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Biochemical changes associated with fast fermentation of squid processing by-products for low salt fish sauce

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Abstract

In order to enhance the economical values of squid processing by-products and reduce the environmental problems caused by the wastes from squid processing, the possibility of utilizing squid processing by-products for low salt fish sauce production was investigated. Low salt fish sauce was prepared experimentally from squid processing by-products, according to three different manufacturing techniques (A, B and C) with or without the autolysis process and the addition of flavourzyme, soybean koji. Fish sauce products with similar quality were obtained at 48 °C after 30 days fermentation. The content of total soluble nitrogen in three fish sauce A, B and C were 2.135 ± 0.038%, 1.958 ± 0.041% and 2.038 ± 0.043%, respectively. The content of formaldehyde nitrogen in three fish sauce A, B and C were 1.028 ± 0.038 g/100 ml, 1.000 ± 0.046 g/100 ml, 1.127 ± 0.043 g/100 ml, respectively. The salt content in three fish sauce A, B and C were 8.842 ± 0.138%, 9.058 ± 0.142%, 8.764 ± 0.129%, respectively. At the same time, changes in total soluble nitrogen, conversion of nitrogen, pH, formaldehyde nitrogen, total titration acid, total volatile base nitrogen, salt concentration, protease activity, total plate counts of fish sauce were observed during fermentation. The results suggested that total soluble nitrogen, conversion of nitrogen, formaldehyde nitrogen, total volatile base nitrogen and salt concentration increased throughout the fermentation period. However, pH, total titration acid, protease activity and total plate counts showed different changes in different fermentation stage. The result of amino acids analysis suggested that glutamic acid was the most prominent in three fish sauce samples. The results from quantitative descriptive analysis test showed that all fish sauce samples were tasted and no particularly strong or unpleasant flavor.

Keywords: Fish sauce; Squid by-products; Fermentation; Acceleration

1. Introduction

The exploitation of cephalopods has increased in recent years as a consequence of the declining stocks of commonly exploited fish species. The world demand for cephalopods increased at a rate of 15% per year between 1994 and 1996. The world total catches of cephalopods (squids, cuttlefishes and octopuses) were higher than 3.3 millions of tons between 1999 and 2001 (FAO, 2001). A large amount of squid by-products, which generally amount to 50% of the weight of the fish materials, was generated accompanying fishery processing. Despite international attempts to decrease squid wastes through various kinds of waste treatment systems, the quantity of wastes produced had been increasing annually (Nagai & Suzuki, 2000). These squid by-products could cause serious environmental problems if disposed improperly. Nowadays, the majority of squid by-products were dumped as wastes and caused serious environmental problems. Only a small part squid by-products was utilized for fishmeal and animal feed. And these recovered products are low in market values, making the recovery process uneconomical. It is necessity of converting these by-products into higher value products (Wang & Chang, 1997). In addition, some researcher reported that these squid by-products contained abundant natural protein and minerals that may be used for human

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consumption (Jeon & Kim, 1999; Kristinsson & Rasco, 2002). Therefore, the utilization of squid by-products for fish sauce production are both economical and environmental advantageous (Shi, Chen, Yu, Chang, & Wang, 2003).

Fish sauce fermentation is a common practice in Southeast Asia as a means of preserving and producing value-added products from underutilised fish species (Klomklao, Benjakul, Visessanguan, Kishimura, & Simpson, 2006). In Southeast Asia, fish sauce was not only popular as a condiment, but in some areas and certain social classes in the region, it was the main source of protein in the diet and had become a necessity in the household. Recently, Fish sauce has become more interesting for consumers in Europe, North America and other countries (Brillantes, 1999). Traditional fish sauce was produced by mixing whole fish with salt at a ratio of 1:1–3:1 and fermented for 6–12 months or longer (Tsai et al., 2006). High salt content in fish and shellfish sauces had limited its nutrient value because they could not be consumed in large quantities (Aryanta, Fleet, & Buckle, 1991). Therefore, low salt fish sauce is the urgent demand for society.

At present, although some works have been done on traditional fish sauce process (Dissaraphong, Benjakul, & Visessanguan, 2006; Shih, Chen, & Yu, 2003), there has few report on fast fermentation technology for low salt fish sauce, especially utilized squid by-products as materials. To accelerate the fermentation of fish sauce, soybean koji was used as inocula. The reason of koji used as inocula is that it was nonpathogenic and frequently used in food processing. In addition, it could add to the aroma, nutrition, and color of the fermentation product (Shih et al., 2003). The objective of the study is aimed at investigating fast fermentation technology for low salt fish sauce, in which squid byproducts were used as materials. The changes on biochemical and microbial were compared in different fish sauce fermentation technology process. It was hoped to obtain a fast fermentation technology for low salt fish sauce with better flavor and nutrition value.

