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Study on Use of Commercial Proteolytic Enzymes in Production of Fish Sauce

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Abstract

Sardine, mackerel, and ami were used for fish sauce making by adding commercial enzymes of papain, bromelain and trypsin. Fermentation temperature was varied as room temperature, 37°C or 50°C. Initial pH was adjusted to 4.5, 6.5 or unadjusted (5.2). Addition of salt was carried out either by adding total amount of salt (25% based on fish weight) or by adding part of salt (5% or 15%) on first day and adding the remaining after 24 h keeping at 37°C or 50°C. Minced samples were prepared by using hand-mincer. Weight of papain was varied as 0.2, 0.3 or 0.5% based on fish weight. Fermentation was proceeded to 340–350 days.

Total soluble nitrogen, amino-type nitrogen, volatile base nitrogen, total volatile acid and individual volatile organic acids were determined at occasions during fermentation period. Papain was satisfactory for proteolysis of unminced sardines under the processing conditions of fermentation temperature of 37°C, natural initial pH, 25% salt based on fish weight, added in once together with enzyme, and enzyme weight of 0.3% based on fish weight. The taste and colour of papain-added sample was satisfactory. The aroma did not appear in both control samples without added enzyme and enzyme-added samples. Aroma was detected in matured control sample while it was not detected in matured enzyme-added sample.

Fish sauces are fermented condiments traditionally produced and extensively consumed by over 250 million people in South-east Asia and some other parts of the Far East[1]. Traditionally fish sauces are prepared by mixing small, uneviscerated tropical fish with salt in the ratio of one 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 6–9 months at temperatures between 30°C and 40°C during which time, the fish disintegrates to have a brown liquid. The liquid is run off and allowed to mature for about 3–6 months before shipping to the market. Production of fish sauce takes usually one year or more than one year.

It has been generally considered that, it would be advantageous if the production period of fish sauce could be shortened so that the production rate will be increased and consequently the capital costs will be reduced. Different ways of increasing the fermentation rate have been investigated, among which addition of commercial proteolytic enzymes got favoured by many researchers since proteolysis is the fundamental reaction that takes place during fermentation of fish sauce. Use of bioprase, pronase, molsin, protease AJ, papain, bromelain and ficin was investigated by some

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researchers[2,3,4,5]. However, the results varied widely and most workers did not specifically mention about the organoleptic properties of the product, which are important qualities of fish sauce. This report describes the effect of addition of some commercial proteolytic enzymes, papain, bromelain, and trypsin, on the rate of fermentation of fish sauce at various operating conditions and on the organoleptic properties of the fish sauce products.

Materials and Methods

Fish

Sardines, mackerel, caughtin May, and frozen ami, were bou ght from the market.

Enzyme

Papain, bromelain (both were food additives of Nagase Co.) and trypsin (Laboratory chemicals, Wako Co. product) were used.

Salt

Kitchen salt was bought from the market.

Fish sauce production

Schematic presentation of fish sauce processing is shown in Table 1. For each sample, 400 g of fish was processed. Sardines were divided into four without evisceration. Mackerel was beheaded and cut into pieces approximately equal to the size of sardine pieces, without evisceration. For ami, frozen block was cut into smaller blocks.

In Table 1, where there is no specific description, the general processing conditions would be regarded as follows: — fish used was sardines, unminced; amount of salt added was 25%, based on fish weight, and all amount of salt was added in one time together with enzyme; enzyme used was papain, and the amount was 0.3% based on fish weight, fermentation temperature was 37°C; initial pH was not adjusted. Cysteine (110 mg) was also added to the samples with added papain or bromelain. Fish, salt and additives were well mixed and the mixture was put into 500 ml-glass jar, capped with screw-type plastic cover and placed under the prescribed temperature. Fermentation was carried out for 340–350 days when the samples were filtered through Toyo No. 2 filter paper.

Chemical analysis of fish sauce

At appropriate occasions, fish sauce samples were filtered through Toyo No. 2 filter paper and chemical analyses were carried out in duplicates.

Total soluble nitrogen (T. S. N) was determined by slightly modified Kjeldahl method. For 0.5 ml of fish sauce, 3 ml of conc. H_2SO_4 was used in decomposition

Table 1. Scheme for the fish sauce processing.

