (protein solution), 100 μℓ of ACE solution and 100 μℓ of sodium borate buffer (pH 8.3, containing 400 mM NaCl) were mixed and preincubated at 37 ℃ for 5min. 100 μℓ of 12.5 mM Hip-His-Leu was added as a substrate, and the solution was incubated at 37℃ for 1 hr. Then, the reaction was stopped by adding 300 μℓ of 1N HCl (borate buffer was used instead of sample as blank test, and in control test, 300 μℓ of 1N HCl was previously added before ACE solution was added). 1.5 mℓ of ethyl acetate was added and mixed for 15 sec, and then the solutin was centrifuged at 1,200 g for 10 min. 1 mℓ of the supernatant was dried at 140℃ for 20 min. After holding it 5 min at room temperature, 3mℓ of 1M NaCl was added and mixed for 15 sec. The absorbance at 228 nm was measured, and the ACE inhibitory activity was calculated as follows.

$$\text{ACE inhibitory activity} = (1-\frac{A}{B})\times 100$$

where A is sample absorbance and B is blank absorbancy in which control absorbance was subtracted.

Results and discussion

1. Solubility of hydrolysates

Salmon FPC hydrolysates (0.1 g) were dispersed in 0.2 M NaCl (15 mℓ) and pH was adjusted to 2, 4, 6, 8, 10 with 0.2 M HCl or 0.2M NaOH to determine the solubility as a function of pH. The results are shown in Fig. 1.

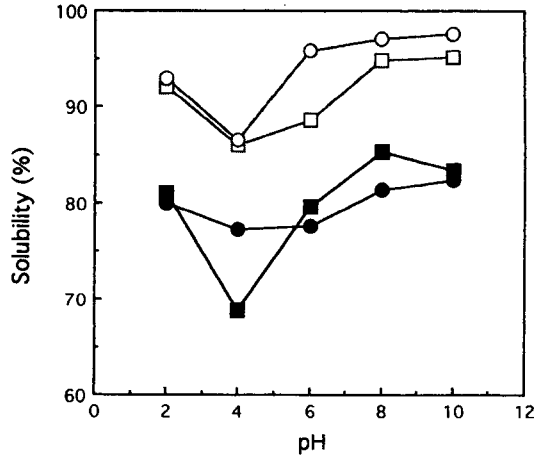

Fig. 1. Solubility of enzymatic hydrolysates of salmon FPC as a function of pH. Hydrolysis was performed at 40℃, pH 8 with trypsin and [E/S]=1 : 100
−■− Hydrolysis time: 2 hrs
−●− Hydrolysis time: 4 hrs
−□− Hydrolysis time: 6 hrs
−○− Hydrolysis time: 8 hrs

Protein solubility is one of the most important factors when protein functionality is evaluated, because it depends to a great extent on the amount of soluble protein present in the system (Kinsella, 1976). In this experiment, the results showed that salmon FPC hydrolysates were soluble over 70% in 0.2 M NaCl solution. Solubility of hydrolysates tended to increase with increase in the degree of hydrolysis (or hydrolysis time). After 8 hrs hydrolysis the solubility was nearly 100% over pH 6. This trend indicated that increase in the solubility was attributed to the increase of low molecular weight peptides and the increase of ionizable groups during hydrolysis. Similiar findings were reported for hydrolysates of heat-denatured whey protein isolate with various proteases (Mutilangi, 1996). Around pH 4, solubility was slightly lower than other pH range, so that one may concluded that pI (isoelectric point) of salmon FPC hydrolysate is pH 4. In the high pH range, solubility value of hydrolysates was steadily increased in every cases. This was probably due to the fact that the net charge of pretein was negative and solubility was enhanced at above the isoelectric point. Because most applications in food system require good solubility, salmon FPC hydrolysate would be a good ingredients in this point of view.

2. Emulsifying properties

Emulsifying Activity: Emulsifying properties are useful functional parameters which play an important role in food system and for food application (Aoki et al., 1989). In the formation of emulsion, the protein molecules diffuse to and are absorbed at the oil-water interface and finally stabilize the interface. In this experiment, emulsion were prepared from salmon FPC hydrolysates and from controls (egg white and sugar ester), and the emulsifying activity index (EAI) was photometrically measured by a turbidimetric technique at 500 nm so as to examine the possibility of applicaion as a food ingredient. And the results are shown in Fig. 2.