2. Materials and methods

2.1. Raw materials

Squid (*Symplectoteuthis oualaniensis*) processing by-products used was obtained from China Aquatic Zhoushan Marine Fisheries Corporation, Zhoushan, China. Soy bean koji (*Aspergilus oryzae*) used was obtained from Qingdao Brewing Limited Corporation, Qingdao, China. Flovourzyme used was obtained from Novozyme Corporation (Danmark). Fig. 1 was a flow chart of fermented fish sauce process from squid by-products.

2.2. Fish sauce samples preparation

The squid (*S. oualaniensis*) processing byproducts was composed of heads, viscera, skin and fins. The by-products was thawed by tap water, cut into pieces and chopped into pastes. Fish sauce mush was prepared by fermenting the mixture of squid by-products pastes, soy sauce koji (20%, koji/pastes, w/w), distilled water (80%,water/pastes,w/w), salt (8%, salt/mixture, w/w) and with or without flovourzyme (0.2%, enzyme/pastes, w/w). The mixture was placed in conical flask (1000 ml) and the conical flask were covered tightly with triple-layer gauze clothe to maintain a semiaerobic condition. The mixture was fermented at 48 °C for 30 days and exhibited characteristic, favorable taste of fish sauce. Then the fish sauce mush was sterilization at 115 °C in autoclaves sterilizer for 10 min. The fish sauce mush cooled was centrifuged at 5000 r/min for 15 min. The lipid was removed with a spoon in the supernatant. The defatted fish sauce obtained was filtered with a filter paper (No. 5) and subjected to chemical analysis. During fermentation, the liquid formed was taken for analysis on days 1, 5, 10, 15, 20, 25 and 30.

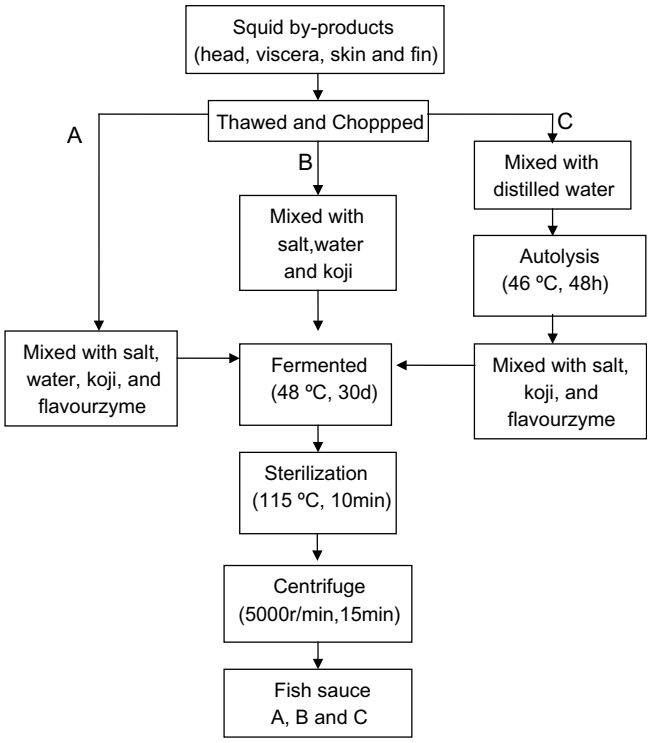

Fig. 1. Flow chart of fermented fish sauce from squid by-products.

2.3. Collection of liquid

At the designated time, the liquid used for protease activity detection was only centrifuged and filtrated.

2.4. Chemical analysis

2.4.1. Determination of pH, formaldehyde nitrogen and total titratable acid

The pH of liquid was determined directly using a digital pH meter (HM-5 S; TOA Electric Industrial Co. Ltd., Tokyo, Japan). Formaldehyde nitrogen was determined by the titration (Beddows, Ismail, & Steinkraus, 1979).

Diluted samples (20 ml) were mixed with 60 ml H_2O and titrated to pH 9.6 with 0.05 mol L^{-1}NaOH before 10 ml formalin solution (37%) was added. The volume of consumed was recorded in order to determine the total titratable acid of samples. The samples were finally titrated to pH 9.2 with 0.05 mol L^{-1} NaOH. All analyses were carried out in triplicate.

