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Chemical Composition and Protein Quality of Fish Sauces (*Kecap Ikan* and *Nampla*)

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Abstract- Chemical analyses were carried out on two commercial fish fermented products; *kecap ikan* which made from Indonesia and *nampla* from Thailand. Chemical composition, colour and amino acid composition were determined and the result shows the significant difference (p<0.05) in the parameters between two commercial fish sauces. Protein content in *kecap ikan* and *nampla* were 1.60 and 10.85 % respectively. Degree of protein hydrolysis was determined by precipitating the soluble nitrogen in 10% trichloroacetic acid (TCA) with 64.62% for *kecap ikan* which higher (p<0.05) than 56.10% degree hydrolysis in *nampla*. To assess the protein quality of these products, amino acid composition was determined. Total amino acid constitute for 707.8 mg/100mL protein in *kecap ikan* and *nampla* containing 8480.3 mg/100mL protein. Chemical score, amino acid score and essential amino acid index were 0.00, 0.00 and 0.56 for *kecap ikan* and 4.22, 5.88 and 7.32 for *nampla*. Protein digestibility is high in *kecap ikan* and *nampla* with 66.51 and 66.26% respectively. Protein digestibility-corrected amino acid score value was only 10.70 % in *kecap ikan* while 100% in *nampla*. The quality of protein in *nampla* indicates that it can provide complete essential amino acid to human. However, the quantity of protein and amino acid was not significant as source of essential amino acid in human diet because of limited daily intake.

Keywords— Fermented fish product, fish sauce, kecap ikan, nampla, protein quality

INTRODUCTION

Fish sauces are clear brown liquid produce by fermentation of underutilized fish species with high salt content in about 1:1-3:1 ratio (Tsai et al., 2006). Fermentation is one of the techniques used in preserving perishable fish due to its high salt concentration. In Thailand, they are well known as *nampla*,while in Philippines they are known as *patis* , follow by *kecap ikan* or *bakasang* in Indonesia, *budu* in Malaysia, *nuocnam* in Vietnam and Cambodia, and *nganpya ye* in Myanmar (Putro,1993; Ismail, 1977; Baens-Arcega, 1977; Phithakpol et al., 1995; Tyn, 1993; Tran, 2002). Fish sauces are also commonly produce from small pelagic species, such as anchovies and sardines (Amano, 1962; Gildberg, 2001; Saisithi, 1994).

In addition, fish sauces are popular among the consumer in most of Southeast Asian country as food condiment and also as good sources of protein supply in the diet (Brillantes, 1999). Based on studies report by Mizutani et al. (1992), the nitrogen content in fish sauces is about 20 g/l and 80% of this value is in form of amino acid compound. This advantage is due to natural fermentation involved in production of various types of fish sauces in their respective country. During fermentation, proteins which are the major component found in meat fish will be hydrolyzing into short amino acid chain which contributes in development of the flavor, aroma, texture and unique product characteristics of this product (Caplice et al., 1999). Therefore, it causes subsequence increase in total soluble nitrogen and the nutritional quality of food when providing all essential amino acid including mineral such as sodium (Na) , calcium (Ca), and magnesium (Mg) (Lopetcharat et al., 2001).

The process of fermenting the traditional fish sauce is conducted by combination of some reactions, which are salting, enzyme hydrolysis, and bacterial fermentation (Saisithi, 1994). However, the degradation of protein is primarily responsible by the endogenous fish enzyme. Bacterial activity is limited by high salt content which are the main ingredient in preparing fish sauces (Orejana and Listen, 1982). Fish enzyme such as chymotrypsin has high catalytic activity and thus it can hydrolyse more peptide bonds in protein substrates. As the major changes in fermentation process are the conversion of protein into small peptides and amino acid, the total nitrogen value become important indicator in determining the quality of fish sauces.

In any food product with high protein content, assessment on protein quality is very important as their function become more diverse in human health (Millward et al., 2008) .The method for protein assessment is protein digestibility-corrected amino acid score (PDCAAS) approach (FAO/WHO/UNU, 2007). This method was introduced by The Food and Agriculture Organization of the World Health Organization (FAO/WHO) during year of 1991.

Protein quality is defined by protein digestibility-corrected amino acid score (PDCAAS) as a measure of a protein's ability to provide sufficient amount of essential amino acids for human needs. The PDCAAS method combines the reference on age-related amino acid pattern which represent human requirement and the estimation on protein digestibility. Comparison of limiting amino acid with the respective reference pattern will be conducted to find amino acid score. This score indicate the ability of the protein being absorbed to fulfil human consumption on amino acid and corrected by its digestibility which then provide the net protein utilization or PDCAAS value (FAO/WHO/UNU, 2007).

The main objective in this study is to determine the chemical composition (moisture, protein, salt, water activity, pH, degree of hydrolysis) and colour of commercial *kecap ikan* fish souce from Indonesia and commercial *nampla* from Thailand as well as to determine the protein quality of *kecap ikan* and *nampla* by measuring amino acid composition and digestibility value.