	No	Description
Samples without commercial enzymes	1	Control, Sardine
	2	Mackerel
	3	Ami
	4	Initial pH adjusted to 4.5 with 2N citric acid
	5	Initial pH adjusted to 6.5 with 2N $NaHCO_3$
	6	Fermentation at room temperature
	7	Fermentation at 50°C
	8	Minced with hand mincer
	9	5% salt added on first day, placed at 37°C for 24h followed by addition of remaining salt
	10	The same as No 9 except that 15% salt was added on first day
	11	The same as No 9 except that the sample was placed at 50°C for 24 h
	12	The same as No 10 except that the sample was placed at 50°C for 24 h
Samples with added enzyme	13	Counterpart of No 1
	14	〃 No 2
	15	〃 No 3
	16	〃 No 4
	17	〃 No 5
	18	〃 No 6
	19	〃 No 7
	20	〃 No 8
	21	〃 No 9
	22	〃 No 10
	23	〃 No 11
	24	〃 No 12
	25	0.3% bromelain was added
	26	0.3% trypsin was added
	27	0.2% papain was added
	28	0.5% papain was added

step and 15 ml of alkali solution (40% NaOH, 5% $Na_2S_2O_3$) was used in evaporation step. Amino acid type nitrogen (A. N) was determined by Ninhydrin method. Volatile basic nitrogen (V. B. N) was determined by Conway's microdiffusion method[6] and pH was measured by Horiba pH meter.

Volatile organic acids (V. O. A)

V. O. A was analysed by slightly modified Dougan's method[7]. Five ml of fish sauce was acidified to pH 2.5 with conc. phosphoric acid and was steam distilled in Kjeldahl still. One hundred ml of distillate was collected and titrated with 0.1 N

NaOH solution using phenolphthalein as indicator. Titration volume was noted as total volatile acid (T. V. A). Then 1.5 ml of alkali was added in excess. The solution was evaporated to small volume and was transferred to an oven at 40°C where it was left overnight to evaporate to dryness. Next day, the dry residue was dissolved with 1 ml of 0.1 N phosphoric acid solution containing 0.5 ml n-valeric acid/100 ml solution; the latter served as internal standard.

Portions (2–8 μl) of this solution were injected into gas chromatograph (Shimadzu, GC-3BF), pyrex glass column of 3 mm dia × 2.6 m, packed with Chromosorb 101 (80–100 mesh) under the conditions of oven temperature, 210°C; ignition temperature, 5.5; carrier gas pressure, 1.0 kg/cm^2; H_2 gas pressure, 0.5 kg/cm^2; air pressure, 1.0 kg/cm^2. Peak areas were determined by triangulation. A series of standard synthetic solutions were prepared by using standard organic acids. Five ml each of standard solutions were steam distilled without adjusting pH and the same procedure as in the case of fish sauce was followed. Portions of the same standard solutions, after adding n-valeric acid as internal standard, were directly injected into gas chromatograph. From the peak areas of chromatograms of steam-distilled and non-distilled standard solutions, the recovery of acetic acid was calculated as 86% and the other acids as almost 100%. These values of recovery were used in the calculation of the volatile organic acid concentrations of fish sauce to correct for the losses.

Results and Discussion

Type of enzymes used

Fig. 1 to 4 shows the change in some properties of fish sauce during fermentation according to the type of enzymes used. Both for control and enzyme-added samples, pH did not markedly change. The values of T. S. N, and A. N for all enzyme-added samples were higher than the corresponding values for control through out the fer-