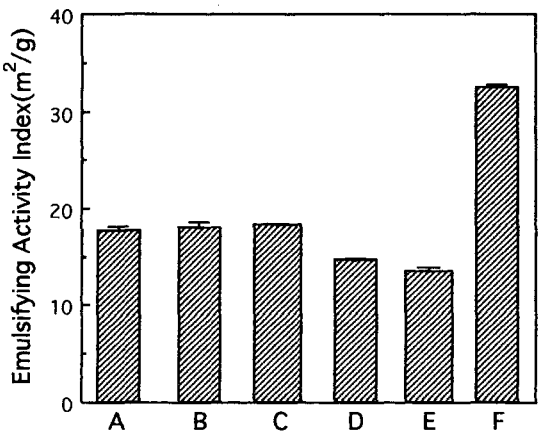

Fig. 2. Effect of hydrolysis of salmon FPC on the emulsifying activity index (EAI) compared with controls.
A : hydrolysis time; 2 hrs
B : hydrolysis time; 4 hrs
C : hydrolysis time; 6 hrs
D : hydrolysis time; 8 hrs
E : Egg white
F : Sugar ester

The EAI value of hydrolysates tended to slightly increase until 2~6 hrs, from 17.7~18.3m^2/g, then it decreased at 8 hrs as 18.3. Although sugar ester had the highest EAI value of 32.5 among samples, all hydrolysates had higher EAI values (14.6~18.3) than that of egg white (13.5). It is known that FPC had poor emulsifying activity, so it is obvious that enzymatic hydrolysis improve their emulsion property. Chobert et al. (1988) reported that hydrolysates of casein and whey protein concentrate (WPC) digested with trypsin, improved their emulsifying activity compared to the controls. In case of casein hydrolysate, the increase of EAI was significant compared to the control casein at pH 2.0. But in case of WPC, in the ranges pH 1.0~3.5 and 5.0~11.0, the EAI values showed a large big increase compared to control whey protein. And also they observed that EAI value of trypsin-treated casein increased when degree of hydrolysis (DH) increased until the optimum DH and then decreased. Similiar trend was observed in our experiment.

Similiar finding was reported by Mutilangi et al. (1996). They reported that enzymatic hydrolysis of heat-denatured whey protein (HDWPI) increase the EAI value for 3~10 m^2/g, and especially EAI values of trypsin hydrolysates were generally higher than those of other hydrolysates such as hydrolysis from chymotrypsin, alcalase and neutrase treatment. Several reasons were suggested to explain why hydrolysis of protein increases the emulsifying activity. Hydrolysis of protein increases the number of charged groups because extra terminal carboxyl and amino groups are generated. It causes a decrease in molecular weight, increase in hydrophilicity, and a change in molecular configuration. All these changes affect the solubility of the modified protein, and afterall, it changes emulsifying properties (Arai and Watanabe, 1988; Chou et al., 1985; Nakai and Li-Chan, 1988). In conclusion, we assumed that salmon FPC hydrolysates could be applied in food system such as sausages, soups and cakes in which emulsifying property is very important.

Emulsifying stability (ES): After 24 hrs storage and heating of the emulsion, ES was measured. Sugar ester had the best ES value (9.65), and hydrolysates (2, 6 hrs) had lower ES values than that of egg white while salmon FPC has higher ES value at 4, 8 hrs of hydrolysis (Fig. 3).

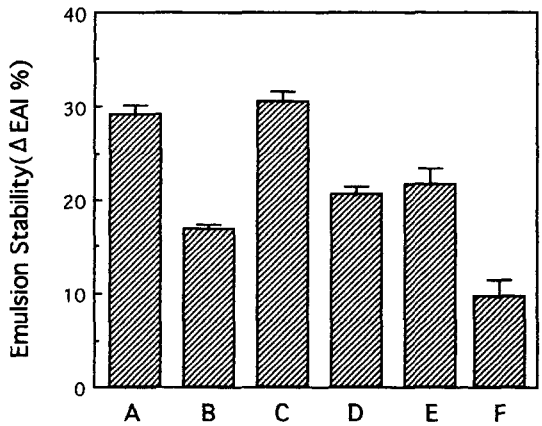

Fig. 3. Emulsion stability of salmon FPC hydrolysates and control after 24 hr at room temp. and 30 min at 80℃.
A : hydrolysis time; 2 hrs
B : hydrolysis time; 4 hrs
C : hydrolysis time; 6 hrs
D : hydrolysis time; 8 hrs
E : Egg white
F : Sugar ester

There are some reports showing that solubility of protein was related with emulsifying properties, but other factors affect the emulsifying properties such as adsorption kinetics, interfacial load, reduction of oil/water interfacial tension, rheology of the interfacial film, and surface hydrophobicity of protein (Das and Kinsella, 1991).