MATERIALS AND METHODS

Material

Commercial fish sauces product from Indonesia (*kecap ikan*) and Thailand (*nampla*) were purchased from local market in Indonesia and local market in Malaysia respectively. Ten bottles of each fish sauce has been used in this study. *Kecap ikan* and *nampla* samples are stored at room temperature (26-28 ^{o}C) during analysis.

Determination of Moisture and Protein content

Analysis of moisture, protein and salt content was determined based on AOAC Method (AOAC, 2000).

Determination of water activity.

The water activity of the *Kkecap ikan* and *nampla* were checked using an Aqualab Water Activity Meter (Series 3, Decagon Devices, Inc., Pullman, WA, USA). A small amount of samples were placed in a disposable cup and water activity values were recorded when equilibrium water vapour was reached at the temperature of 25 ^{0}C. Result was reported as average of duplicates measurements.

Measurement of pH

The pH measurement was carried out using a pH meter (Mettler Toledo Delta, USA). Measurements were analyzed in triplicate for *Kecap ikan* and *nampla*.

Measurement of degree of hydrolysis

The degree of hydrolysis was estimated according to the method established by (Hoyle & Merritt, 1994). One volume of 20% trichloroacetic acid (TCA) was added to the supernatant, followed by centrifugation at 10000 rpm at 4 °C for 10 minutes to collect the 10% TCA-soluble materials. Total nitrogen in the 10% TCA soluble material and the substrate was estimated by Kjeldahl method (AOAC, 2000).

Measurement of colour

The colour parameters, L* (lightness), a* (redness), and b* (yellowness), of *kecap ikan* and *nampla* were measured using colourimeter (Minolta Spectrophotometer model CM-3500d, USA).

Amino acid composition

Amino acid composition was determined according to method of Sarwar *et al*., (1983). Samples were analyzed with three hydrolyses (6N HCl, performic acid + 6N HCl and 4.2 N NaOH). Samples were hydrolyzed with 6N HCl to obtain hydrolysates suitable for analysis of all amino acids except cystine+cysteine and tryptophan and methionine. Samples oxidized with performic acid for the determination of cysteine+cysteine and methionine. Samples hydrolyzed with 4.2N NaOH for the determination of tryptophan. The hydrolysates were then applied to an amino acid analyser (MLC-703; Atto Co., Tokyo, Japan).

Chemical score, Amino acid score and Essential amino acid index

The chemical score was determined by comparing the essential amino acid content (EAA) of samples to the reference protein pattern of egg (FAO/WHO, 1991). The amino acid score was determined by comparing the essential amino acid content (EAA) of the sample with suggested pattern of amino acid requirements for human nutritional needs (Sawar & Mcdonough, 1990). The amount of suggested for preschool children aged 2-5 years old was used in this study by (FAO/WHO, 1991). Next, the essential amino acid index (EAAI) was obtained from the chemical score data. The score was determined by calculating the $\log_{10}$ for every amino acid. The mean was converted to antilog as the amino acid index score (Acton & Rudd, 1987).

Protein digestibility assay

Protein digestibility is an important parameter in the determination of protein quality. *In vitro* method has been carried out as described by the (Hsu *et al*., 1977; Satterlee., *et al*., 1979) to estimate protein digestibility using a three-enzyme solution (trypsin, chymotrypsin and peptidase). The protein digestibility-corrected amino acid scores (PDCAAS) scores were stated in percantage terms (El & Kavas, 1996). PDCAAS of the samples were calculated by multiplying the lowest amino acid ratio (mg of an essential amino acid in 1.0 g test protein/mg of the same amino acid in 1.0 g reference pattern of the 8 essential amino acids plus tyrosine, cystine and histidine) by the *in vitro* protein digestibility.

Statistical analysis of data

The collected data were analyzed with Statistic Package for Social Science (SPSS) software version 16.0

and Excel (Microsoft Inc.). One-way analysis of variance (ANOVA) was used to determine the significance difference between samples with a significant level of α = 0.05. Tukey's test was used to perform multiple comparisons between means.

RESULTS AND DISCUSSIONS

Chemical Composition and Colour of *Kecap Ikan* and *Nampla*.

Table 1 shows the chemical composition and colour of *Kecap ikan* and *Nampla*. There was significant difference (p<0.05) in their chemical composition and colour. The percentage of moisture in *Kecap ikan* was higher compared to *Nampla*. However, total crude protein and salt content were higher in *nampla*. On the other hand, degree of protein hydrolysis was higher in both fish sauces sample. The colour of kecap ikan was lighter compared to *nampla*. The liquid fish sauce of *nampla* has darker colour and has lower viscocity properties compared to *kecap ikan*.