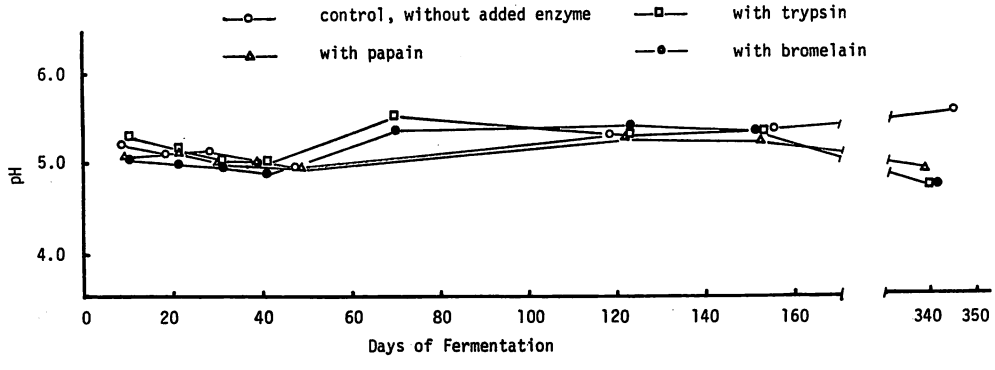

Fig. 1. Change of pH of fish sauce samples during fermentation.

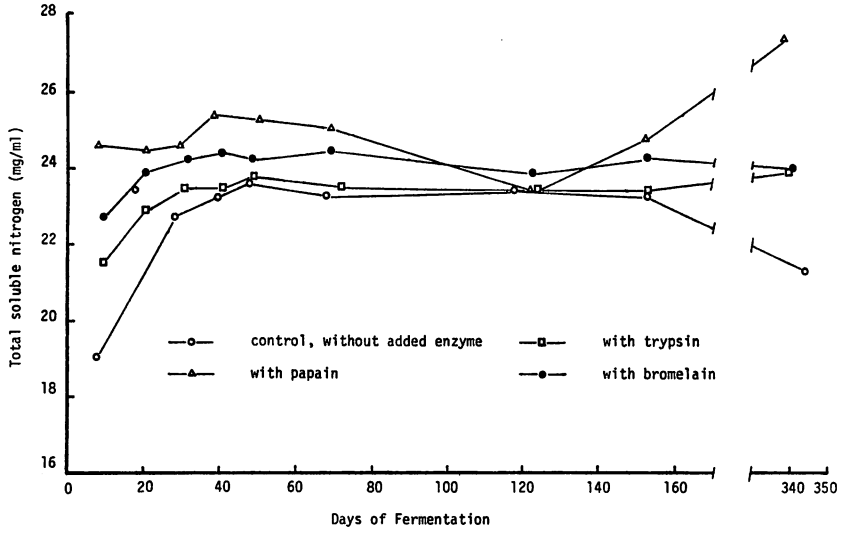

Fig. 2. Change of T. S. N of fish sauce samples during fermentation.

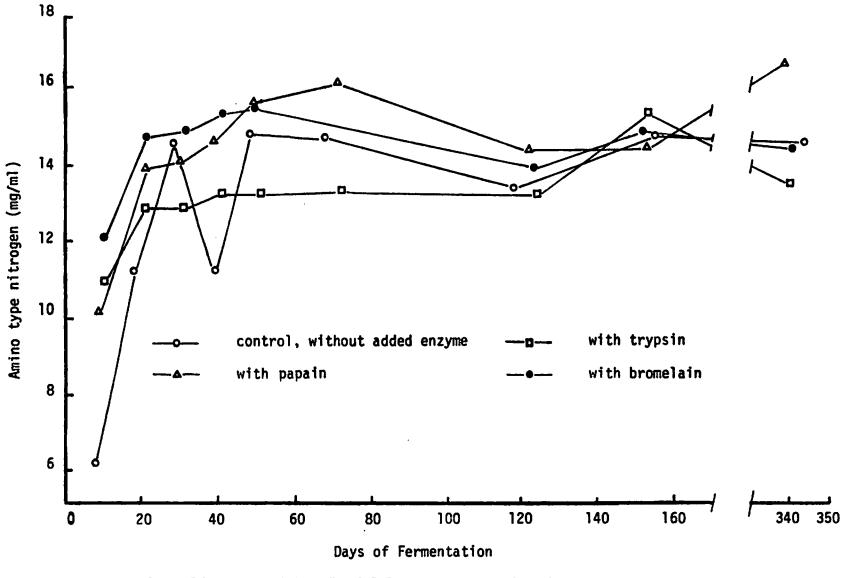

Fig. 3. Change of A. N of fish sauce samples during fermentation.

mentation period. The same was true in the case of V. B. N except for trypsin-added sample. For fish sauce, V. B. N is considered as one of the notes of fish sauce aroma[7]. Of the enzymes used, papain resulted in higher values of properties, although the difference in the values were not so great. It is needed to point out that the effect of added-enzyme was seen sharply only for the early few days of fermantation period, after which the enzyme activity became weak due to the high salt concentration.

