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Effects of Soy Sauce Koji and Commercial Proteolytic Enzyme on the Acceleration of Fish Sauce Production

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Abstract

The possibility of the use of soy sauce koji and commercial proteolytic enzyme for the acceleration of fish fermentation without affecting its characteristic flavor and nutritional quality inherent to the final products was investigated. Fish sauces were prepared experimentally from small horse mackerel under sixteen kinds of conditions and the chemical composition of those were examined, individually. The amino type nitrogen content, ratio of amino type nitrogen to total nitrogen and protein conversion ratio were the highest in the fish sauce product treated with soy sauce koji, of which 10% salt was added to the minced raw fish at the start and additional 10% salt was added to the mixture after 48 hrs. incubation.

Key words: acceleration, fish sauce, soy sauce koji, proteolytic enzyme.

Introduction

Seafoods constitute a major source of protein in many oriental countries. Fermentation is one of the methods commonly used to preserve these foods. Fish sauces are traditional fermented fish products that are used extensively in Southeast Asia and some other parts of the Far East[1-3]. According to Westenberg[4], fish sauces first came into use in regions where the climate was too rainy to allow sun-drying of the fish and where sufficient fuel could not be found for artificial drying. Therefore, a number of fish sauces are well known in Southeast Asia.

Fish sauces are prepared by mixing small uneviscerated fish with salt in the ratio of 1 part of salt to 1.5-3 parts by weight of fish. The mixture is placed in a concrete vat where it is allowed to ferment for about 9 months at temperature between 20 and 35 °C during which time the fish disintegrates to give a brown liquid. The liquid contains soluble nitrogen which is mainly derived from the breakdown of the fish protein by the viscera enzymes of the fish.

Technical process so far employed for the production of fish sauce is rather simple and needs no more complicated treatment than adding salt to fish. The proteolysis results from a combination of the autolytic action of the natural fish enzymes and the effects of microbial fermentation. However, the salted fish has to be preserved in a vessel as long as 6 to 12 months at ambient temperature before it becomes ready to eat and to use.

It would be advantageous with the fish sauce, (a) if the fermentation period could be shortened to reduce the capital costs and the increase the throughput, (b) if a maximum conversion of insoluble to soluble protein could be achieved, (c) if the flavor and aroma components could be established and their production accurately could be controlled, (d) if the high concentration of salt could be reduced to allow for a higher individual consumption of fish sauce and (e) if fish, normally discarded, could be incorporated.

Therefore, it has been attempted to accelerate fish fermentation without affecting its characteristic flavor and nutritional quality inherent to the

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final products.

Amano[5] used an antibacterial agent instead of salt to accelerate fish fermentation. Digestion of insoluble protein was complete within a few days and a yield of solubilized nitrogen was increased by 50%, however the sauce did not have a desirable aroma or flavor. Oishi *et al.*[6] and Suzuki *et al.*[7] investigated the development of fish sauce from skipjack head paste by adding koji of soy sauce. Skipjack head paste was fermented at 30 °C for 90 days. During the fermentation period, the chemical analysis and sensory test of the fish sauce were performed. Beddows and Ardeshir[8,9] investigated the production of a fish hydrolysate, using plant protease, which could be added to traditionally fermented fish sauce to increase the total volume without affecting the overall nutritional quality. Bromelain was reported to give better results than papain or ficin in that greater amount of fish protein are solubilized using minced fish in 18 to 21 days at 33 °C. The distribution and concentration of nitrogeneous compounds were similar to traditional fish sauce but very little aroma was present. Ooshira *et al.*[10] also examined the use of papain, bromelain and trypsin to produce a fish sauce from sardine, mackeral and opossum shrimp, with 25% salt added directly, or in 3 stages over 24 hrs. However, the fermentation takes 340 to 350 days but the best results are obtained with 0.3% papain with whole sardine at pH 5.2, 37 °C and 25% salt.

The present work was designed to see the possibility of use of the soy sauce koji inoculated *Aspergillus oryzae* and commercial proteolytic enzyme for the acceleration of fish sauce production.

Materials and Methods

Materials

Small horse mackerel was used for the preparation of fish sauce as raw fish. Body size and weight of the raw fish were about 10.5 cm and 23.5g, respectively.