Table 1: Chemical composition and colour of *Kecap Ikan* and *Nampla*

Parameters	*Kecap ikan*	*Nampla*
Moisture (%)	71.87±0.33[b]	62.86±0.55[a]
Protein (%)	1.60±0.13[a]	10.85±0.50[b]
Water activity (Aw)	0.81±0.00[b]	0.79±0.00[a]
Salt (%)	26.66±0.82[a]	27.00±0.40[b]
pH	5.30±0.05[a]	5.42±0.05[b]
Degree hydrolysis (%)	64.62±5.74[b]	56.10±1.88[a]
Lightness(L*)	72.77±0.01[b]	62.36±0.01[a]
Redness (a*)	11.49±0.01[a]	28.45±0.01[b]
Yellowness(b*)	57.29±0.02[a]	85.56±0.04[b]

[ab]Means with different letter(s) of *kecap ikan* and *nampla* within the same column are significantly different (p<0.05).

The result shows that *kecap ikan* contains 71.46 to 71.87% moisture values while *nampla* has about 62.17 to 63.60 % moisture from their overall composition. *Kecap ikan* contains average total value of 71.87% moisture while *nampla* contains 62.86% moisture. From statistical analysis, moisture in *kecap ikan* was significantly higher (p<0.05) compared to *nampla*. The percentage moisture of *nampla* was very near to the moisture of commercial fish sauce reported by Lopetcharat and Park (2002) which is 64.51%. Moisture value of *kecap ikan* was within the two type of *kecap ikan*, where *kecap ikan* A has 66.67% moisture and *kecap ikan* B has 76.89% moisture (Poernomo et al., 1984).

Amount of protein in *nampla* was significantly higher (p<0.05) compared in *kecap ikan*. The protein value in *nampla* was follow the requirement stated in Thai Industrial standard which cannot less than 2.0% of total nitrogen or 12.5% protein. Park et al. (2001) reported that *nampla* contains 10.5% protein content which was slightly lower compared to average protein of 10.85 obtains during the experiments. However, amount of *kecap ikan* was much lower compared to the value of 10.17 and 10.51% protein in *Kecap ikan* A and B as reported by Poernomo et al. (1984). Protein determination is variety in fish sauces sample because of different type of raw material used and also the processing method (salt concentration and fermentation condition). Different composition of protein in the fish as the raw material therefore produce different amount of protein in these fish fermented products. So, the lower protein content in *kecap ikan* can be due to loss of soluble protein during the washing steps and dilution of the liquid fish extract.

Salt analysis in *kecap ikan* result in high amount of salt was present with 25.49 to 27.82%. On the other hand, *nampla* also have high salt content with value of 26.39 to 27.55%. The average salt content in *kecap ikan* and *nampla* were 26.66 and 27.00% respectively. As refer to Standard Nasional Indonesia (SNI) (1996), salt content in *kecap ikan* was slightly higher compared to the value outline which was between 19 to 25% salt. Amount of salt in *nampla* sample was 27.00%, which was an acceptable value as it cannot be less than 20% salt content according to Thai Industrial Standard (1983). A report by Mizutani et al. (1992) support this result when found 25.9% of salt in commercial fish sauces from South East-Asia.

From statistical analysis, the amount of salt in *nampla* was significantly higher (p<0.05) compared to *kecap ikan*. Salt was important ingredients in fish sauce production as it can help to accelerate fermentation and inhibit the spoilage bacteria at concentration of 18% salt (Sikorski et al. 1995).The bacterial and enzymatic activity was changed at different salt concentration, therefore effect the effectiveness of fermentation process. So the concentration of salt must be at optimum so that it neither inhibits the growth of fermenting organism nor deteriorate the quality of final products.

Kecap ikan has water activity value between 0.81 and 0.82 while *nampla* has value 0.79. The average values of water activity were 0.81 and 0.79 for *kecap ikan* and *nampla* respectively. Salt plays very important role in determining the stability and quality of finish products. It preserves the food product by lowering the water activity (Aw) and creates a condition that was less favourable for microbial activity (Barbut et al., 1986). Davidson (1997) reported that water activity 0.90 can be achieved by approximately 15.5% amount of salt which can inhibit most of bacteria. A fish fermented product called as Naniura, which mostly consume by community in North Sumatra has water activity 0.8 (Huda, 2012). High salt concentration in fish sauces products reduce the water activity (Aw) and provides an advantage as bacteriostatic against gram negative putrefactive bacteria and other spoilage microorganisms. It will give stability and longer the shelf-life of food products.

