Fisheries Science 65(4), 588-591 (1999)

Recovery of Amino Acid from the Protein in the Head and Viscera of Frigate Mackerel by Autolysis*

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(Received July 27, 1998)

The digestive conditions were investigated to recover amino acids and peptides from the protein by autolysis from the viewpoint of the effective utilization of the wastes of frigate mackerel.

Protein in the head-viscera mixture was efficiently and easily autolyzed at 15°C for 24 h at neutral pH. Eighty-seven percent of protein in the mixture were recovered as extractive nitrogen and the autolytic extract obtained was rich in free amino acids and peptides. The hot water extract obtained from the autolyzate had umami taste. These results suggested that this autolytic extract can be used as a seasoning material.

Key words: frigate mackerel, wastes, autolysis, protein, amino acid

The development of traffic network and the advanced technology to transport food at low temperature have made it possible to transport fish as fillet to the urban area after filleting the fish in processing plant near the fishing port. Recently, such transportation network has grown rapidly in Shikoku Island, Japan.

In processing plants, the head and the viscera of fish are automatically cut off from the trunks and are disposed of as fish wastes or used for the production of fish meal and fertilizer. The fish wastes are rich in nutrients such as lipid and protein. A better utilization of the fish waste is required not only from the viewpoint of effective utilization of the fish waste, but also to prevent the environmental pollution.

In a previous study,[1] we examined the seasonal variations of lipid content and fatty acid composition of the lipid from frigate mackerel from the viewpoint of the effective utilization of fish by-products. We found that the head portion, which accounted for about 40% of fish by-product, contained 15.1% crude protein and 11.7% crude fat. Most of the proteins in the head portion are presumed to be water-insoluble proteins such as myofibrillar and stroma proteins and are difficult to collect. These protein fractions would be easily recovered as amino acids by proteolysis. Miyake[2-5] reported the solubilization of fish scraps by enzyme treatment. In those studies, Miyake used the commercial enzymes for proteolysis. However, the high cost of commercial enzymes makes their use economically unviable for the proteolysis of the fish by-products. As suggested by Mohr,[6] autolysis may provide the most promising way to produce fish protein concentrate rather than proteolysis by using commercial enzymes. Therefore, we made attempts to recover amino acid from the protein in the fish head and viscera by autolysis. This method would be advantageous to the effective utilization of fish by-products. In this study, the autolysis conditions of wastes from frigate mackerel were examined.

Materials and Methods

Materials

Frigate mackerel *Auxis rochei* was purchased from the Kochi central wholesale market. Head and viscera were excised from the fish and minced with knife. The sexual organ and the content of the digestive organ were removed from viscera before mincing. Minced head and viscera were kept at −80°C before use.

Fractionation of Protein

Ten grams of the minced sample (head, viscera) were homogenized with 50 m*l* of distilled water. The homogenate was centrifuged at 10,000 × *g* for 15 min and this step was repeated twice. The supernatants were combined and used for the determination of water-soluble nitrogen and non-protein nitrogen. Non-protein nitrogen was prepared from the supernatant by adding an equal volume of 10% trichloroacetic acid (TCA) solution. The final precipitate was homogenized with 10 m*l* of 0.1 N NaOH and stirred overnight in cold room at 5°C. After stirring, the homogenate was centrifuged at 10,000 × *g* for 15 min. This extraction process was repeated and the supernatants were combined, and was used for the determination of alkali-soluble protein nitrogen. Nitrogen content of each fraction and total nitrogen of the sample was determined by the Kjeldahl method. The content of water-soluble protein nitrogen was estimated by subtracting the content of non protein nitrogen in TCA extract from the content of water soluble nitrogen.

Autolysis Procedure

Minced head and viscera were mixed in the ratio 3:1(w/w) and the mixture was homogenized with 2 volumes of ice

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deionized water. The homogenate was transferred to a 100 m*l* Erlenmeyer flask and incubated in water bath at fixed temperature with shaking. After incubation, 10 g of the autolyzate was added to 10 m*l* of 10% TCA solution to stop the further autolysis and homogenized at 10,000 rpm for 5 min. The TCA solution obtained was kept for 30 min at room temperature and centrifuged at 10,000×*g* for 20 min. The supernatant was used for the determination of nitrogen content and amino acid composition. Nitrogen content was determined by the same method as mentioned above. To check the taste of the autolytic extract, autolysis was terminated by boiling for 10 min instead of adding the 10% TCA solution. After boiling, the autolyzate was centrifuged at 10,000×*g* for 20 min. The supernatant obatined was used for checking the taste.