Soy sauce koji was offered from Kubota Shoyu Co., Japan. Pronase E of commercial proteolytic enzyme having an activity of 1,000,000 PUN/g was purchased from Kaken Seiyak Co., Japan.

Preparation of fish sauce

The composition of materials for the preparation of fish sauces are shown in Table 1. The methods of the preparation of fish sauces are shown in Fig. 1 and Table 2.

Chemical analysis

Yield of fish sauces was determined by filtration of each mashes after fermentation.

Total soluble solid was calculated as a value that the moisture content was subtracted from the weight of sample. The moisture content was determined by the method of Karl-Fisher reagent titration by MCI Karl-Fisher Titrater, Mitsubishi Chemical Ltd., Japan[11].

Specific gravity was measured by an hydrometer. Sodium chloride content were determined by

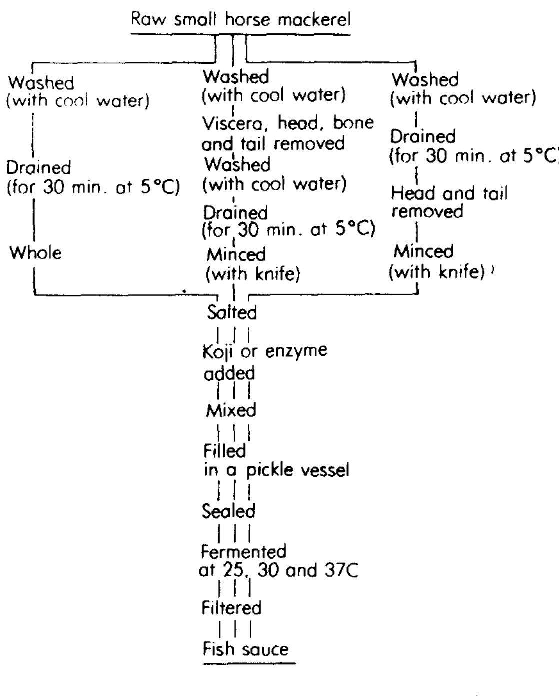

Fig. 1. Flow diagram of preparation of fish sauce.

Table 1. Composition of materials for the preparation of fish sauce

Sample No.	Material treatment	Koji[a] (%)	Enzyme[b] (%)	Viscera	Salt (%)	Temperature (°C)
C-1	whole	0	0	+	25	37
C-2	minced	0	0	+	20	30
C-3	minced	0	0	-	20	30
K-1	whole	10	0	+	20	30
K-2	minced	10	0	+	20	30
K-3	minced	10	0	+	20	25
K-4	minced	10	0	+	15+5	30
K-5	minced	10	0	+	10+10	30
K-6	minced	10	0	-	10+10	30
K-7	minced	20	0	+	20	30
P-1	minced	0	0.2	-	20	30
P-2	minced	0	0.2	-	20	25
P-3	minced	0	0.2	+	20	30
P-4	minced	0	0.2	-	5+15	30
P-5	minced	0	0.2	+	5+15	30
P-6	minced	0	0.1+0.1	-	20	30

a) Soy sauce koji produced by inoculation of *Aspergillus oryzae*
b) Pronase E of commercial proteolytic enzyme

potentiometric titration with 0.1N silver nitrate[12].

pH was measured by T0A pH meter, Model Hm20E.

Acidity I and II were determined by the experimental methods of Japanese soy sauce[13]. To 10 ml of the sample, 40 ml of deionized water was added. It was titrated with 0.1 N sodium hydroxide to pH 7.0 to pH 8.3. The volume of 0.1 N sodium hydroxide for the first titration was noted as acidity I and that of second titration was acidity II.

The contents of amino type nitrogen and total nitrogen were determined by the methods of formol titration and Kjeldahl, respectively.

The ability of the process to convert the insoluble protein into the soluble form was calculated as following equation; (8)

$$\text{Percentage of protein conversion} = \frac{\text{Volume of liquid produced} \times \text{its N content (g/g)}}{\text{Weight of fish used} \times \text{its N content (g/g)}} \times 100$$

The nitrogen content of the raw fish was found to be 0.0243 g/g wet weight.