The pH of *kecap ikan* was in between 5.22 and 5.37 while *nampla* had pH value of 5.39 to 5.47. Average pH value of *kecap ikan* was 5.30 and *nampla* was 5.42. All value was acceptable as lie between range value stated in Thai Industrial Standard and Standard Nasional Indonesia (SNI). These standards state that the pH must be between 5.0 and 6.0. Average pH value shows the significant different (p<0.05) between *kecap ikan* and *nampla*. pH was important parameter that affect the fermentation process. At the beginning of fermentation, the digestive enzyme responsible for autolysis (trypsin and chymotrypsin) was stable at neutral condition (Lopetcharat et al., 2001). This would help in increasing protein hydrolysis. The lower (more acidic) pH of *kecap ikan* was observed compared to *nampla*. This was probably due to minor ingredient added in *kecap ikan* during fermentation such as glucose. According to Frazier and Westhoff (1988), lactic acid bacteria might be present in the product solution which then able to ferment glucose as a carbon source into lactic acid, consequently lowering the pH. The pH observed by the Lopetcharat and Park (2002) on commercial fish sauces samples were higher than the observe pH of both sample which was 5.48. This product may have been seasoned with food grade additive such as citric acid and sorbic acid to lower the pH and adjust the colour of fish sauces product.

As shown by Table 1, *kecap ikan* has high degree of hydrolysis value which in the range of 60.36 and 67.86% compared with a slightly lower value of *nampla* which about 54.10 to 57.82%. Average degree of hydrolysis for *kecap ikan* was 64.62%, significantly higher ($p<0.05$) than 56.10% for *nampla*. High degree of hydrolysis shows that percentage of peptides bonds cleaved was also high (Jin et al., 2007). Degree of hydrolysis represents the extent of the hydrolytic degradation of the protein in sample. A study conducted by Orejana and Liston (1982) stated that endogenous enzymes are the major agents responsible for protein digestion during the fish sauce production. Degree of hydrolysis of fish sauce was measured by precipitating the nitrogen compound released by protein hydrolysis in the presence of trichloroacetic acid (TCA), a precipitating agent (Hoyle and Merrit, 1994). It also refers to the percentage of peptide bonds cleaved (Jin et al., 2007). TCA was used because it was effective although at lower concentration. High degree of hydrolysis indicates much greater amount of complex compound will cutting down into smaller compound. A study conducted by Haslaniza et al., (2010), show that 55.00 % degree of hydrolysis produce about 0.70% of nitrogen in meat wash water. In *nampla*, they contain about 1.69% of nitrogen which was much higher compared to protein of meat wash water. Although the degree of hydrolysis is high, the amount of nitrogen produce can be different because the differences in protein composition of fish and also fermentation time and method.

High lightness intensity or L* value can be observed in *kecap ikan* sample with value of 70.63 to 74.59 compared with *nampla* with only 58.69 to 66.27. However, b* hue represent the redness was dominant in *nampla* with value of 27.28 to 29.07 but lower in *kecap ikan* with value between 10.64 and 13.29. Yellowness was imparted greater on *nampla* product with value range about 83.27 to 87.24. *Kecap ikan* consist of 56.69 to 59.18 b* hue value. Average L*, a* and b* value of *kecap ikan* was 72.77, 11.49, 57.29 while *nampla* was 62.63, 28.45 and85.56. Colour of *nampla* was near to colour of commercial fish sauces reported by Lopetcharat and Park (2002) with lightness value was 58.24, redness 20.17 and yellowness 71.80. No report about the colour of kecap ikan from previous study. Measurement of browning colour can be used as quality indicator to observe the formation of total nitrogen in fish sauces product. It is true for *kecap ikan* because the low nitrogen and protein content illustrate by their light yellow colour. *Nampla* constitute much higher protein content presenting its dark brown colour. Most of nitrogenous compound in fish sauce such as free amino acid and small peptides were contribute to brown colour development especially at temperature 50^0C, where the rate of development was high. This browning effect is also due to the non-enzymatic reaction, mainly the Maillard reaction. The substrate of carbohydrate derivate such as glucose-6-phosphate might come naturally from the fish through metabolic pathway (Kawashima and Yamanaka, 1996) and might be due to addition of spices and other minor ingredient.

Amino Acid Composition and Protein Quality

The amino acid compositions of *kecap ikan* and *nampla* have shown in Table 2. The essential amino acid content in *nampla* was higher than in *kecap ikan*. Glutamic acid was dominant in both samples. From the result of amino acid composition, chemical score, amino acid score and essential amino acid index of *kecap ikan* and *nampla* are calculated and the value are also presented in the Table 2. Digestibility and protein digestibility-corrected amino acid value have been determined to evaluate the protein quality of *kecap ikan* and *nampla*.

Table 2: Amino acid composition (mg /100mL) of fish sauces sample.