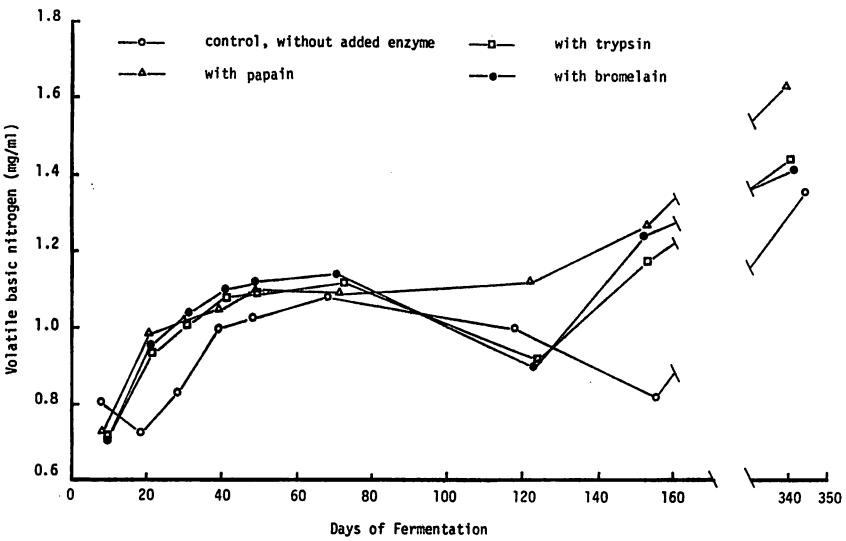

Fig. 4. Change of V. B. N of fish sauce samples during fermentation.

On the other hand, the control, although the values were low for first few days, followed the enzyme-added samples later because of the endogeneous enzymes of fish.

Type of fish used

The properties of fish sauce samples from different fish are shown in Table 2, together with those of commercial fish sauce from Burma and China for comparison. As can be seen from the table, T. S. N, A. N and V. B. N were somewhat increased for sardine while these properties were unaffected or decreased for mackerel and ami. It would be deduced that the effect of added papain was more pronounced for sardine than for mackerel and ami.

Table 2. Some properties of fish sauce from three kinds of raw materials.

Fish sauce sample	pH		T.S.N (mg/ml)		A.N (mg/ml)		V.B.N (mg/ml)	
	without added enzyme	with added enzyme	without added enzyme	with added enzyme	without added enzyme	with added enzyme	without added enzyme	with added enzyme
Sardine	5.6	4.9	21.29	27.34	14.57	16.69	1.36	1.73
Mackerel	5.6	4.9	25.54	25.61	17.80	13.20	1.51	1.35
Ami	6.5	7.0	12.23	18.42	6.34	6.16	1.67	1.81
Burma	6.9	—	19.41	—	15.61	—	2.92	—
China	5.6	—	17.97	—	12.66	—	2.66	—

Effect of initial pH

The final values of T. S. N, A. N, and V. B. N of the samples with different initial

pH at the time of filtration are plotted with initial pH in Fig. 5. It is noted that the sample without adjustment of initial pH (initial pH 5.2) would be regarded as the best for enzyme-added samples since it resulted in highest values of properties. The results were random for samples with no added enzymes.

Effect of fermentation temperature

Fig. 6 shows the properties of fish sauces at different fermentation temperatures