The contents of volatile basic nitrogen and trimethylamine type nitrogen were determined by the Conway microdiffusion method.

Results and Discussion

Changes in chemical components of fish mashes during fermentation

The changes in amino type nitrogen content of fish mashes during fermentation are shown in Figs. 2 and 3. Amino type nitrogen content was increased with a progress of fermentation in all samples. No. K-5 showed the highest amino type nitrogen levels in samples treated with soy sauce koji and No. P-5 showed the highest amino type nitrogen levels in samples treated by pronase. No. K-5 is the sample that 10% salt is added at the beginning and additional 10% salt is added after 48 hrs. incubation. No. P-5 is the sample that 5% salt is added at the beginning and 15% salt is added after 7 hrs. incubation.

The changes in total nitrogen content of fish mashes were similar to those of amino type nitrogen content as shown in Figs. 4 and 5.

From the above results, it was found that the

Table 2. The methods of preparation of fish sauces

Sample No.	Method of preparation
C-1	The whole body of raw fish was used. To 1 kg of whole fish 25% salt was added and mixed thoroughly. The mixture was put into plastic pickle jar and incubated at 37°C.
C-2	Head and tail of raw fish were removed and then it was minced with knife. To 1 kg of the minced raw fish 20% salt was added and mixed thoroughly. The mixture was put into plastic pickle jar and incubated at 30°C
C-3	At first, raw fish was eviscerated. Head and tail of the eviscerated raw fish were removed and then it was minced with knife. To 1 kg of the minced raw fish 20% salt was added and mixed thoroughly. The mixture was put into plastic pickle jar and incubated at 30°C.
K-1	The whole body of raw fish was used as sample No. C-1. To 1 kg of whole fish 20% salt was added and mixed thoroughly. 10% soy sauce koji was added to the mixture. The mixture was put into plastic pickle jar and incubated at 30°C.
K-2	Same as sample No. C-2 except that 10% soy sauce koji was added to the mixture.
K-3	Same as above sample No. K-2 except that the mixture was incubated at 25°C.
K-4	Same as sample No. K-2 except that 15% salt was added to the minced raw fish at the start and after 48 hrs. incubation 5% salt was added to the mixture.
K-5	Same as sample No. K-2 except that 10% salt was added to the minced raw fish at the start and after 48 hrs. incubation 10% salt was added to the mixture.
K-6	Same as above sample No. K-5 except that the eviscerated raw fish was used.
K-7	Same as sample No. C-2 except that 20% soy sauce koji was added to the mixture.
P-1	Same as sample No. C-3 except that 0.2% Pronase E was added to the mixture.
P-2	Same as above sample No. P-1 except that the mixture was incubated at 25°C.
P-3	Same as sample No. C-2 except that 0.2% Pronase E was added to the mixture.
P-4	Same as sample No. P-2 except that 5% salt was added to the minced raw fish at the start and after 7 hrs. incubation 15% salt was added to the mixture.
P-5	Same as above sample No. P-4 except that the uneviscerated raw fish was used.
P-6	Same as sample No. P-1 except that 0.1% Pronase E was added to the mixture at the start and after 15 days incubation 0.1% Pronase E was added again to the mixture.

addition of small amount of salt at the first stage was better for the digestion of fish protein, whereas a larger amount of it disturbed the enzyme activity. In the case of pronase treatment, considered that the enzyme activity might be inhibited by the bile acid which was in fish viscera, fish viscera was removed before mashing of samples treated by pronase. But uneviscerated No. P-3 was better for the digestion of fish protein rather than eviscerated No. P-1. Therefore, it was found that the enzyme activity might not be inhibited by the viscera constituents and the autolytic enzyme which was contained in fish viscera was more effective for the digestion of fish protein.

The changes in the ratio of amino type nitrogen (A.N.) to total nitrogen (T.N.) contents of fish mashes during fermentation are shown in Figs. 6 and 7. All samples showed a slight fluctuation in the ratio of amino type nitrogen to total nitrogen contents during fermentation. After 15 days of fermentation, the ratio of A. N./T. N. in No. K-5 treated with soy sauce koji was 0.515 and it was the highest value in all samples. The ratio of A. N./T. N. in No. P-5 treated by pronase was 0.424 after 15 days of fermentation.

