Effect of Heating Condition on Protein Quality of Retort Pouched Fish Meat

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Effect of heat processing on fish meat in retort pouches has been examined in connection with its protein quality. Bigeye tuna and halibut meat were heat-processed at two different temperatures (115°C and 124°C) varying in F_0 values from 8 to 32. At the F_0 value of 8 or 21, non-specific degradation of peptide bonds in the fish meat proteins was observed by electrophoresis to occur more slightly at 124°C than at 115°C and their amino acid compositions were not significantly changed during the heat processing.

Glucose, glucose-6-phosphate, available lysine and *in vitro* digestibility of the fish meat were also determined in order to reveal the changes of protein quality from the standpoint of amino-carbonyl reaction. Losses of glucose, glucose-6-phosphate and available lysine followed the first order reaction kinetics during the heat processing, but were smaller at 124°C than at 115°C when compared at equivalent F_0 values. On the other hand, *in vitro* digestibility of the fish meat decreased only slightly in the course of heat processing.

From these combined results, it was concluded that when compared at the equal lethality, the protein quality of heat-processed fish meat in retort pouches could be retained more effectively at 124°C than at 115°C and that at least no remarkable loss in protein quality was observed under the heat processing at 124°C with $F_0=8$.

The flexible laminated retort pouch which withstands thermal processing has a number of advantages compared to conventional metal cans and glass containers. It has been shown that the alteration of heating temperature or retort structure does not significantly affect the heating time required to gain a given lethality in conduction-heating foods.¹⁾ On the contrary, modification in container geometry is more effective in reducing the heating time.²⁾ Retort pouched foods usually have a slab-type shape, so that the surface to volume ratio of retort pouch is much higher than that of metal or glass counterpart of equal volume. Therefore, the retort pouch requires less energy in container manufacture, delivery, food processing, transportation, and storage. Additionally, retort pouched foods generally exhibit high retention of flavor, color, texture, and nutrient content.³⁾

There has been few studies on the retort pouched seafoods in terms of their quality.⁴⁻⁶⁾ In this study, the effect of heating on the quality of fish meat in retort pouches is examined especially from the standpoint of protein quality.

Materials and Methods

Materials and Heat Processing

Bigeye tuna *Paratunnus sibi* and halibut *Hippo-*

glossus stenolepis were used throughout this study. The sliced meat with the thickness of about 8 mm (weight: about 100 g) was vacuum-packed in flexible retort pouches (hiRP-F, 13×17 cm, Toyo Seikan Kaisha, Ltd.) and heat-processed to the core temperature of 115°C or 124°C with an F_0 value (F_{121}^{10}) ranging from 8 to 32. For the heat processing, the simulator retort (H60-C50-120/150-SWAR, Toyo Seikan Kaisha, Ltd.) was used. Heat processing conditions employed in this study are given in Table 1.

Analytical Procedures

To prepare the samples for SDS-polyacrylamide gel electrophoresis and amino acid analysis, 20 g of raw or heat-processed fish meat was first homogenized in 120 ml of ethanol and centrifuged at 5,000 rpm for 6 min. The resulting precipitate was again homogenized in 120 ml of 80% ethanol and centrifuged to recover the protein fraction. This treatment with 80% ethanol was repeated to remove non-protein components. The residue thus obtained was defatted and dehydrated by immersing in the mixture of acetone and ether overnight and then dried in air to prepare powdered protein samples.

Electrophoresis was carried out, in principle, according to the method of Weber and Osborn⁷⁾

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Table 1. Heat processing conditions

F ₀	Heating time (min)	
	115°C	124°C
8	60	3.5
12	83	—
21	143	12.5
32	—	18

on 2.5% polyacrylamide gel in 0.02 M phosphate buffer (pH 7.2) containing 0.1% SDS and 3.5 M urea at 8 mA/gel for 3–4 h. The powdered protein sample (10 mg) was dissolved in 4 ml of 0.02 M phosphate buffer, pH 7.2, containing 1% SDS, 6 M urea and 0.02 M dithiothreitol. After electrophoresis, the gels were stained with Coomassie Brilliant Blue and destained with 10% acetic acid containing 10% isopropyl alcohol.