Amino acid	***Kecap ikan***	***Nampla***
Essential		
Histidine	10.20±0.00 [a]	392.20±0.01 [b]
Isoleusine	61.00±0.00 [a]	310.80±0.02 [b]
Leucine	92.70±0.00 [a]	388.30±0.02 [b]
Lysine	48.50±0.02 [a]	943.20±0.05 [b]
Methionine	4.50±0.00 [a]	209.70±0.01 [b]
Phenylalanine	42.00±0.00 [a]	304.70±0.02 [b]
Threonine	9.00±0.00 [a]	606.70±0.04 [b]
Tryptophan	0.00±0.00 [a]	77.20±0.01 [b]
Valine	78.20±0.00 [a]	567.00±0.03 [b]
Non-essential		
Alanine	60.00±0.00 [a]	644.70±0.04 [b]
Arginine	26.00±0.00 [a]	157.70±0.01 [b]
Aspartic acid	40.70±0.00 [a]	932.30±0.07 [b]
Cysteine	0.00±0.00 [a]	47.50±0.01 [b]
Glutamic acid	128.50±0.02 [a]	1449.70±0.04 [b]
Glysine	20.00±0.00 [a]	558.20±0.98 [b]
Proline	51.30±0.01 [a]	395.70±0.03 [b]
Serine	8.70±0.00 [a]	407.20±0.20 [b]
Tyrosine	26.50±0.00 [a]	87.50±0.05 [b]
∑ **Essential**	346.10	3799.80
∑ **Non-essential**	361.70	13160.80
Chemical score	0.00(Trp)	4.22(Phe+tyr)
Amino acid score	0.00(Trp)	5.88(Leu)
EAA index	0.56	7.32
***Protein digestibility (%)**	66.51	66.26
PDCAAS (%)	10.70	100

*Reference protein: casein 89.33% digestibility.

Amino acid profile in *kecap ikan* shows that leucine and valine were dominant with 92.70 and 78.20

mg/100mL protein in their respective value. Lysine constitutes the 4th place among essential amino acid with amount 48.50 mg/100mL protein after isoleusine 61.00 mg/100mL protein. Tryptophan, methionine and threonine were the lowest essential amino acid present in *kecap ikan* with amount 0.00, 4.50 and 9.00mg/100mL protein respectively. The concentration of amino acids present in *nampla* was relatively near to the amino acid profile of commercial fish sauce reported by Cho and Choi (1999). Overall composition of amino acid shows that *nampla* contain high essential amino acid especially lysine with 943.20 mg/100mL protein. Among of nine essential amino acid component, *nampla* was rich with threonine and valine with value of 606.70 and 567.00 mg/100mL respectively. Glutamic acid and alanine were major component found among non-essential amino acid. The lowest essential amino acids were tryptophan, methionine and phenylalanine with amount 77.20, 209.70 and 404.7 mg/100mL protein respectively.

Based on the study of commercial fish sauce (Thailand) by Park et al., (2001), the total amino acid concentration was 6456.5 mg/100mL protein. This value is much lower compare to the total amount of amino acid in *nampla* which contains 16960.6 mg/100mL protein. The dominant essential amino acid in the commercial product and *nampla* was lysine with relatively similar amount of 956.5 and 943.2 mg/100mL protein. However the amino acid threonine (606.7 mg/100mL protein) was higher in *nampla* compared to commercial product (379.4 mg/100mL protein) reported by Park et al. (2001). Amino acid valine in *nampla* and commercial products were 567.0 and 476.1 respectively.

The non-essential amino acid such as glutamic acid was higher in *nampla* with value 1449.7 mg/100mL protein compare to commercial product which has 1205.1 mg/100mL protein. Arginine in commercial product constitutes only 6.8 mg/100mL protein from total amino acid compare to 157.7 mg/100mL protein in *nampla*. These values contribute to a great different total amino acid in *nampla* and the commercial products studies by Park and others. In addition, amino acid cysteine was not determined by Park and others. There is not much analysis on amino acid in *kecap ikan* in previous study. However, in study of amino acid in *bakasang*- a fish fermented food from eastern part of Indonesia- total amino acids have range in between 43.26 and 46.65 mg/ml and they contribute to supply essential amino acid. They were good in supplying valine, isoleucine, phenylalanine and lysine same with *kecap ikan*

Amino acid composition was important especially in region that consume fish sauce as dietary protein. They were produce from the enzyme reaction and the action of halotolerant microorganism which hydrolyze fish protein into amino acid, peptides and ammonia. The difference in amino acid composition of *kecap ikan* and *nampla* produced from same type of fish species may affected by the type of feed and also the source of fish itself. Predominan amino acid for example alanine and glutamic acid were important to impart taste in fish sauce products (Park et al., 2002). Alanine and lysine were high in long-fermented products. They also contribute to unique characteristic of taste such as glutamic acid which imparts umami taste.

A complete amino acid composition was required in order to calculate the chemical based measures of protein quality. Result in Table 2 shows that tryptophan has the lowest chemical score value compared to another essential amino acid and therefore become the limiting amino acid in *kecap ikan*. Otherwise, *nampla* was limited with phenylalanine and tyrosine with 4.22 chemical score. Sikorski et al., (1990) reported that tryptophan was the limiting amino acid in ten fish protein during their study. Other than tryptophan, phenylalanine can also become one of limiting amino acid. The difference in limiting amino acid shows that different composition of protein and amino acid was present in each fish species. The process of washing can also contribute to these differences. This method was scoring the protein based on chemical composition of amino acid and not related to the digestion of protein and therefore not reflect the use of protein in human body. The lowest chemical score value help to identify the limited amino acid supply to human. This result indicates that protein in fish sauce contains lower amount of tryptophan in *kecap ikan* while phenylalanine and tyrosine in *nampla* therefore need to get supplement or food that rich in these limiting amino acid.