Figs. 8 and 9 show the changes in volatile basic nitrogen content of fish mashes during fermentation. All samples showed an increasing tentency in the volatile basic nitrogen content as the fermentation was made a progress. The fish sauces treated with soy sauce koji showed more remarkable increasing tendency than the fish sauces treated by pronase.

To investigate the effect of accelerated fish fer-

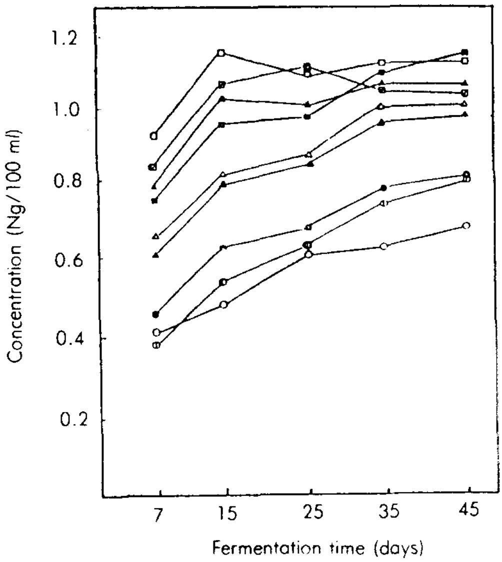

Fig. 2. Changes in amino type nitrogen content of fish mash treated with soy sauce koji during fermentation.
○—○ : Sample No. C-1, ⌽—⌽ : Sample No. C-2, ●—● : Sample No. K-1, △—△: Sample No. K-2, ⧊—⧊ : Sample No. K-3, ▲—▲: Sample No. K-4, □—□ : Sample No. K-5, ⊠—⊠ : Sample No. K-6, ■—■ : Sample No. K-7

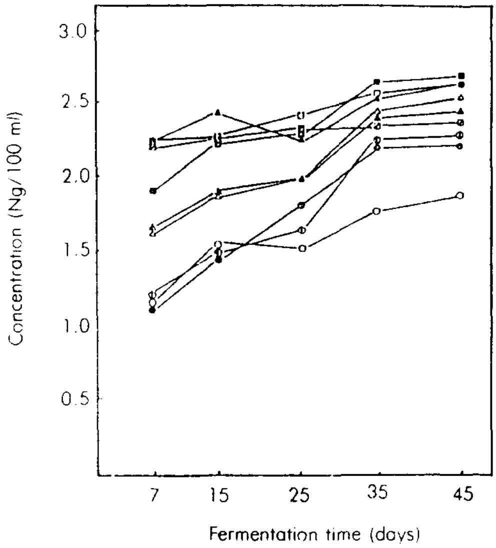

Fig. 4. Changes in total nitrogen content of fish mash treated with soy sauce koji during fermentation.
○—○ : Sample No. C-1, ⌽—⌽ : Sample No. C-2, ●—● : Sample No. K-1, △—△ : Sample No. K-2, ⧊—⧊ : Sample No. K-3, ▲—▲: Sample No. K-4, □—□ : Sample No. K-5, ⊠—⊠ : Sample No. K-6, ■—■ : Sample No. K-7.

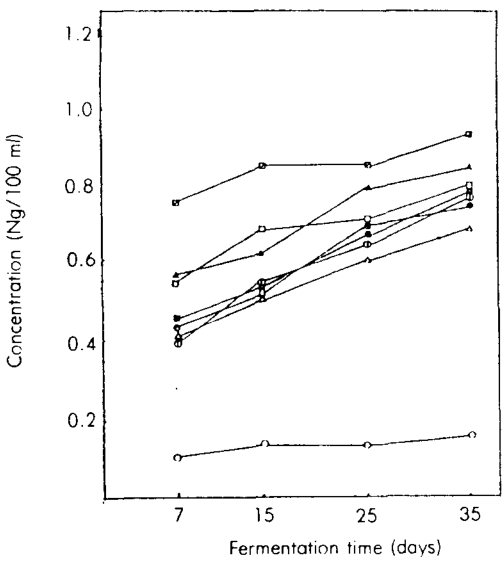

Fig. 3. Changes in amino type nitrogen content of fish mash treated by pronase during fermentation.
⌽—⌽ : Sample No. C-2, ○—○ : Sample No. C-3, ●—● : Sample No. P-1, △—△: Sample No. P-2, ▲—▲: Sample No. P-3, □—□ : Sample No. P-4, ⊠—⊠ : Sample No. P-5, ■—■ : Sample No. P-6