Amino acid composition was determined with a single-column system on a JEOL amino acid analyzer (JLC-3BC). The hydrolysis was carried out by the method of Simpson *et al.*⁸⁾ in order to analyze all constituting amino acids including tryptophan and cysteine simultaneously. Five mg of samples were hydrolyzed *in vacuo* in 2 ml of 4 N methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole at 115°C for 22 h. Cystine and cysteine were determined as S-sulfocysteine by treating the hydrolyzate with dithiothreitol followed by an excess of tetrathionate. The hydrolyzate thus treated was dissolved in 20 ml of citric acid buffer (pH 2.2) for amino acid analysis (sample load: 1 ml).

Available lysine, as an index of protein quality, was measured by the method of Kakade and Liener⁹⁾ using 2,4,6-trinitrobenzenesulfonic acid. *In vitro* protein digestibility of raw or heat-processed fish meat was determined according to the modified method of Akeson and Stahmann.^{10,11)} A sample meat (500 mg) was incubated with 15 ml of 0.1 N HCl containing 4.5 mg pepsin at 37°C for 3 h. After neutralization of the pepsin digest with 5 ml of 0.2 N NaOH, 10 ml of 0.1 M phosphate buffer (pH 8.0) containing 6 mg pancreatin and 2 mg thimerosal were added and incubated at 37°C for additional 24 h. The digestion was terminated by the addition of 20% trichloroacetic acid (10 ml). The digest was then filtered through a No. 5C filter paper (Toyo Roshi Co., Ltd.) and total nitrogen in the filtrate was determined by the Kjeldahl method.

For the determination of glucose and glucose-6-phosphate, samples were homogenized with 6%

perchloric acid according to the procedure of Tarr.¹²⁾ Free glucose in the perchloric acid extracts (neutralized with 20% KOH solution) was measured by the Glucostat reagent (Worthington Chemical Corp.). Free glucose-6-phosphate in the perchloric acid extracts was enzymatically assayed by Hohorst's procedure.¹³⁾

Results and Discussion

Electrophoretic Pattern and Amino Acid Composition of Fish Meat Proteins

Protein samples prepared from raw fish meat were first examined by SDS-polyacrylamide gel electrophoresis (F₀=0, Fig. 1). The major components of myofibrillar proteins such as myosin heavy chain (200 K), actin (43 K), and tropomyosin (34 K) were detected as clear bands, but distinct differences in electrophoretic patterns were not observed between bigeye tuna and halibut. When heat-processed in retort pouches, raw fish meat was coagulated as a result of heat denaturation leaving the exudate which corresponded to 20–25% of the fish meat weight. Since the heat-processed meat was completely dissolved in SDS-urea solution, their electrophoretic patterns were also examined to elucidate the changes of protein components due to heating.

In the case of 115°C heating, electrophoretic patterns of bigeye tuna and halibut meat proteins were quite similar to each other (Fig. 1) in the bands of myosin heavy chain disappeared at any F₀ value examined in this study. Bands corresponding to actin and tropomyosin decreased with the increase of F₀ values; at F₀=21, they were only faintly observed. On the other hand, the whole gels were stained with Coomassie Brilliant Blue, indicating that a number of peptide fragments with different molecular weights were formed during heat processing as a consequence of non-specific degradation of peptide bonds. Moreover, bands for extremely high molecular weight substances were also detected at the top of 2.5% polyacrylamide gels. It is assumed that those substances which could not even penetrate into the gels would be polymers resulting from the recombination of heat-degraded peptide fragments.

In the case of 124°C heating, the degree of fish meat protein degradation was smaller as compared with that of 115°C heating, because the processing time at 124°C required to attain the equal lethality was far shorter than at 115°C processing (Table 1). It is clear from Fig. 1 that both bigeye tuna

Fig. 1. SDS-polyacrylamide gel electrophoresis patterns of raw and heat-processed fish meat proteins.

Table 2. Amino acid composition of raw and heat-processed meat proteins from bigeye tuna and halibut (g amino acid/100 g protein)