The value of amino acid score represent deficiency or adequacy of essential amino acid in food product for consumption of human body. Based on the result in Table 2, tryptophan was the limiting amino acid in *kecap ikan* with scoring value of 0.18 while *nampla* was limited in amino acid leucine with 5.88 score value. Sikorski et al. (1990) reported that the limiting amino acid in ten fish protein was also tryptophan. From the result, amino acid tryptophan from *kecap ikan* and leucine from *nampla* cannot be supply in adequate amount for human consumption. They must be derived from other sources such as protein from cereal or nuts. This combination of protein from cereal and fish can provide the amount of amino acid needed for human body. In addition, the amount of essential amino acid in fish sauce is much lower than the amount needed by children aged 2-5 years old. This would indicate that they should take other foods to fulfil their requirement of essential amino acid for growth. Fish sauces are not suggested to be the nutritional food for them.

Index essential amino acid has greater correlation with *in vivo* measure of protein quality compared to chemical scores. However the limiting amino acid cannot be determine by this index value. As the result, *kecap ikan* has lower index amino acid with only 0.56 while *nampla* has index value 7.32. Acton and Rudd (1987) reported that essential amino acid of fish protein was 79-90 score and average value of 85. The lower scoring value of *kecap ikan* and *nampla* were due to lower protein as well as amino acid content.

Protein quality determination by *in vitro* method can estimate the nutritional value for human diet. As the result, *kecap ikan* has average value of digestibility of 66.51% and *nampla* constitute 66.26%. This protein digestibility value is lower compared to soy protein which having digestibility value of 75 to 85% by using the same method (Clatterbuck et al., 1980). The high value of *in vitro* digestibility shows high predicted digestibility and more easily to be absorbed in human intestinal. Fermentation process aimed to prolong the shelf- life and improves the palatability of food. Other than that, it also improves the digestibility of food due to partial degradation of complex protein into more simple and soluble products. Reduction in pH during fermentation period was believed to enhance the native proteolytic enzyme activity and consequently promotes the breakdown of protein into smaller peptide which is easily to be digested by enzyme (Monawar, 1983). Fish protein has high digestibility than protein derived from vegetables and poultry (Suzuki 1981, Mc Donough et al. 1990, Damodaran 1996). However the lower digestibility obtains from experiment was probably due to high stroma protein in the fish meat and therefore reduces the percentage of protein digestibility for fish sauces sample.

However, *in vitro* assay method usually comes with underestimated digestibility compared to *in vivo* assay. The reason was describe by Satterlee et al., (1977). This is due to highly sensitive of this method towards small chemical changes in protein structure. A slightly increase in peptide bond for enzymatic attack would result in large increase in *in vitro* digestibility compared to *in vivo* assay (Eicher, 1982). The calculation of *in vitro* are based on pH value and any error during the measurement at the beginning would affect the final result. Autolytic enzyme would cause rapid changes in freshness of fish meat after harvesting. Changes in freshness will increase the released of amino acid while decrease the protein substrate for enzyme action. This will let the poor freshness of fish having smaller changes of pH compared to the fresh one therefore underestimate the protein digestibility.

Protein digestibility – corrected amino acid score (PDCAAS) was an approved method for evaluating protein quality of food products. Based on the protein digestibility estimation and amino acid score data, *kecap ikan* have PDCAAS value between 10.52 and 10.88% while *nampla* have value greater than 100% and then truncated to 100% value. The 100% PDCAAS value indicate that the protein from fish sauces can provide adequate amount of all essential amino acid and become a complete protein for growth. However, the limited amount of fish sauces intake in daily consumption will reduce the effectiveness of fish sauce to provide complete amino acid for human requirement.

CONCLUSION

The result from chemical composition analysis showed that *nampla* has high protein quality compared to *kecap ikan*. The protein content in *nampla* and *kecap ikan* were 10.85 and 1.60% respectively. Amino acid composition analysis shows that *nampla* has complete amino acid. The chemical score, amino acid score, and essential amino acid index for *nampla* were 4.22, 5.88 and 7.32 and for *kecap ikan* were 0.08, 0.18 and 0.56. Digestibility by *in vitro* method shows that *kecap ikan* and *nampla* have high protein digestibility and therefore easy to absorb as nutrient for human.

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REFERENCES

Acton, J.C.and C.L. Rudd, (1987) Protein Quality Method for Seafood. In: Seafood Quality Determination, Kramer, D.E. and J. Liston (Eds.). Elsevier, Amsterdam, pp: 453-473.