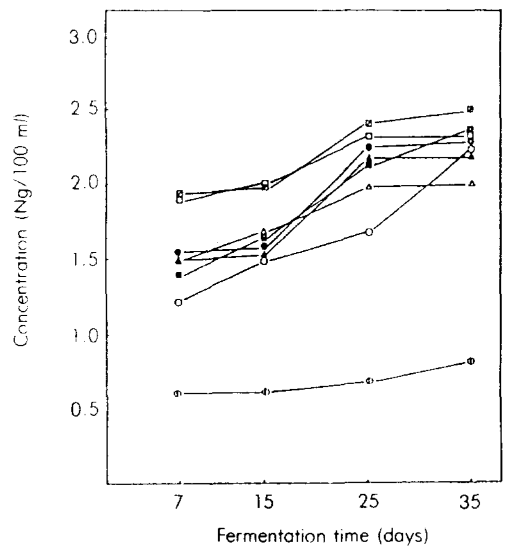

Fig. 5. Changes in total nitrogen content of fish mash treated by pronase during fermentation.
○—○ : Sample No. C-2, ⌽—⌽ : Sample No. C-3, ●—● : Sample No. P-1, △—△ : Sample No. P-2, ▲—▲: Sample No. P-3, □—□ : Sample No. P-4, ⊠—⊠ : Sample No. P-5, ■—■ : Sample No. P-6

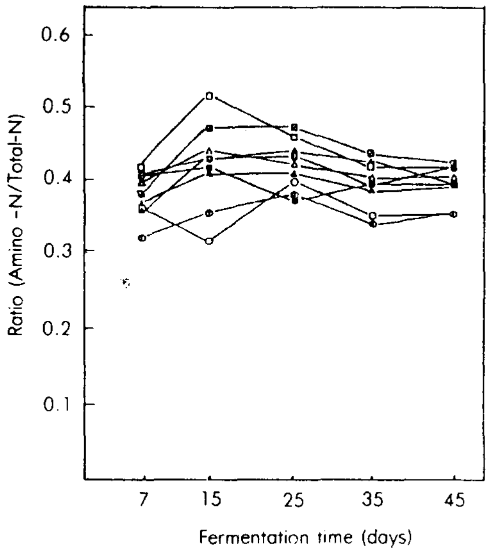

Fig. 6. Changes in the ratio of amino type nitrogen to total nitrogen content of fish mash treated with soy sauce koji during fermentation.
○—○ : Sample No. C-1, ◐—◐ : Sample No. C-2, ●—● : Sample No. K-1, △—△ : Sample No. K-2, ⧊—⧊ : Sample No. K-3, ▲—▲ : Sample No. K-4, □—□ : Sample No. K-5, ⊠—⊠ : Sample No. K-6, ■—■ : Sample No. K-7.

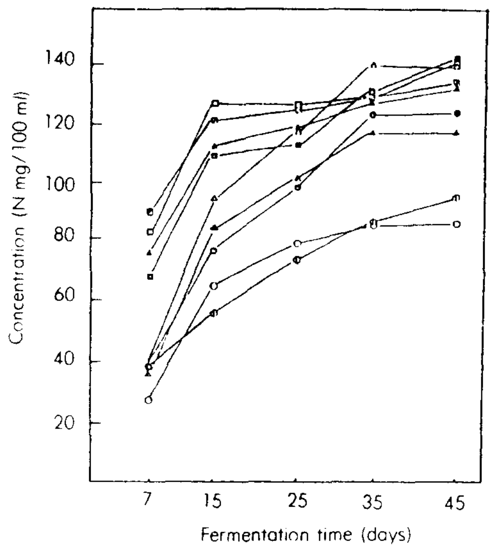

Fig. 8. Changes in volatile basic nitrogen content of fish mash treated with soy sauce koji during fermentation.
— : Sample No. C-1, ◐—◐ : Sample No. C-2, ●—● : Sample No. K-1, △—△ : Sample No. K-2, ⧊—⧊ : Sample No. K-3, ▲—▲ : Sample No. K-4, □—□ : Sample No. K-5, ⊠—⊠ : Sample No. K-6, ■—■ : Sample No. K-7.