Amino acid	Bigeye tuna					Halibut				
	115°C					124°C				
	Raw	F ₀ 8	F ₀ 21	F ₀ 8	F ₀ 21	Raw	F ₀ 8	F ₀ 21	F ₀ 8	F ₀ 21
Asp	11.8	11.9	12.0	12.0	12.1	12.0	12.0	12.0	12.4	12.3
Thr	5.5	5.6	5.5	5.7	5.6	5.5	5.4	5.4	5.6	5.6
Ser	4.9	4.8	4.7	5.0	4.8	5.1	5.0	4.7	5.1	5.1
Glu	17.4	17.3	17.1	17.3	17.2	17.6	17.1	17.1	18.0	17.5
Pro	4.4	4.1	4.2	4.1	4.2	3.7	3.9	3.8	3.8	3.9
Gly	4.8	5.1	5.1	5.0	5.1	4.9	4.9	5.1	4.7	4.7
Ala	7.1	7.2	7.3	7.2	7.2	6.8	6.9	6.8	6.7	6.6
Cys	1.5	1.3	1.3	1.3	1.3	1.5	1.5	1.3	1.5	1.5
Val	6.4	6.6	6.6	6.3	6.4	6.6	6.8	7.0	6.4	6.4
Met	3.3	3.5	3.3	3.5	3.3	3.4	3.3	3.3	3.4	3.5
Ile	5.4	5.5	5.5	5.4	5.5	5.5	5.8	5.7	5.4	5.5
Leu	10.0	10.0	10.0	10.0	10.0	10.0	10.0	9.9	10.1	10.0
Tyr	4.7	4.7	4.9	4.7	4.7	4.8	4.8	4.8	4.7	4.8
Phe	5.0	4.9	4.9	5.0	5.2	5.1	5.3	5.3	5.0	5.0
Try	1.8	1.8	1.8	1.7	1.7	1.8	1.8	1.8	1.6	1.8
Lys	11.2	11.2	11.1	11.0	11.2	11.2	11.0	11.2	11.4	11.4
His	3.6	3.6	3.7	3.7	3.5	3.2	3.2	3.6	3.0	3.0
Arg	7.4	7.3	7.3	7.4	7.3	7.4	7.6	7.4	7.4	7.5
Total	116.2	116.4	116.3	116.3	116.3	116.1	116.3	116.2	116.2	116.1

and halibut proteins heated at $F_0=8$ have a clear band for myosin heavy chain and that the formation of extremely high molecular weight substances detected at the top of gels is not so appreciable. At $F_0=21$ and 32, a myosin heavy chain band mostly disappeared; the electrophoretic patterns of both fish proteins heated at 124°C closely resembled

those at 115°C.

Table 2 presents amino acid composition of raw and heat-processed fish meat proteins. Although slight differences were noted for individual amino acids, the overall compositions did not significantly vary before and after heat processing. Taking into account the differences in portions

Fig. 2. Changes in glucose content in bigeye tuna and halibut meat during thermal processing.

- : bigeye tuna at 115°C
- : halibut at 115°C
- : bigeye tuna at 124°C
- : halibut at 124°C

of fish meat used and the accuracy of analysis, it can be said that the degradation of amino acids due to heating at 115°C or 124°C was negligible.

It has already been reported that non-specific degradation of peptide bonds and formation of extremely high molecular weight substances took place in fish meat products which were heat-processed at high temperatures of 100°C–120°C for a prolonged period.^{14–16)} The present study confirmed this observation and also revealed that myosin heavy chain was less stable to heat than actin and tropomyosin. Further, the heat processing at 124°C with $F_0=8$ is noteworthy from the viewpoint of protein degradation; an appreciable amount of myosin heavy chain remained and the formation of extremely high molecular weight substances was not significant.

Protein Quality from the Standpoint of Amino-carbonyl Reaction

It is well known that severe thermal processing results in decreased protein quality of foods due to the amino-carbonyl reaction, so-called Maillard browning reaction.^{17,18)} Since the amino-carbonyl reaction is attributed to the reaction between reducing sugars and proteins or amino acids, the

Fig. 3. Changes in glucose-6-phosphate content in bigeye tuna and halibut meat during thermal processing.

- : bigeye tuna at 115°C
- : halibut at 115°C
- : bigeye tuna at 124°C
- : halibut at 124°C

contents of typical reducing sugars, glucose and glucose-6-phosphate in fish meat were examined to reveal the extent of this reaction.

The glucose content of raw bigeye tuna was 1.70–1.81 mg/g meat (dry base), which was apparently lower than 2.23–2.33 mg/g of raw halibut meat. Changes in glucose contents in bigeye tuna and halibut during thermal processing are shown in Fig. 2. It is evident from this figure that concomitant with the decrease of glucose content was an increase in the sterilization effect and that the degree of glucose loss was higher at 115°C than at 124°C when compared at the equivalent F_0 value. The loss of glucose content followed the first order reaction kinetics at both 115°C and 124°C. At 115°C heating, the loss rate constants of glucose in bigeye tuna and halibut meat were 1.34×10^{-2} and 1.36×10^{-2} (min^{-1}), respectively. This result showed that the rate of glucose loss was almost the same in both fish meat at 115°C, though their initial glucose levels were somewhat different. At 124°C heating, the loss rate constant of bigeye tuna was 4.97×10^{-2} (min^{-1}) and that of halibut 2.95×10^{-2} (min^{-1}), indicating that glucose was lost faster in bigeye tuna at 124°C processing than at 115°C. However, it is obscure why this fish species difference in glucose loss rates occurred at 124°C.