Determination of Amino Acid Composition of the Extract

A portion of TCA extract was hydrolyzed in 6 N HCl at 110°C for 24 h to determine the total content of free and bound amino acids. The content of bound amino acid in TCA extract was estimated by subtracting the content of free amino acid in TCA extract from the total content of amino acid in the hydrolyzed one. Amino acids were determined by an automatic amino acid analyzer (Hitachi-model 835).

Results and Discussion

Fractionation of Protein in Head and Viscera

The content of protein in the fish head and viscera was examined prior to the determination of autolysis conditions. As reported previously,[1] the head and viscera portions in frigate mackerel accounted for 21% and 8% of whole body weight, respectively. The head portion contained about 15% crude protein and 12% crude fat and the viscera contained about 21% crude protein and 5% crude fat. These results indicated that head portion, which accounted for about 40% of fish wastes, is rich in nutrients, such as protein and lipid. Table 1 shows nitrogen content in the head and viscera. The head contained 8.7% non-protein nitrogen and 91.3% protein nitrogen. The alkali-soluble protein nitrogen, which is assumed to be derived from muscle protein, accounted for 24% of the total nitrogen. This result indicated that a large quantity of the muscle protein was contained in the head. Most of the muscle proteins in the head are presumed to be water-insoluble protein and are difficult to collect. However, the degradation of fish head muscle protein by proteinase would facilitate the recovery of amino acid from the protein. In general, fish viscera have a high proteinase activity.[7-9] Attempts were made to recover amino acid from the protein in the head and viscera by proteolysis.

Effect of Temperature on the Autolysis

The effect of temperature on the autolysis of the head and viscera mixture was examined. Fish digestive proteases have high activity at low temperature.[10] In this experiment, therefore, the reaction temperature was set at low temperature between 10 to 25°C at 5°C intervals. The results are shown in Fig. 1. The ordinate shows the increase in non-protein nitrogen content. The nitrogen content in the extract before autolysis was defined as 100%.

Table 1. Fraction of protein in the head and viscera from frigate mackerel

Portion	Non-protein nitrogen	Protein nitrogen			Total nitrogen
		Water-soluble fraction	Alkaline-soluble fraction	Alkaline-insoluble fraction	
Head	226*1	885	624	864	2599
	(8.7)*2	(34.1)	(24.0)	(33.2)	(100.0)
Viscera	1575	968	359	304	3206
	(49.1)	(30.2)	(11.2)	(9.5)	(100.0)

*1 mgN/100 g.
*2 Percentage.

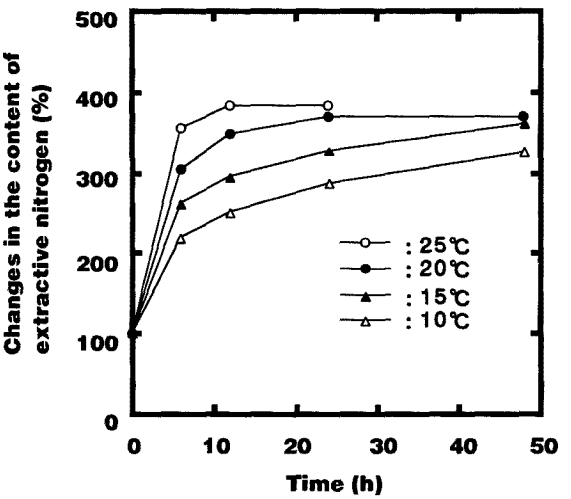

Fig. 1. Effect of temperature on the autolysis efficiency of the mixture of head and viscera from frigate mackerel.