2.4.2. Determination of total soluble nitrogen (TSN) content and conversion of nitrogen

TSN content of fish sauce samples were measured using Kjedahl method (AOAC, 1999). TSN content was expressed as g/100 ml. The conversion of insoluble fish proteins into soluble form was estimated by the following equation:

$$\%\text{N conversion} = \frac{\text{soluble N\%} * \text{volume of fish sauce obtained}}{\text{N\% in squid byproducts} * \text{volume of squid by-products} + \text{N\% in koji} * \text{volume of koji used}}$$

All analyses were carried out in triplicate.

2.4.3. Determination of total volatile base nitrogen (TVB-N) contents and NaCl content

TVB-N content of each sample was measured using the Conway microdiffusion assay according to the method of Conway and Byrne (Conway & Byrne, 1936). The TVB-N were released after addition of saturated K_2CO_3 and then titrated with 0.01 N HCl. TVB-N content were calculated and expressed in mg/100 ml sample. Salt (NaCl) content was determined according to the Volard method (AOCS, 1990). All analyses were carried out in triplicate.

2.5. Measurements of proteinase activity

At the time designated, fish sauce sample was taken and centrifuged at 4 °C (5000 r/min × 15 min). The supernatant obtained was used for the enzyme solutions. Protease activity was assayed at 40 °C with casein (Merck, Germany) as a substrate. 1.0 ml of 2% casein in phosphate buffer (pH 7.2) was incubated for 5 min at 40 °C followed by adding 1.0 ml of the enzyme solution. After 20 min, the reaction was terminated by adding 2 ml of a 0.4 mol L^{-1} trichloroacetic acid (TCA) solution. The reaction was kept at 40 °C for 20 min and filtered through Whattman No. 5 filter paper. 1.0 ml of the filtrate was added with 5.0 ml of 0.4 mol L^{-1} Na_2CO_3 solution and 1.0 ml of Folin reagent. After mixed completely, the reaction mixture was kept at 40 °C for 20 min. The control test was identical with the sample except that TCA solution was added into the preheated enzyme solution immediately. The absorbance of the solution was measured at 660 nm. One unit (U) was defined as the amount of enzyme that released 1 μg equivalent of tyrosine per minute. All analyses were carried out in triplicate.

2.6. Microbiological analysis

Microorganisms in fish sauce are important to the quality of fish sauce. An aliquot of the fermented fish sauce were taken for microbial counts. Samples were taken aseptically from the conical flask and homogenized. Serial dilutions of homogenates were made and total viable counts were determined by the pour plate method, using Plate Count Agar (PCA, Oxoid, CM463) containing 0.5%NaCl as the medium. Plates were incubated at 35 °C for 48 h (Harrigan & Mccanee, 1976). Microbiological data were transformed into logarithms of the number of colony forming units (CFU/ml).

2.7. Free amino acid composition analysis

The fish sauce samples were used to determine the free amino acid composition using amino acid analyzer (835–50 model amino acid analysis apparatus, Japan). The concentration of amino acid in fish sauce samples were calculated by calibrating with standard amino acids.

2.8. Sensory evaluation

Quantitative descriptive analysis (QDA) was performed to determine the sensory characteristics of fish sauce samples. Analysis was carried out with an internal panel of nine members (five man and four women, age 20–35). Each sample was coded and presented to the panelists in isolation in a sensory laboratory. Members were asked to compare sensory attribute of each treatment (bitter, savory "umami," fishy, meaty, sour, caramel, ammonia, rancid) on a 10-point scale, "0" accounts for a not perceivable intensity while "9" accounts for an extreme intensity of an attribute.

2.9. Statistical analysis

Analysis of variance (ANOVA) was used to search for significant differences between mean values of the different results. The results are presented as means ± SD. The parallels $N = 3$ were used for all analyses.

3. Results and discussion

3.1. pH, formaldehyde nitrogen and total titratable acid

The changes of pH showed a similar trend among three manufacturing technique of A, B and C (Fig. 2). The pH of liquid decreased during the first 5 days. However, the pH of