Table 3. Changes in available lysine and *in vitro* protein digestibility of bigeye tuna and halibut meat during thermal processing

Fish	Heating temperature	F ₀	Available lysine (μg/mgN)	Protein digestibility (%)
Bigeye tuna	115°C	0	641	96.8
		8	598	95.7
		12	580	94.2
		21	532	90.8
		32	452	94.9
	124°C	0	634	96.7
		8	592	96.2
		21	510	95.9
		32	452	94.9
		32	452	94.9
Halibut	115°C	0	604	96.1
		8	559	94.9
		12	541	94.0
		21	513	93.0
	124°C	0	595	96.1
		8	559	96.4
		21	490	93.4
		32	426	94.0

The glucose-6-phosphate content of raw bigeye tuna was around 150 μg/g (dry base), while that of raw halibut meat was about 240 μg/g. Overall changes of glucose-6-phosphate content during the thermal processing are quite similar to those of glucose as shown in Fig. 3. The loss rate constants of glucose-6-phosphate at 115°C heating were 1.32×10^{-2} (min⁻¹) for bigeye tuna and 1.39×10^{-2} (min⁻¹) for halibut, which were very close to those of glucose. On the contrary, at 124°C processing that for bigeye tuna was 9.70×10^{-2} (min⁻¹) and halibut 7.17×10^{-2} (min⁻¹). It is noteworthy that the loss rate constant of glucose-6-phosphate at 124°C was approximately double that of glucose; these results may indicate that the activation energy for glucose-6-phosphate loss is larger than that for glucose loss. Moreover, it is obvious that the loss of glucose-6-phosphate was larger at the lower processing temperature (115°C) when compared at the equal lethality (Fig. 3). From the changes of glucose and glucose-6-phosphate contents in fish meats, it seems likely that amino-carbonyl reaction was initiated during the thermal processing.

Protein sources processed for long of time often lose their nutritional value due to the amino-carbonyl reaction, which involves the interaction of reducing sugars with the ε-amino group of lysine. This reacted lysine is unsusceptible to *in vitro* enzymatic hydrolysis and thus becomes nutritionally unavailable. Therefore the available

lysine content in processed foods can be an appropriate indicator of protein quality.^{19,20)} Available lysine contents of bigeye tuna and halibut meat are given in Table 3. Both fish meat contained comparable amounts of available lysine. During the thermal processing, available lysine was lost following the first order kinetics. The loss rate constants of available lysine were 1.30×10^{-3} (min⁻¹) at 115°C and 1.88×10^{-2} (min⁻¹) at 124°C for bigeye tuna; 1.14×10^{-3} (min⁻¹) at 115°C and 1.86×10^{-2} (min⁻¹) at 124°C for halibut. Those values were fairly smaller than those for losses of glucose and glucose-6-phosphate. This is because glucose and glucose-6-phosphate react with other reactive amino acid residues such as histidine and arginine during the course of amino-carbonyl reaction. Although the loss rate constants of available lysine were larger at 124°C than at 115°C, its loss was not, on the whole, significant during the thermal processing. For instance, about 84% of available lysine was retained even after the sterilization at F₀=21 (115°C), and about 71% at F₀=32 (124°C) in both fish meat. Since fish meat generally contains a fairly large amount of available lysine, this level of loss is not probably serious from the standpoint of protein nutrition.

In vitro protein digestibilities of fish meat are also shown in Table 3. Though it is known that digestibility of proteins is generally lowered by the development of amino-carbonyl reaction, *in vitro* digestibility of bigeye tuna and halibut meat did not only vary significantly during the thermal processing, but also was not influenced by the processing conditions. This observation is quite similar to the finding of Tanaka *et al.*²¹⁾ on canned fish products. Thus, it is assumed that the amino-carbonyl reaction taken place during sterilization is still at the initial stage.

These composite results have demonstrated that the protein quality of heat-processed fish meat in retort pouches can be retained more effectively at 124°C than at 115°C when compared at the equal lethality. At least, under the heat processing at 124°C with F₀=8 which has the sufficient sterilization effect for foods, there appeared no remarkable loss in the protein quality of fish meat.

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