A.O.A.C (1990) Official Methods of analysis of the association of official analytical chemistry (15th ed.). Arlington, VA: Association of Official Analytical Chemists.

A.O.A.C (2000) Official Methods of analysis of the association of official analytical chemistry (17th ed.) AOAC International: Gaithersburg, MD.

Amano, K. (1962). Influence of fermentation of the nutritive value on fish with special reference to fermented fish products of Southeast Asia. In E. Heen & R. Kreuzer (Eds.), Fish in nutrition (pp. 180–200). London: Fishing News

AOAC International. (1995) Official methods of analysis of AOAC International. 2 vols. 16th edition. Arlington, VA, USA, Association of Analytical Communities.

Badan Standarisasi Nasional. (1996) Standar Nasional Indonesia (SNI). SNI 01-4271-1996.Produk Kecap Ikan. Jakarta : Dewan Standarisasi Indonesia.

Baens-Arcega, L (1977) Patis, a traditional fermented fish sauce and condiment of the Philippines. Symposium of Indigenous Fermented Foods, Bangkok, Thailand.

Barbut, S., Tanaka N., Maaurer A.J.,(1986) Effect of varying levels of chloride salt on *Clostridium botulinum* toxin production in Turkey Frankfurters, *J Food Sci*, 51(5) 1129-1131.

Brillantes, S. (1999) Histamine in fish sauce-health and safety considerations, *Infofish International*, 4, 51–56.

Clatterbuck, K.L., Kehrberg, N.L.,and Marable, N.L. (1980) *Journal of food science* 45: 935

Damodaran, S. (1996). Amino acids, peptides and proteins. In: Fennema O.R. (Ed.) Food Chemistry (pp. 321-429) 3rd edition, New York: Marcel Dekker.

Davidson, P.M.,(1997) Chemical preservatives and natural antimicrobial compounds, In: *Food Microbiology-Fundamentals and Frontiers*, edited by Doyle MP, Beuchat LR & Montville TJ, (ASM Press, Washington DC).

Eicher, N., (1982) The chemical and nutritional characteristics of great northen bean protein as affected by pH and heat, MS thesis, University of Nebraska –Lincoln.

El, S. N., and Kavas, S.N. 1996. Determination of protein quality of rainbow trout (*Salmo irideus*) by *in vitro* protein digestibility – corrected amino acid score (PDCAAS). Food Chemistry, 55(3): 221-223).

Caplice, E., (1999) Food fermentations: role of microorganisms in food production and preservation, *International Journal of Food Microbiology* 50, 131–149.

FAO/WHO (1991) Protein Quality Evaluation; Food and Agricultural Organization of the United Nations: Rome, Italy,p 66

FAO/WHO/UNU WHO (1985). Energy and Protein requirement. Report of a Joint FAO/WHO/UNU Expert Consultation, Tech. Rept.Series No. 724, WHO, Geneva, Switzerland.

FAO/WHO/UNU WHO (2007). Protein and amino acid requirements in human nutrition. Report of a Joint FAO/WHO/UNU Expert Consultation, Tech. Rept.Series No. 935, WHO, Geneva, Switzerland.

Frazier, W.C., Westhoff, D.C., (1988) Food Microbiology. McGraw Hill, New York.

Gildberg, A. (2001). Utilization of male Arctic capelin and Atlantic cod intestines for fish sauce production–evaluation of fermentation conditions. *Bioresource Technology*, 76, 119–123.

Haslaniza H., Maskat, M.Y., Wan Aida, W.M., Mamot, S. (2010) The of enzyme concentration, temperature and incubation time on nitrogen content and degree of hydrolysis of protein precipitate from cockle(Anadara granosa) meat wash water, Int. Food Research Journal 17:147-152

Hoyle, N.T.and Merritt, J.H. (1994) Quality of fish protein hydrolysates from herring (Clupea harengus). Journal of food Science 59(1): 76-79.

Hsu, H.H., Vavak, D. L., Satterlee L.D., Miller., G.A. (1977) A multienzyme technique for estimating protein digestibility, *J.F.S*. 42: 1269-1273.

Huda, N., (2012) Indonesian Fermented Fish Products In: Handbook of Animal-Based Fermented Food and Beverage Technology, Second Edition,Y . H . Hui and E . Özgül. Evranuz CRC Press 2012 Pages 717–738.

Ismail, M.S., (1977) Accelerated fermentation of fish soy paste and fish soy-sauce by using Aspergillus oryzae NRRL 1989. Symposium on Indigenous Fermented Foods, Bangkok, Thailand.

Kawashima Kand Yamanaka H. (1996) Free amino acids responsible for the browning of cooked scallop adductor muscle. Fisheries Science 62(2): 293-296.