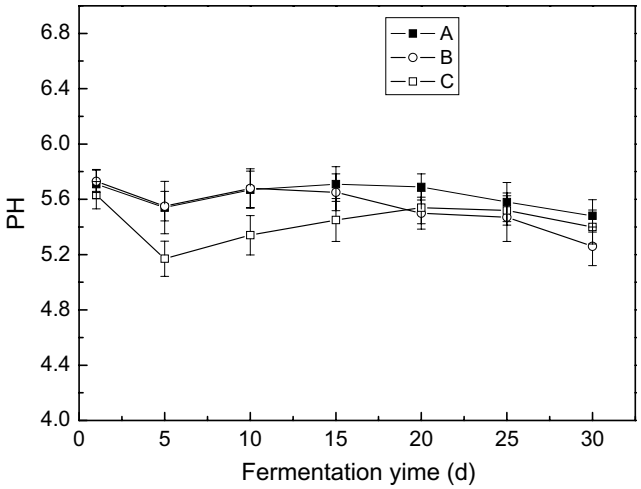

Fig. 2. Changes of pH during fermentation process.

liquid increased little during the 5–15 days and decreased again thereafter. The change of pH was probably due to produce acids and alkaline VBN during the fermentation. The pH fish sauce of A, B and C were 5.48 ± 0.12, 5.26 ± 0.14 and 5.40 ± 0.13 after 30 days fermentation, respectively. The pH was suitable for the sour taste in the final fish sauce.

Formaldehyde nitrogen is an important index used to classify the quality of fish sauce in China. In fermented foods, formaldehyde nitrogen content plays an important role as the indicator of degree of aging or putrefaction and optimum taste (Byun et al., 2000). Formaldehyde nitrogen content showed a similar change trend among three manufacturing technique of A, B and C (Fig. 3). The formaldehyde nitrogen content in liquids increased quickly during the first 20 days fermentation but increased a little thereafter. The formaldehyde nitrogen content in fish sauce A, B and C were 1.028 ± 0.038 g/100 ml, 1.000 ± 0.046 g/100 ml, 1.127 ± 0.043 g/100 ml after 30 days fermentation. Formaldehyde nitrogen content in fish sauce C was higher than one of fish sauce A, B. It was probably due to the difference in manufacturing technique because the manufacturing technique C included an autolysis procedure. The increase in formaldehyde nitrogen content suggested that protein was hydrolyzed gradually by the endogenous and koji proteases.

The changes of total acids content showed a similar trend among three manufacturing technique of A, B and C (Fig. 4). The content of total acids in liquids increased in all samples during initial 5 days fermentation and thereafter decreased gradually. The total acids content of liquid from three manufacturing technique A, B and C were 1.3894 ± 0.0407 g/100 ml, 1.2998 ± 0.0519 g/100 ml and 1.2101 ± 0.0540 g/100 ml, respectively after 5 days fermentation and thereafter fell gradually to 0.6843 ± 0.0499 g/100 ml, 0.6338 ± 0.0456 g/100 ml and 0.7243 ± 0.0494 g/100 ml, respectively after 30 days fermentation. The change of total acids content in liquids was probably due to the change of quantity of acid compounds and alkaline VBN produced during fermentation.

3.2. Total soluble nitrogen (TSN) content and conversion of nitrogen

The total soluble nitrogen content in liquid was one of the most important quality factors for fish sauce. It is the only objective index used to classify the quality of the Thai fish sauce nampla (Wilaipan, 1990). High quality nampla and patis must have 1.5% or higher total soluble nitrogen content, based on the Kjeldahl method (Lopetcharat, Choi, Park, & Daeschel, 2001; Lopetcharat & Park, 2002; Wilaipan, 1990). The total nitrogen content in the fish sauces samples (B) exceeded the minimum value for second grade fish sauce (1.5–2.0%) set by the Thai Industrial Standards Institute after 30 days fermentation and other two kind of fish sauce samples (A and C) exceeded the first grade limit (2%) after 30 days fermentation (Fig. 5). The total sol-

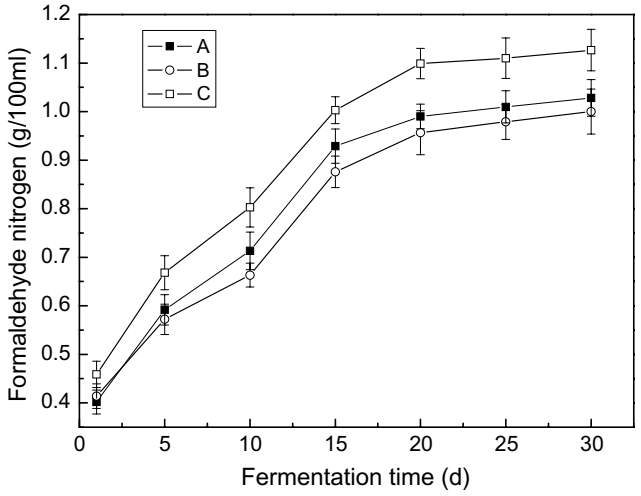

Fig. 3. Changes in formaldehyde nitrogen content during fermentation process.