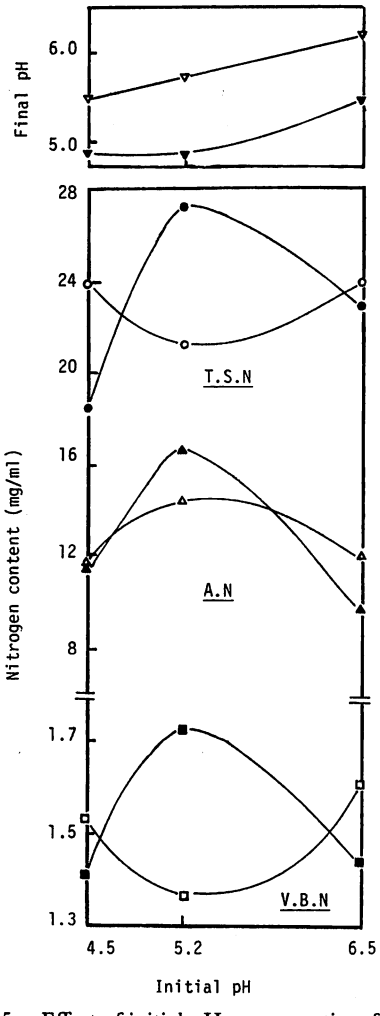

Fig. 5. Effect of initial pH on properties of fish sauce.

—○— —△— —□— —▽—
samples without added enzyme
—●— —▲— —■— —▼—
samples with added enzyme

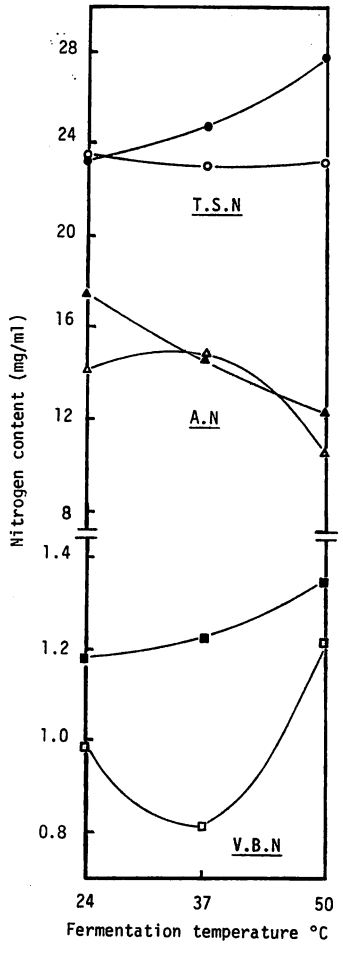

Fig. 6. Effect of fermentation temperature on properties of fish sauce.

—○— —△— —□—
samples without added enzyme
—●— —▲— —■—
samples with added enzyme

on their 153th day since fermentation started. Although room temperature varied greatly throughout the prolonged processing period, the data of sample at room temperature are arbitrarily plotted on the ordinate of 24°C for convenience. The data on the final day of filtration were not available since the samples at 50°C lost all of their moisture content at that time. Even on 153th day, loss of moisture due to evaporation would be great enough so that all of the various nitrogen contents were concentrated. Even on this assumption, it is quite notable that lowest A. N content was observed for samples at 50°C either with or without added enzyme. The temperature of 50°C is high enough to depress the activity of either endogeneous or added enzyme.

On the other hand, the difference between room temperature and 37°C was not clearly observed. The processing of fish sauce samples started in May when the average room temperature was far less than 37°C. During this early period, the concentrations of various nitrogen in fish sauce at room temperature were far less than the corresponding values of fish sauce at 37°C (data not shown). However the values of properties increased and approached to those of sample at 37°C, as room temperature increased in mid summer. Beyond this period, the values of both samples (room temperature and 37°C) fluctuated.

Effect of delayed addition of salt

It was clear that, from Fig. 7, delayed addition of salt did not promote the proteolysis of fish sauce and even worsened both for control and enzyme-added counterparts. Delayed addition of salt plus 24 h keeping at 50°C further made decrease in various N concentrations in fish sauce.

Effect of mincing

As can be seen from Table 3, mincing made increase in T. S. N and V. B. N but decrease in A. N for samples without added enzyme. However, it is quite interesting that mincing resulted in inferior results for enzyme-added sample. One notable advantage of mincing was that the highest yield in volume of fish sauce were obtained (data not shown).

Effect of amount of enzyme used

From Fig. 8, it can be seen clearly that, all of the properties were highest for the sample with 0.3% papain. Sample with 0.5% papain showed better results than that with 0.2% enzyme. It is notable that sample with 0.2% enzyme had lower content of A. N even than control with 0% enzyme.