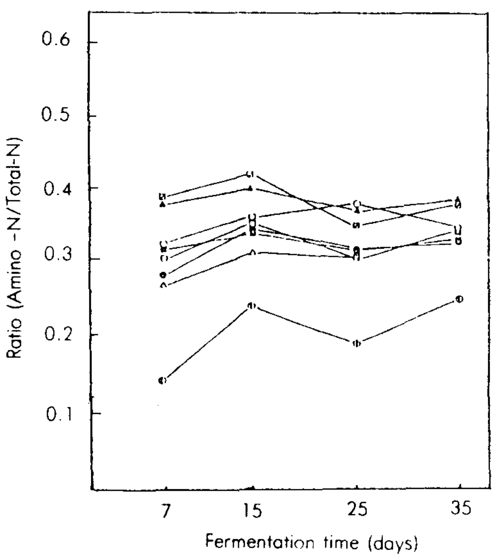

Fig. 7. Chanes in the ratio of amino type nitrogen to total nitrogen content of fish mash treated by pronase during fermentation.
○—○ : Sample No. C-2, ◐—◐ : Sample No. C-3, ●—● : Sample No. P-1, △—△ : Sample No. P-2, ▲—▲ : Sample No. P-3, □—□ : Sample No. P-4, ⊠—⊠ : Sample No. P-5, ■—■ : Sample No. P-6

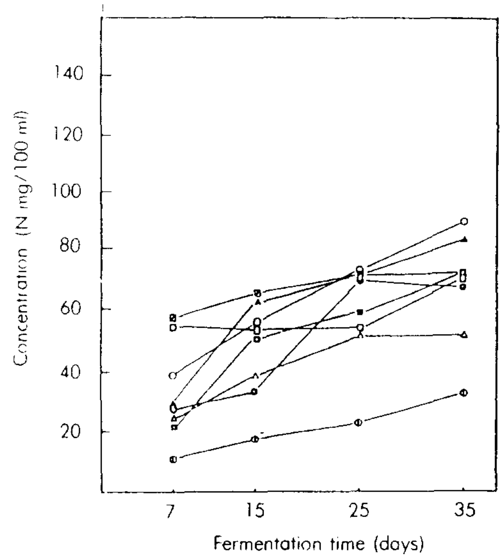

Fig. 9. Changes in volatile basic nitrogen content of fish mash treated by pronase during fermentation.
—○ : Sample No. C-2, ◐—◐ : Sample No. C-3, ●—● : Sample No. P-1, △—△ : Sample No. P-2, ▲—▲ : Sample No. P-3, □—□ : Sample No. P-4, ⊠—⊠ : Sample No. P-5, ■—■ : Sample No. P-6

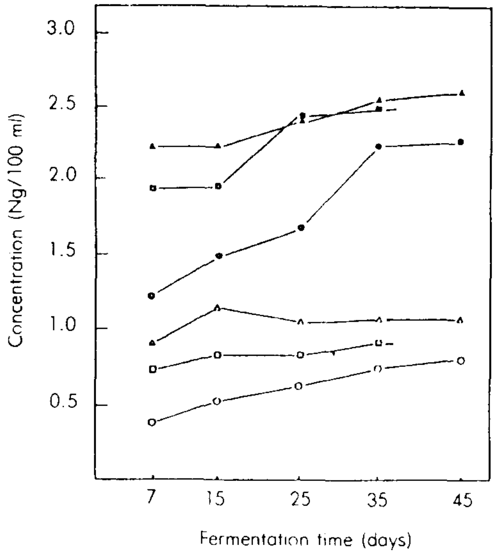

Fig. 10. Effect of the soy sauce koji and pronase treatment on the amino type nitrogen and total nitrogen content during fermentation.