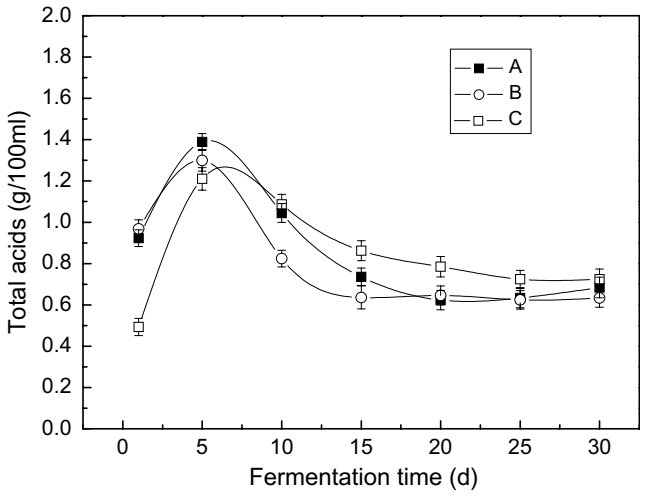

Fig. 4. Changes in total titratable acid content during fermentation.

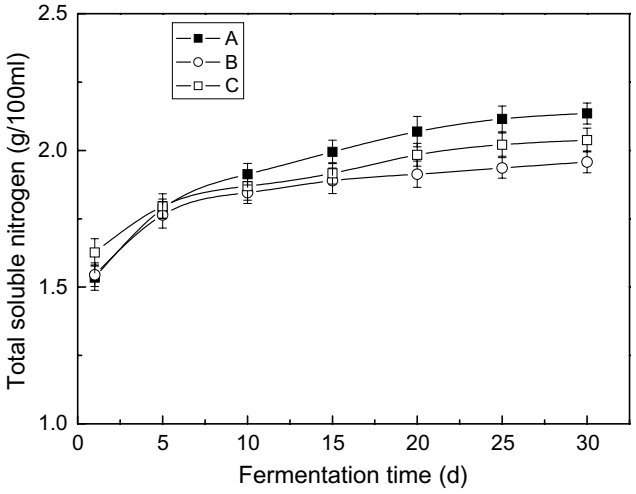

Fig. 5. Changes in total soluble nitrogen content during fermentation.

uble nitrogen content in three fish sauce samples (A, B and C) was $2.135 \pm 0.038\%$, $1.958 \pm 0.041\%$ and $2.038 \pm 0.043\%$, respectively after 30 days fermentation. The extraction of total soluble nitrogen from manufacturing technique (A) was slightly faster because proteases activity was higher in liquid. During the fermentation process, total soluble nitrogen content in the liquid increased, due to a breakdown of the fish proteins. The increase in total soluble nitrogen content during the initial fermentation stage had been connected to osmosis that leaded to the replacement of water and soluble nitrogen compounds from the fish cells and koji. Lopetcharat et al. (2001) reported 1.57% (15.7 g n/l) total protein for Pacific whiting, which had been mixed with salt after grinding and fermented for 10 days. They concluded that the fast fermentation of fish sauce from Pacific Whiting may have been caused by the combined effects of high enzymatic activity and grinding.

The changes in conversion of nitrogen showed a similar trend among three manufacturing technique of A, B and C (Fig. 6). The conversion of nitrogen increased quickly during the initial 15 days fermentation but increased a little thereafter. The conversion of nitrogen in fish sauce A, B and C were $81.943 \pm 3.564\%$, $80.882 \pm 3.013\%$, $83.687 \pm 3.344\%$, respectively after 30 days fermentation. The recovery of nitrogen was high.

3.3. Total volatile base nitrogen (TVB-N) contents and NaCl content

The changes in TVB-N contents of fish sauce samples (A, B and C) during fermentation were presented in Fig. 7. The changes of TVB-N value showed a similar trend among three manufacturing technique of A, B and C and increased gradually during 30 days fermentation. In addition, TVB-N content of fish sauce samples (C) was higher than samples (A and B). It might be due to the difference in manufacturing technique because there was an autolysis process in manufacturing technique C. Some volatile base compounds were produced during the autolysis process.