Lopetcharat, K.and Park, J. W. (2002). Characteristics of fish sauce made from Pacific whiting and surimi by-products during fermentation storage. *Journal of Food Science*, 67, 511–516.

Lopetcharat, K., Choi, Y.J., Park, J.W., Daeschel, M.A. (2001). Fish sauces products and manufacturing –a review. *Food International*, 17(1), 65-88.

McDonough, F. E.; Steinke, F. H.; Sarwar, G.;Eqqum, B.O.;Bressani, R.; Huth, P.J.;Barbeau, W.E.;Mitchell,G.V.;Philips, G.J.(1990) *In vitro* rat assay for true protein digestibility: Collaborative study. *J Assoc. Off. Anal. Chem*.73:801-805

Millward D. J., Layman D. K., Tome D., Schaafsma G. (2008) Protein quality assessment: impact of expanding understanding of protein and amino acid needs for optimal health. *Am. J. Clin. Nutr*. 87, S1576–S1581.

Mizutani, T., Kimikuza, A., Ruddle, K., Ishige, N. (1992). Chemical components of fermented fish products *Journal of Food Composite Analysis*, 5, pp. 152–159

Monawar, L.Y., (1983) Food value of Sudanese indigenous cereal grains. Ph.D. Thesis, University of Khartoum, Sudan.

Orejana, F. M., Liston, J. (1982). Agents of proteolysisand its inhibition in Patis (Fish sauces) fermentation, *J of Food Sci*, 47, 198-203.

Park, J.N., K. Ishida, T. Watanabe, (2002) Taste effects of oligopeptides in a Vietnamese fish sauces. Fisheries Science 68:921-928.

Park, J.N., Y. Fukumoto, E.Fijita, (2001) Chemical composition of fish sauces produced in Southest and East Asian countries. Journal of Food Composition Analysis 14:113-125.

Phithakpol,B,Varanyanond,W.,Reungmaneepaitoon, S., Wood, H (1995) The traditional fermented foods of Thailand. *ASEAN Food Handling Bureau*, p 3.

Poernomo, A. , Suryaningrum, T.D. Ariyani, F., Putro, S. (1984) Studies on the nutritive value and microbiology of traditional fishery products. Laporan Penelitian Teknologi Perikanan 30: 9-19 (*in Indonesian)*

Putro, S., (1993) Fish Fermentation Technology in Indonesia. In: Fish Fermentation Technology. Lee, CH, Steinkraus, K H and Alan Reilly, P J (Eds). United Nations University Press, Tokyo, Japan p 107-128.

Saisithi, P. (1994). Traditional fermented fish: fish sauce production. In A. M. Martin (Ed.), Fisheries processing biotechnology application (pp. 111–131). London: Chapman and Hall.

Sarwar, G.; Christiansen, D.A.; Finlayson, A.T.; Friedman, M. ; Hackler, L.R.; Mc Keinzie, S.L.; Pellet, P.L.; Tkachuk, R.(1983) Intra- and inter- laboratory variation in amino acid analysis. J. Food Sci. 48, 526-531.

Satterlee, L.D., Kendrick, J.G., Miller, G.A., (1977) Rapid *in vitro* assays for estimating protein Quality, *Food Technol.*, 31(6) 78-81.

Satterlee, L.D. Marshall, H.F. and J.M. Tennyson. 1979. Measuring protein quality. J.A.O.C.S. 56:103-109.

Sikorski, Z.E., Gildberg,A., Ruiter, A. (1995). Fish products, pp. 315-346. In A. Ruiter, (ed.). Fish and Fishery Products: Composition, Nutritive Properties and Stability. CAB International. Wallingford, UK.

Sikorski, Z.E., Lolakowska, A., Pan, B.S., (1990) The Nutritive Composition of the Major Groups of Marine Food Organisms. In: Resources Nutritional Composition and Preservation, Sikorski, Z.E. (Ed.). CRC Press-Inc., Boca Raton, Florida, pp: 30-52.

Suzuki, T., (1981) Fish and Krill Protein Processing Technology, pp. 10-13. Applied Science Publishers, London.

Thai Industrial Standard. 1983. Local Fish Sauces Standard, Department of Industry: Bangkok, Thailand, 1983.

Tran, T. D., (2002). Country Report-Vietnam. In: Third Regional Workshop on the application of HACCP in the fish processing industry in Southeast Asia. Yeap, S E and Hariono, I (Eds) p 40.

Tsai, Y.-H., Lin, C.-Y., Chien, L.-T., Lee, T.-M., Wei, C.-I., Hwang, D.-F. (2006) Histamine contents of fermented fish products in Taiwan and isolation of histamine-forming bacteria, *Food Chemistry*, 98, 64–70.

Tyn, M. T., (1993) Trends of fermented fish technology in Burma. In: Fish Fermentation Technology. Lee, C H, Steinkraus, K H and Alan Reilly, P J (Eds). United Nations University Press, Tokyo, Japan p 129-153.

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