Organoleptic properties

At the early days of fermentation, the colour of fish sauce samples was amber yellow but it gradually darkened as the time proceeded, and became dark-red at the time of final filtration. There was no remarkable difference between the colour of samples

Table 3. Properties of fish sauce using minced and unminced fish.

Properties (mg/ml)	samples without added enzyme unminced	minced	samples with added enzyme unminced	minced
T.S.N	21.29	24.82	27.34	21.87
A.N	14.57	10.71	16.69	11.30
V.B.N	1.36	1.75	1.73	1.38

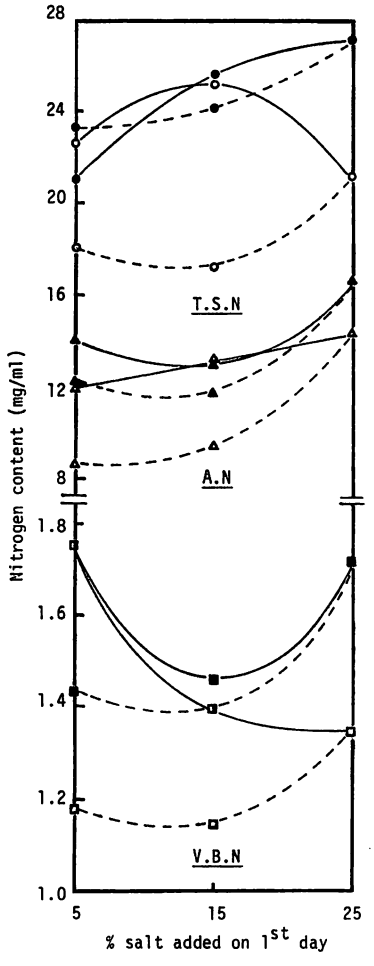

Fig. 7. Effect of delayed addition of salt on properties of fish sauce.

—○— —△— —□—
samples without added enzyme
—●— —▲— —■—
samples with added enzyme
——— 24 h kept at 37°C
····· 24 h kept at 50°C

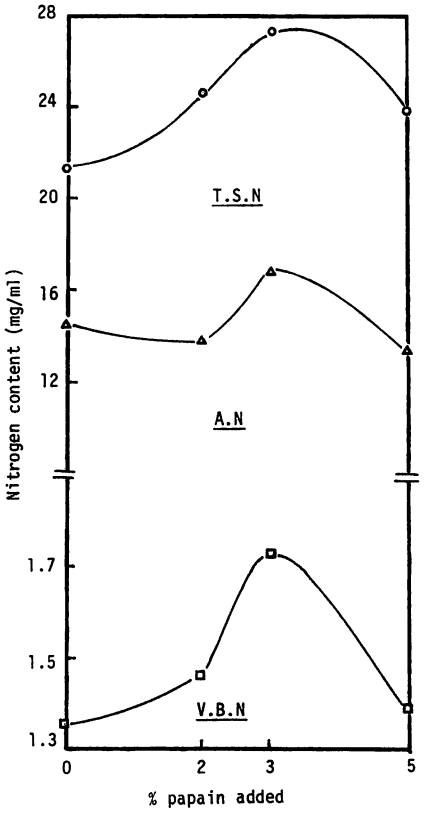

Fig. 8. Effect of % papain added on properties of fish sauce.

with no added enzyme and that of enzyme-added counterparts. Mackerel sauce were more reddish and ami sauce were darker than sardine sauce. As expected, the degree of darkness was in direct relationship with fermentation temperature — the highest temperature resulted in the darkest product. The colour of Burmese fish sauce was dark-brown and that of Chinese fish sauce was yellowish brown.

The taste of all sardine samples were stronger than that of commercial fish sauce products of Burma and China, and all were acceptable. However, the taste of enzyme-added product (eg. No. 13) was slightly sour, which might be the consequence of low pH (final pH of No. 13 was 4.90 in contrast to 5.55 of No. 1). No bitter taste was detected in all enzyme-added products. The taste of ami sauce was unsatisfactory.