One of the goals of this research was to produce fish sauce with lower salt content. Salt content in fish sauce A, B and C were approximately $8.014 \pm 0.135\%$ after 1days fermentation and increased gradually to $8.837 \pm 0.138\%$, $9.063 \pm 0.142\%$, $8.758 \pm 0.129\%$, respectively after 30 days of fermentation (Fig. 8). The salt content observed in fish sauce samples A, B and C were lower than what was generally found in commercial fish sauce. Mizutani, Kimizuka, Ruddle, and Ishige (1992) found 25.9 ± 3.7 g/dl of salt in commercial fish sauce samples from south east-Asia. The low salt content might have favorable effects on fermentation process. These include an increased rate of protein breakdown, increased in nutritional value, and an acceleration of fermentation process. The activation of proteases in fish has been shown to be highly dependent on salt concentration in the environment. If the initial salt content was too high, it would not only retard enzymatic activity, but also cause the fish tissue to harden too much due to an

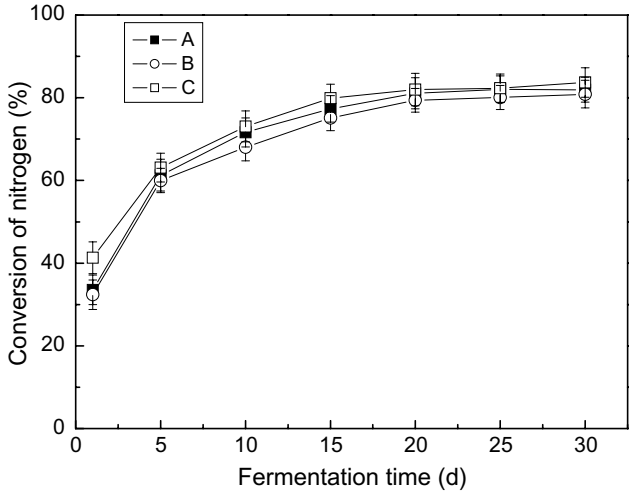

Fig. 6. Changes in conversion of nitrogen during fermentation.

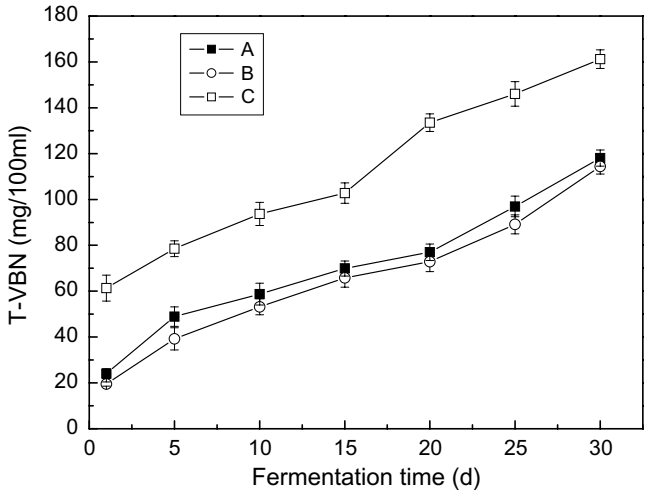

Fig. 7. Changes in volatile basic nitrogen (VBN) during fermentation.

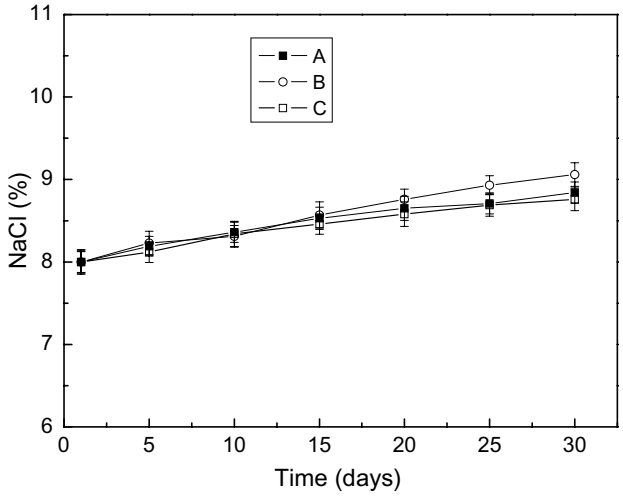

Fig. 8. Change in salt (NaCl) content during fermentation.

increase in osmotic pressure, thus further inhibited attack by proteolytic enzymes. Orejana et al. (1981) reported that pepsin was particularly sensitive to high salt concentration and was inhibited at 5% salt (Orejana & Liston, 1981). The initial salt content in the fish-salt mass in our study was 8.0% (w/w). Therefore, it is unlikely that pepsins played a major role during fermentation process.