3. Foaming properties

Foaming activity (FA): Foaming property is of importance in food system since it endows food with unique physical property, controls textural property of food, and eventually determines the shape of food. Foams are mostly generated by protein, and protein must be capable of migrating rapidly to the air-water interface, unfolding and rearranging at the interface to have a good foaming property (Halling, 1981). Foams are commonly generated by whipping, injection, and sparging (bubbling) or shaking, and foam stability is commonly measured as drainage or collapse of foam (Mangino. 1994; Phillips et al., 1994).

In this study, foams were generated by whipping the protein dispersion (hydrolysates) in a homogenizer because this method was accord best with industrial process (Halling, 1981). And their results are shown in Fig. 4. Generally, hydrolysates of salmon FPC showed much higher FA values (346.6~433.3) than those of controls (40, 151.6). And value of FA was increased with the increase of degree of hydrolysis (DH), and reached the maximum value of 433 (%) at 8 hrs of hydrolysis. Dickinson (1985) suggested that FA of a protein tended to be influenced by its flexibility, hydrophobicity and average molecular weight. Mutilangi (1996) reported that foams were generated mainly by peptides less than molecular weight of 10,000 in hydrolysates of HDWPC. Similiar trend was found in our study, hydrolysis time was positively correlated with FA value, which indicated that the lower molecular weight the hydrolysate, the better the FA value.

Foaming stability (FS): After foam was held 30 min at room temperature, drainage was measured. Foam generated from hydrolysates showed more stable FS value than controls (Fig. 5). No significant differences were observed in FS value of hydrolysates according to their hydrolysis time, which ranged from 0.32~0.33. FS is known to be influenced by the nature of the generated peptides and their interactions in the protein film at the interface. It was obvious that DH did not have correlationship with FS value but it is predominant that hydrolysis of salmon FPC improved its foaming properties.

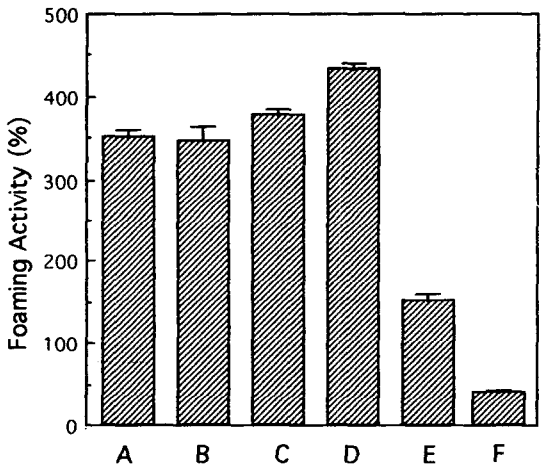

Fig. 4. Foaming activity of salmon FPC hydrolysates and controls.
A : hydrolysis time; 2 hrs
B : hydrolysis time; 4 hrs
C : hydrolysis time; 6 hrs
D : hydrolysis time; 8 hrs
E : Egg white
F : Sugar ester

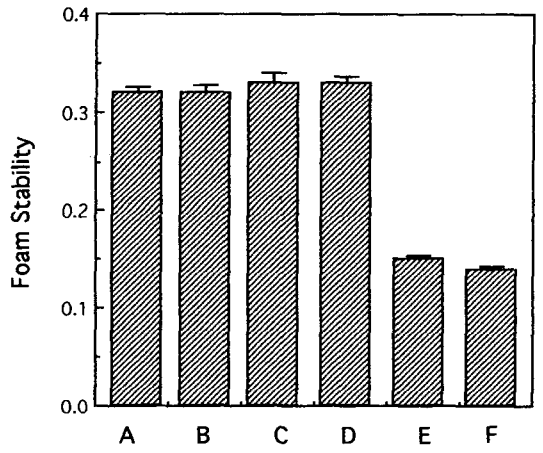

Fig. 5. Foam stability of salmon FPC hydrolysates and controls.
A : hydrolysis time; 2 hrs
B : hydrolysis time; 4 hrs
C : hydrolysis time; 6 hrs
D : hydrolysis time; 8 hrs
E : Egg white
F : Sugar ester

4. Osmolality

Osmolality is defined as the number of osmoles of solute per 1,000 grams of solvent, and osmoles represent a measure of the osmotically effective amount of solute in a solution.

With feezing point depression osmometry, osmolalities of hydrolysates were measured and the results are shown in Fig. 6. Osmolality increased with the increase of concentration of protein. Matsubara et al. (1994) reported that the osmolality of coelomic fluid was much lower than that of serum in same species of salmon, and they considered that this seemed to occur by the scarce amounts of protein in coelomic fluid, which indicated that the higher the protein concentration, the higher the osmolality. The DH also positively correlated with osmolality. This was probably due to the generation of small peptides during hydrolysis, which resulted in the increase of the amount of osmoles.