The nitrogen content of all autolyzates increased rapidly up to 6 h incubation. The nitrogen content of autolyzate at 25°C reached the maximum value after 12 h incubation and thereafter remained constant. The autolyzate at 25°C developed a weak foul smell after 24 h incubation, suggesting the propagation of microorganisms. The extractive nitrogen of autolyzate at 20°C increased slowly after 6 h incubation, reaching the maximum value at 24 h incubation. The autolyzate at 20°C didn't show foul smell after 24 h incubation, but showed a weak foul smell after 48 h incubation. In the autolyzates at 10 and 15°C, such foul smell was not found. Therefore, in the next experiment, autolysis of the head and viscera mixture was performed at 15°C for 24 h to prevent the sample from decaying despite the slightly lower recovery of protein at 15°C than that of the autolysis at 20 and 25°C.

Effect of Shaking Treatment on the Autolysis Efficiency

The effect of shaking treatment on the recovery during autolysis was investigated. The head and viscera mixture (head:viscera:water=3:1:8) was homogenized and then autolyzed at 15°C for 24 h. The increase in extractive nitrogen content are shown in Fig. 2. The result showed that the shaking treatment during autolysis had no significant effect on the extractive nitrogen content. However, the con-

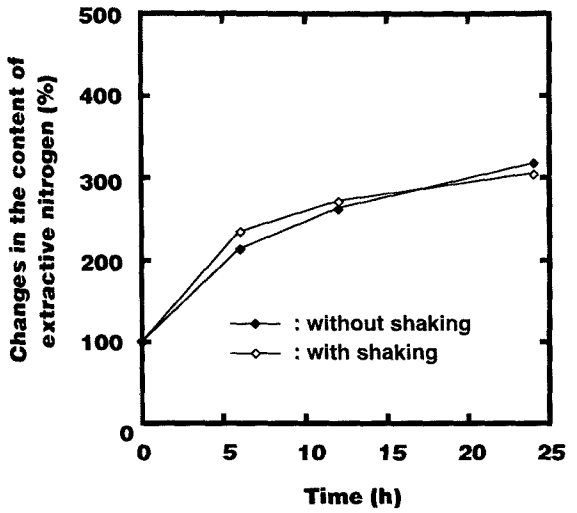

Fig. 2. Effect of shaking treatment during autolysis on the autolysis efficiency of the fish wastes.

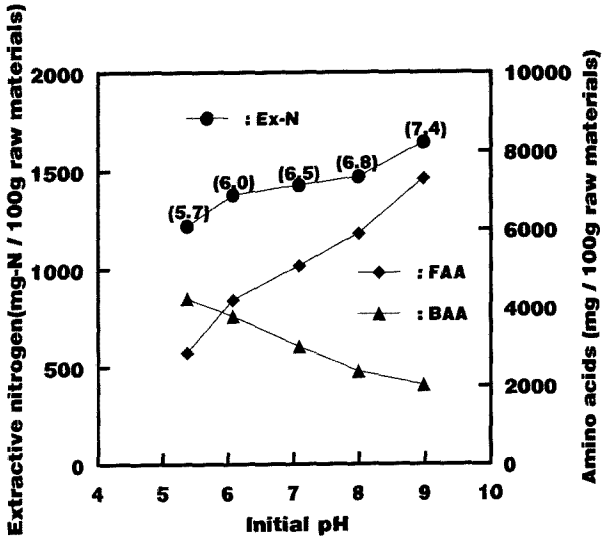

Fig. 3. Effect of pH on the extractive nitrogen, the cotent of free and bound amino acids in the autolyzate of the fish wastes.
Abbreviations in the figure are as follows: Ex-N, extractive nitrogen; FAA, free amino acids; BAA, bound amino acids.

Table 2. Free amino acid compositions of autolytic extract prepared from the wastes with and without shaking
(mg/100 g wastes)

Amino acids	Before autolysis	After autolysis	
		Without shaking	With shaking
Tau	343	354	342
Asp	84	465	393
Thr	59	280	241
Ser	70	313	276
Glu	141	605	518
Gln	66	228	196
Gly	52	225	180
Ala	104	433	395
Val	73	359	319
Met	47	200	162
Ile	61	337	285
Leu	110	556	491
Tyr	61	297	263
Phe	61	278	252
Lys	124	550	448
His	138	252	199
Arg	104	322	333
Pro	59	247	213
Total	1758	6300	5506

tent of the free amino acids except taurine (Tau) and Arg were lower in the autolyzate with shaking than without shaking (Table 2). In addition, the color of the autolyzate which was shaken was much more brown than the autolyzate without shaking and the bitterness of the former was stronger than the latter. This implies that the bitterness of the extract was enhanced by shaking the sample during autolysis. These results suggest that autolysis should be done without shaking. The reason for the enhanced bitterness by shaking during autolysis remains unclear and needs to be clarified further.