3.4. Protease activity

Changes of protease activities in liquids from three different manufacturing techniques (A, B and C) showed a similar trend during 30 days fermentation (Fig. 9). The protease activities decreased during the first 10 days fermentation. However, the protease activities increased gradually during the 10–20 days fermentation and decreased again thereafter. The change of protease activities was probably due to salt concentration, growth and yield proteases of *Aspergilus oryzae* during fermentation process. The decrease during the initial fermentation was due to that salt concentration directly restrained protease activity. Generally, the activity decreased with increasing NaCl concentration (Klomklao et al., 2006). The apparent increase of protease activity was compositive effect of protease amount produced from koji and the restraining of protease activity result from salt concentration (Su, Wang, Kwok, & Lee, 2005). The decrease of protease activity in last period might be due to the denaturation of enzymes. The loss in activity was also thought to be due to the inhibition by end-products, such as amino acid and short chain peptides (Orejana & Liston, 1982).

3.5. Microbiological counts

Change of microbiological count was showed in Fig. 10. A continuously decrease in microbiological counts occurred during the first 10 days and then increased a little during 10–15 days fermentation and decreased thereafter in manufacturing technique A and B. There was a little difference between technique C and A, B. Microbiological counts in fish sauce C increased continuously during 5–20 days fermentation and decreased thereafter. The reason of difference might be due to microorganism growth and propagate during autolysis process in manufacturing technique C. With regard to the changes of bacterial count during fermentation, it should be explained that non-halotolerant bacteria decreased in the initial fermentation. But after 5 days fermentation, halotolerant bacteria began growth and propagate and lead to increase in microbiological counts (Paludan-Müller, Madsen, & Sophanodora, 2002). With the development of fermentation, due to absent in nutrition ingredient and accumulated in metabolite, microbiological counts decreased gradually. It has been reported that these halophiles and halotolerant bacteria mostly were LAB and yeasts.

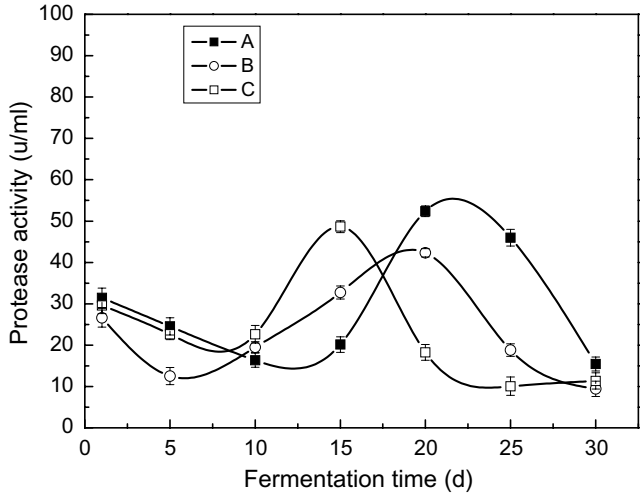

Fig. 9. Change in protease activities during fermentation.

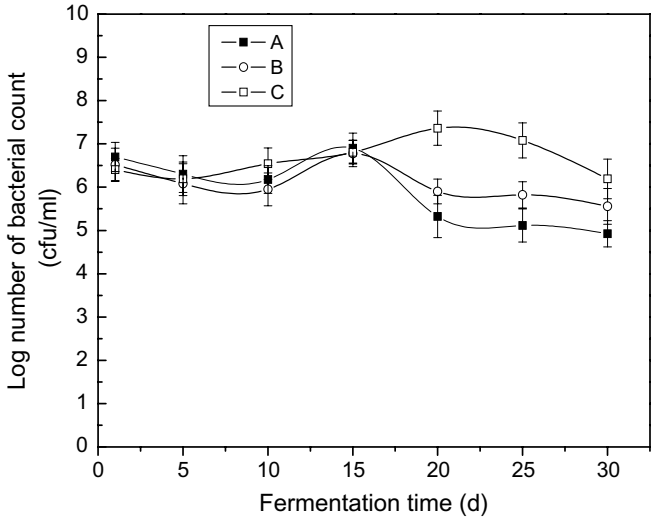

Fig. 10. Change in viable bacterial count during fermentation.