Only very faint aroma appeared in all samples until the day of final filtration (340–350 days since fermentation started). Rancid smell was detected in mackerel sauce especially in the sample with added enzyme. This fact is in agreement with the result of some investigators[3]. The typical aroma of fish sauce is generally considered as comprised by three notes, namely, meaty aroma due to amino acids and amines, ammoniacal aroma due to volatile basic compounds, and cheese aroma due to volatile organic acids[7]. Total volatile acid content of samples were determined on their 153th day of fermentation (part of the results shown in Table 4) and found out that the values were far less than those of commercial products. When the individual volatile acids were analysed by gas chromatography, it was observed that only small amount of acetic acid appeared in the samples (Table 5). That the samples had very

Table 4. Total volatile acid content of some fish sauce samples.

Samples without added enzyme			Samples with added enzyme		
No	Description	T.V.A	No	Description	T.V.A
1	control	0.4	13	papain added	0.6
1*	ripened No 1	0.8	13*	ripened No 13	0.6
	Burmese sauce	8.0	25	bromelain added	0.5
	Chinese sauce	5.0	26	trypsin added	0.6

Note: 1) T.V.A is expressed in m*l* of 0.1 N NaOH titrated.
2) Explanation of ripened samples are referred to the text.

Table 5. Individual volatile organic acids of fish sauce samples.

Sample	volatile organic acids (mg/100 m*l*)				
	acetic	propionic	iso-butyric	n-butyric	iso-valeric
Burmese sauce	783.1	93.9	78.0	79.3	237.1
Chinese sauce	636.7	83.1	4.5	152.8	10.5
Sample No 1	60.6	—	—	—	—
Sample No 13	89.8	—	—	—	—

low V. B. N values was also partly attributable to their faint aroma. V. B. N increased considerably as the fermentation proceeded, but the values were still less than those of commercial fish sauce products (Table 2).

The synthetic aroma solutions (volatile organic acids) were prepared based on the results of commercial fish sauce products and added to the fish sauce samples in various ratios. It was found out that if sufficient amount of volatile organic acids solution was added to the fish sauce sample, either with or without added enzyme, the typical fish sauce aroma appeared distinctly. Hence, it is sure that volatile organic acids are important components for the development of fish sauce aroma.

One interesting point was that part of the fish sauce sample, without added enzyme, (No. 1), which was filtered in its 7th month of fermentation, and ripened at room temperature, was found that fish sauce aroma appeared when total processing time reached 344 days. Total volatile acid content of this ripened sample, (No. 1* in Table 4) became doubled than that of unripened sample, (No. 1), although it was still far less than that of commercial fish sauce products. The detection of individual volatile acids by gas chromatography was not carried out because of the insufficient amount of this ripened sample. From this result, it would be considered that the ripening or maturing period is essential for the development of fish sauce aroma. Most investigators on fish sauce did not mention the significance of ripening stage which, in fact, is practically established in production of fish sauce in Bruma. There may be colloidal compounds in the sludge of fish sauce mass which interferes with the development of aroma compounds.

On the other hand, when part of the sample with added papain, (No. 13), was filtered in its 7th month of fermentation and ripened till the total processing time reached 339 days, the aroma did not appear. This result was supported by the fact that total volatile acid content of this ripened sample was the same as that of unripened sample (No. 13* and No. 13 in Table 4). This fact was in alignment with what revealed by some researchers that the quality of fish sauce with added enzyme was poor[8].

Conclusions and Recommendations

1. Of the three types of enzymes tested, papain was the most effective in view point of proteolysis for the production of fish sauce from sardines. Mackerel resulted in rancid smell and the taste of ami was unsatisfactory.
2. Fermentation with 0.3% papain at 37°C without adjusting initial pH was the best condition for maximum proteolysis. Delayed-addition of salt and mincing before fermentation did not promote proteolysis and, even worsened for enzyme-added samples.
3. The taste and colour of papain-added samples were acceptable, and were not different from those of control sample without added enzyme.
4. The typical aroma of fish sauce did not appear in all of the samples, even though

the fermentation time proceeded to more than 340 days. Ripening or maturing period was found to be essential for development of aroma compounds.

5. Aroma did not appear in sample with added enzyme even though matured.

Determination of free amino acids content and ripening of samples at different ripening conditions are being carried out. It would be recommended that evolution of aroma compounds from fish sauce during its ripening period should be further investigated so that the significance of ripening period is well established.

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