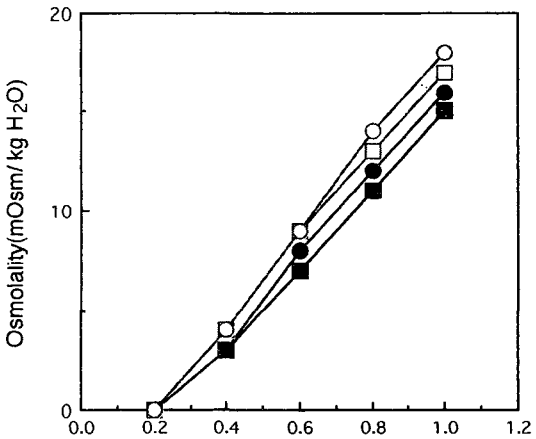

Fig. 6. Osmolality of salmon FPC hydrolysates in different protein concentration.
—■— Hydrolysis time: 2 hrs
—●— Hydrolysis time: 4 hrs
—□— Hydrolysis time: 6 hrs
—○— Hydrolysis time: 8 hrs

Osmolality play important role in food system since it induce a diarrhea, a collapse of balance of ions in the human body and a nauseation if it is extreamly high in body fluids (Macburney, 1984). Osmolality of 1% NaCl determined by trial runs was 326 mosmol/kg H_2O, which was much higher than that of hydrolysates. So, we thought that hydrolysates can be applied in food system without any side-effect.

5. Rheological properties

The flow property of protein dispersions is of importance in food processing (Ma, 1993) and closely related to the textural quality of products (Hermansson, 1975). It provides essential information for optimal process design of unit operation such as pumping, mixing, heating and cooling and spray drying (Tung, 1978).

The flow behavior of salmon FPC hydrolysates (0.5%) was evaluated measuring shear rate and shear stress from 0 to 100 rpm. The results are shown in Fig. 7. Although similiar flow properties were observed at different hydrolysis time, hysteresis loop (textural breakdown) tended to increase with the increase of hydrolysis time. Hysteresis loop, which is typical phenomenon occurs in newtonian flow when the fluid is warmed, showed the great difference between 2 hrs and 8 hrs hydrolysis, since the upward and the downward flow curve did not overlap at 8 hrs hydrolysis. We assumed that this trend probably due to the weakness of peptide bonds which was effected by enzyme during hydrolysis.

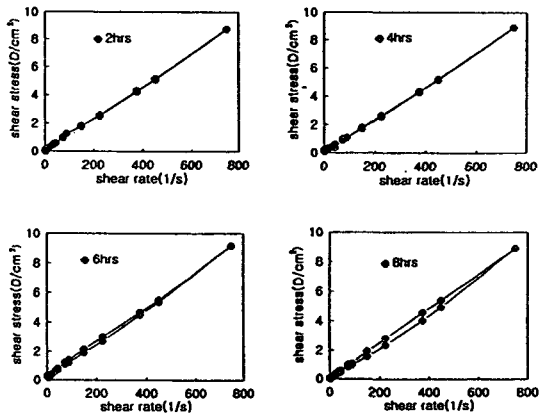

Fig. 7 Typical upward and downward flow curves for salmon FPC hydrolysates (0.5%).

6. Angiotensin I-coverting enzyme (ACE) inhibitory activity

Proteins from natural resources are known to have a physiological function which can inhibit action of Angiotensin I-converting enzyme (ACE: peptidyl dipeptidase, E. C. 3.4.15.1), which catalyzes both the production of angiotensin II and the inactivation of bradykinin. They

involve in blood pressure regulation (Maruyama et al., 1987). Table 1 shows the ACE inhibitory activity of salmon FPC hydrolysates. There was no significant difference in ACE inhibitory activities and it ranged from 21~24%. Matsui et al. (1993) reported that inhibitory activity of hydrolysates from sardine muscle increased with increasing proteolysis time, and peptides generated during hydrolysis contributed the activity. Although our data are not sufficient and results show relatively lower activity, we thought that hydrolysates had considerable physiological function.

Table 1. Angiotensin I-converting enzyme (ACE) inhibitory activity of salmon FPC hydrolysates

Hydrolysis time	2 hrs	4 hrs	6 hrs	8 hrs
ACE inhibitory activity (%)	21.1*	21.8	23.6	24.0

* Values are measured at protein concentration of 100 μg/mℓ

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