○—○ : Amino-N of control, No. C-2
△—△ : Amino-N of No. K-5 treated with soy sauce koji
□—□ : Amino-N of No. P-5 treated by pronase
●—● : Total-N of control No. C-2
▲—▲ : Total-N of No. K-5 treated with soy sauce koji
■—■ : Total-N of No. P-5 treated by pronase

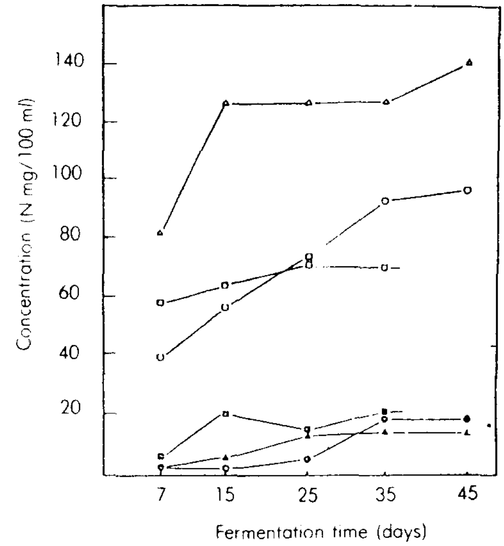

Fig. 11. Effect of the soy sauce koji and pronase treatment on the volatile basic nitrogen and trimethylamine nitrogen content during fermentation.

○—○ : VBN of control, No. C-2
△—△ : VBN of No. K-5 treated with soy sauce koji
□—□ : VBN of No. P-5 treated by Pronase
●—● : TMA of control, No. C-2
▲—▲ : TMA of No. K-5 treated with soy sauce koji
■—■ : TMA of No. P-5 treated by pronase

mentation, the fish sauce treated with 10% soy sauce koji was compared with the fish sauce treated by 0.2% pronase. The effect of the treatments with soy sauce koji and pronase on the contents of amino type nitrogen and total nitrogen in the fish sauces during fermentation are shown in Fig. 10, and the effect of the treatments with soy sauce koji and pronase on the contents of volatile basic nitrogen and trimethylamine type nitrogen in the fish sauces during the fermentation period are shown in Fig. 11. As the results shown in Figs. 10 and 11, the contents of amino type nitrogen, total nitrogen, volatile basic nitrogen and trimethylamine type nitrogen in the fish sauce treated with 10% soy sauce koji were higher than the contents of those in the fish sauce treated by 0.2% pronase.

Chemical composition of final fish sauce products

Sixteen kinds of fish sauces were prepared from small horse mackerel. The yield of fish sauce, total souble solid, specific gravity, salt, pH, acidity, amino type nitrogen, total nitrogen, ratio of amino type nitrogen to total nitrogen, protein conversion ratio, volatile basic nitrogen and trimethylamine type nitrogen of the final fish sauce products were determined. The results are shown in Table 3.

In general, the yields of fish sauces treated by pronase were higher than those treated with soy sauce koji and the yields of fish sauce increased according to an increase of the protein conversion ratio. The salt contents of all fish sauce products were over 25%. The total soluble solid contents of fish sauces treated with soy sauce koji were higher than those treated by pronase. In the amino type nitrogen content and ratio of amino type nitrogen to total nitrogen of fish sauce products, the fish sauces treated with soy sauce koji were hig-

Table 3. Chemical compositions of final fish sauce products prepared from small horse mackerel.