Effect of pH on Autolysis

Optimum pH for the autolysis was investigated. Before onset of autolysis, the initial pH of the head-viscera mixture samples were adjusted to different set values by an addition of 1 N HCl or 1 N NaOH. The original pH of the head-viscera mixture without pH adjustment was around 6.8. The mixture was then autolyzed at 15°C for 24 h without shaking. The contents of extractive nitrogen, free and bound amino acids are shown in Fig. 3. The number in the parentheses shows the pH value of each autolyzate at 15°C for 24 h. The extractive nitrogen gradually increased with an increase of initial pH. The content of free amino acids of the autolyzate at pH9 was much higher than that at pH 5.5, while the content of bound amino acids of the former was lower than that of the latter. The result showed that the pH adjustment before autolysis not only affected the increase in the extractive nitrogen of the autolyzate, but it also affected the content of free and bound amino acids of the autolytic extract. This suggests that, in terms of protein recovery autolysis at pH 9 might be suitable for preparation of extract. However, in terms of organoleptic property the autolytic extract prepared at pH 9 had more bitterness as well as umami taste than that of at pH 7. Therefore, this further implies that the pH adjustment is not necessary for the autolytic extract preparation from the viewpoint of taste and ease of preparation of the extract although the recovery of protein in the autolyzate prepared at neutral pH was a little lower than that of at alkaline pH.

From the results presented here, we concluded that autolysis at 15°C for 24 h at neutral pH is suitable for recovery amino acids from the protein in the head and viscera of frigate mackerel. In the last trial, autolytic extract was prepared under above mentioned conditions and the amino acid composition of the extract was investigated.

As shown in Table 3, extractive nitrogen content of the autolytic extract increased from 315 mg to 1584 mg per

Table 3. Free and bound amino acid compositions of extracts of the wastes before and after autolysis

(mg/100 g wastes)

Amino acids	FAA*1		BAA*1	
	before	after	before	after
Tau	384	397	—	—
Asp	40	373	35	169
Thr	29	215	24	10
Ser	34	261	26	77
Glu	89	516	112	402
Gln	43	154	—	—
Pro	23	173	30	99
Gly	28	163	61	238
Ala	64	404	25	90
Val	35	310	21	89
Cys	—	9	10	43
Met	22	182	6	12
Ile	28	294	10	49
Leu	52	496	17	66
Tyr	31	287	—	—
Phe	30	261	—	—
Lys	54	465	45	142
His	150	577	5	28
Arg	44	213	14	31
Total	1180	5450	441	1636
EX-N*2	315	1584	—	—

*1 FAA, free amino acids; BAA, bound amino acids.
*2 EX-N, extractive nitrogen (mgN/100 g sample).

100 g head-viscera mixture. From the data in Table 1, the content of protein nitrogen except alkali-insoluble protein in 100 g head-viscera mixture was estimated to be 1464 mg. This indicated that 87% of protein nitrogen was recovered as extractive nitrogen by autolysis at 15°C for 24 h. In the extract before autolysis, Tau, a non-proteinious amino acid was the most abundant and accounted for 33% of free amino acid. In the autolyzate, the total content of free amino acid was about 3 times higher than that of bound amino acid. Among the free amino acids, His, Glu, Leu, Lys and Ala were rich (over 400 mg) in the autolyzate followed by Tau, Asp, and Val.

This study reveals that the protein in the fish wastes can be efficiently and easily autolyzed even at low temperature. Organoleptically, the autolytic extract possesses umami taste and weak bitterness. Despite weak bitterness, the umami taste emphasizes its potential to be used as a seasoning material. The taste of the autolytic extract could be improved by reducing the bitterness. Further studies to improve the taste of the autolytic extract are now under progress in our laboratory.

Acknowledgments We thank Mr. D. P. Thakur for his critical reading of the manuscript. This study was partly supported by a Grant-in-Aid for scientific research (B) (No. 08456105) from the Ministry of Education, Science, Sports, and Culture of Japan.

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