Table 1
Free amino acids profiles of fish sauce samples A, B and C

Amino acid	Fish sauce A (mg/g)	Fish sauce B (mg/g)	Fish sauce C (mg/g)
Aspartate	9.335	6.082	2.532
Glutamate	12.099	11.983	15.560
Serine	2.591	2.676	1.867
Glycine	3.581	2.971	5.139
Histidine	0.095	0.171	1.074
Arginine	0.579	0.475	0.259
Threonine	5.347	2.743	4.293
Alanine	7.218	4.958	9.519
Tyrosine	1.470	1.891	2.044
Valine	0.178	7.804	12.639
Methionine	2.374	2.038	2.818
Cysteine	8.442	5.476	2.384
Isoleucine	4.573	3.618	5.945
Leucine	7.315	5.681	9.392
Phenylalanine	2.926	2.456	2.975
Lysine	5.871	4.197	4.520
proline	2.269	1.083	3.093
Total	76.263	66.303	86.053

3.6. Amino acid composition

The amino acid composition of fish sauce may be nutritionally important especially in regions where fish sauce serves as a significant source of dietary protein. The concentration of free amino acids in the fish sauce C was higher than that in fish sauces A and B (Table 1). This difference may be traced to the different manufacturing technique. The manufacturing technique C contained an autolysis process which caused fish proteins broken down into free amino acids and peptides by endogenous proteases from fish viscera. The concentration of free amino acids in the fish sauce A was higher than that in fish sauce B. This was probably due to a higher protease activity in fish sauce A during the fermentation process. As shown in Table 1, Glutamate, Aspartate, Cysteine, Leucine and Alanine were prominent in fish sauce A. Glutamate, Aspartate, Valine were prominent in fish sauce B. Glutamate, Valine, Alanine, Leucine were prominent in fish sauce C. The difference in the amount of free amino acid among the samples seemed to be attributable to differences in the balance of free amino acids produced by autolysis and microbial action, respectively. Amino acids contribute significantly to the taste of fish sauce. For example, the typical aroma of glutamic acid is meaty. Glycine, alanine,serine and threonine taste sweet (Liu,1989). Lopetcharat et al. (2001) reported the contribution of different amino acid to the aroma of fish sauce (Lopetcharat et al., 2001).

3.7. Quantitative descriptive analysis (QDA)

The sensory profiles of fish sauce samples A, B and C based on the QDA test-ten attributes by nine panelists are shown in Fig. 11. Seen from the Fig. 11, there was a similar sensory quality among three fish sauces A, B and C. The scores for Umami, Caramel, Fishy, Meaty were higher and the scores for Bitter, Sour, Ammonia, Rancid were lower. It suggested that fish sauces produced in all treatments were tasted and no particularly strong or unpleasant flavor. It indicated that no apparent spoilage had occurred during 30 days fermentation.

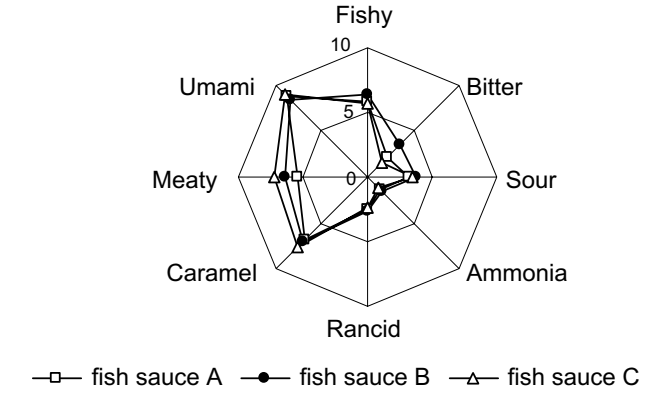

Fig. 11. Sensory profiles of three samples based on the QDA test-eight attributes by 10 panelists.

4. Conclusions

This study suggested that squid by-products could be quickly fermented into low salt fish sauce with acceptable qualities in terms of aroma and nutrition. In addition, the commercial available soybean koji was suitable inocula for the fast fermentation of fish sauce. Reduced salt content in processing may help to increase the fermentation rate and shorten the fermentation time of fish sauce, as well as to improve nutritional properties. Although the fish sauce produced by manufacturing technique C appeared to have slightly better qualities than fish sauce from technique A and B, these fish sauces produced in all treatments were tasted and no particularly strong or unpleasant flavor was found. It indicated that no apparent spoilage had occurred during 30 days fermentation. Therefore, elaboration of fermentation conditions, such as precise control of pH, temperature, time, quantity of koji, quantity of water etc., need to be further attempted. These factors are currently being investigated to achieve a low salt fish sauce product of commercial quality. In conclusion, the use of squid by-products had undoubtedly produced a fish sauce without any loss of nutritional quality and with an increased saving in capital costs.

Acknowledgement

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