Sample No.	Yield of fish sauce (ml)	Total soluble solid (%)	Specific gravity	Salt (g / 100 ml)	pH	Acidity I (ml)	Acidity II (ml)
C-1	406	34.02(5.88*)	1.216	28.14	5.47	6.26	11.98
C-2	422	35.11(8.63*)	1.210	26.48	5.53	8.19	15.70
C-3	244	30.62(0.72*)	1.204	29.90	5.48	4.11	4.00
K-1	355	38.11(10.76*)	1.223	27.35	5.34	8.14	12.77
K-2	412	39.61(14.09*)	1.226	25.52	5.34	11.03	16.88
K-3	416	38.66(14.39*)	1.220	24.27	5.34	9.23	16.35
K-4	447	39.13(14.63*)	1.222	24.50	5.34	11.31	18.00
K-5	443	39.35(14.83*)	1.228	24.52	5.31	11.87	18.45
K-6	472	38.13(13.06*)	1.221	25.07	5.34	10.27	17.35
K-7	397	42.87(17.46*)	1.240	25.41	5.26	13.34	18.96
P-1	457	35.39(9.32*)	1.210	26.07	5.57	7.98	18.60
P-2	417	33.93(8.29*)	1.205	25.64	5.58	7.77	17.68
P-3	508	35.31(9.61*)	1.206	25.70	5.54	7.98	16.39
P-4	507	35.39(9.15*)	1.212	26.24	5.62	7.98	18.72
P-5	478	36.48(9.99*)	1.218	26.49	5.53	8.61	18.25
P-6	443	34.81(9.11*)	1.209	25.70	5.59	8.08	18.58

*: Value subtracted salt content from total soluble solid content.

Table 3. Continued......

Sample No.	Formol-N (g / 100 ml)	Total-N (g / 100 ml)	Formol-N / Total-N	Protein conversion ratio	VBN (mg / 100 ml)	TMA (mg / 100 ml)
C-1	0.676	1.900	0.356	0.332	90.27	19.75
C-2	0.816	2.295	0.356	0.416	95.91	19.75
C-3	0.157	0.769	0.204	0.081	31.03	2.82
K-1	0.804	2.033	0.395	0.310(0.258**)	124.12	16.93
K-2	1.403	2.566	0.408	0.457(0.376**)	138.23	16.93
K-3	0.985	2.460	0.400	0.440(0.366**)	98.74	16.93
K-4	1.064	2.641	0.403	0.507(0.422**)	134.00	13.11
K-5	1.093	2.628	0.416	0.500(0.416**)	141.05	21.16
K-6	1.006	2.380	0.423	0.483(0.401**)	126.95	14.11
K-7	1.131	2.697	0.419	0.460(0.327**)	141.05	22.57
P-1	0.752	2.288	0.329	0.449	67.70	11.28
P-2	0.676	2.016	0.335	0.361	50.78	11.28
P-3	0.845	2.216	0.381	0.484	84.63	14.11
P-4	0.772	2.285	0.338	0.498	70.53	21.16
P-5	0.933	2.500	0.373	0.514	70.53	21.16
P-6	0.746	2.306	0.324	0.439	70.53	14.11

**: Value considered protein content of added soy sauce koji with fish protein content.

her than those treated by pronase, and besides, the fish sauce products treated with soy sauce koji were superior to the Japanese Shottsuru and Thiland Nampla reported by Itoh *et al.*[14]. On the other hand, fish sauce products treated with soy sauce koji showed high contents of volatile basic nitrogen. Generally, fish sauce products treated with soy sauce koji and pronase showed high acidity in comparison with the control fish sauce products.

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速釀 魚醬油 製造에 있어서 醬油코오지와 市販 蛋白分解 酵素의 影響

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최종 제품의 고유 풍미와 영양가에 영향을 미치지 않는 速釀 魚醬油 제조를 위하여 *Aspergillus oryzae*가 접종된 醬油코오지와 시판 단백분해 효소의 이용 가능성을 검토하였다.

전갱이(아지)를 원료로 하여 醬油코오지 또는 시판 단백분해 효소를 첨가하는 등의 조건을 달리한 16종류의 魚醬油를 제조하였고 각각 그들의 화학성분을 조사하였다.

원료 전갱이에 醬油코오지를 첨가하고 초기에 10% 식염을 가하여 48시간 유지한 후 다시 그 혼합물에 10% 식염을 더 가하여 제조한 魚醬油가 아미노태 질소 함량, 총질소에 대한 아미노태 질소 함량 비율및 제품의 단백질 전환율 등에 있어서 기타 조건에서 제조된 모든 魚醬油에 비하여 가장 우수하였다.