Protein Quality of Fish Fermented Product: Budu and Rusip

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Abstract- The commercial Budu and Rusip were determined and differentiated of their chemical compositions and colour. The amino acid compositions and protein digestibility data obtained were carried out for determined the protein quality. To assess the nutritional quality of the proteins in these products, the in-vitro protein digestibility and amino acid content were determined. The results showed significant difference (p <0.05) in colour (L*, a*, b*) between Budu and Rusip. The chemical compositions of Budu were 66.92% of moisture content, 11.39% of protein content, 20.72% of salt content, 0.837 of water activity, 4.98 of pH and 12.81% of degree of hydrolysis. The protein digestibility in Budu and Rusip were 67.29% and 67.82%, respectively. According to amino acid data, the predominant amino acid in Budu and Rusip was glutamic acid. Chemical score, amino acid score, essential amino acid index and protein digestibility-corrected amino acid scores (PDCAAS) were calculated from the amino acid data. The results showed that the Rusip provided a comparable quality of protein than Budu.

Keywords — Fermented fish product, Anchovy, protein quality, budu, rusip

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INTRODUCTION

The famous foods that based from fermented fish product is a fish sauce. Producing fish sauce has the different method in other countries depending on the location and culture. Fish sauces around the world have known with different names and also have the variety aroma and flavour (Beddows et al., 1979). Commonly examples of fish sauce in other countries was known as ‘nuoc-mam’ (Cambodia and Vietnam), ‘nam-pla’ (Thailand), ‘patis’ (Philippines), ‘ngapi’ (Burma) and ‘ishiru’ or ‘shottsaru’ (Japan) (Itoh et al., 1993). Hence, in Malaysia, it is known as budu whereas kecap ikan, rusip, and bakasang in Indonesia (Putro, 1993).

Usually, fish sauce is produced by mixing salt and fish with ratio one part of salt with two or three part of fish and the mixture left for fermentation process at temperatures (30-40°C) for a longer period (Lopetcharat et al., 2001). Budu is the one of the traditional fermented fish sauce that has been produced in the East Coast of Peninsular Malaysia (Kelantan and Terengganu). Budu, a dark brown liquid is commonly consumed as a dipping sauce for daily meals and as flavouring additives in some food (Huda and Rosma, 2006). Budu is produced by mixing fish mainly Stolephorus sp. (ikan bilis) with salt in the ratio 3:2 and undergo fermentation for 6-12 months in covered large earthenware jar underneath sunlight (Beddows, 1985; Klamklao et al., 2006; Rosma et al., 2009). The fish sauce become into a clear liquid solution through a filter process when most of the fish tissue was solubilized (Saisithi et al., 1966).

Traditional fermented fish product of Bangka people known as Rusip and this fish sauce widely consumed from East of Sumatera in Bangka Island, Indonesia. Rusip must undergo fermentation process using anchovies (Stolephorus sp.) with addition of 10% palm sugar and salt about 25%, which then kept for two weeks before it can be serve as side dishes (Susilawati, 1999; Dessi, 1999; Koesoemawardani, 2007). This fish sauce can be eaten directly or with addition of other ingredients to enhance the taste. This fish sauce can is the one of important source of protein. Fish sauce can provide such amounts of protein for human nutrition (Sanceda et al., 1996). Quality of the raw materials used during the production will effect to protein quality of fish sauce. Babji et al. (2010) showed that low quality protein does not contain all eight essential amino acids while almost all essential amino acids can be found in high quality protein.

The objective of this study is to determine the chemical composition, degree of hydrolysis, colour and protein quality of commercial Budu and Rusip.
Material
Samples used in this research are commercial Budu and Rusip. 10 bottles of samples from Budu and Rusip that has been carried out in this study was obtained in Malaysia and from Indonesia. Budu was bought from the local traditional market in Tanah Merah, Kelantan whereas Rusip from Palembang, Sumatera Selatan, Indonesia. Each sample of Budu and Rusip were stored at room temperature.

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 initial and critical water activity of the Budu and Rusip 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 °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 Budu same as Rusip.

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 budu and rusip 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 percentage 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 compositions and colour of Budu and Rusip
Table 1 presents the chemical compositions such as moisture content, protein content, salt content, water activity, pH and degree of hydrolysis and also colour of Budu and Rusip samples. The comparison among the moisture content of Budu and Rusip showed that there was significant difference (p < 0.05). The values obtained of Budu samples were higher than the data of Ghazali et al., (2011) which reported the moisture content of Budu from Tumpat and Bachok were 58.13% and 62.93%, respectively. Meanwhile the results for the Rusip were complied with the data of Madani et al., (2010) which reported moisture content of commercial Rusip ranged from 62.20% to 83.70%). The differences in moisture content between Budu and Rusip might due to the fermentation time and the use of salt added which can influence the moisture content of fish sauce as reported from previous studies by Huda & Herpandi, (2012).
As shown in Table 1, there were differences between protein content of Rusip and Budu (p < 0.05). The protein content obtained from Budu in this study was higher than the data reported Ghazali et al., (2011) and complied with Malaysian Food Act 1983 and Food Regulation 1985 (Anon, 2007) which stated that Budu should contain not less than 5% of protein. The value of protein content obtained for Rusip were complied with Koesoemawardani, (2007) which reported that Rusip contain 10.52% and up to 1% protein. The differences in protein content between Budu and Rusip occurred might be due to different level of salt added during preparation of the products.

The salt content of Budu samples ranged between 20.28% - 20.99% while Rusip varied between 13.82% - 15.36%. The average values of Budu (20.72 ± 0.17) significantly higher compared to Rusip (14.76 ± 0.58). Previously Mohamed et al., (2012) reported the salt content of commercial Budu samples ranged between 11.80% - 22.50%. According to Malaysian Food Act 1983 and Food Regulation 1985 (Anon, 2007), the salt content in Budu must not be lower than 15% of salt. Therefore, the salt content obtained in this study was complied with Food Regulation. According to Koesoemawardani, (2007), the salt content in Rusip ranged from 17.00% - 30.00%. Hence, the salt content of Rusip obtained was lower than the previous study. According to Madani et al., (2010), the amount of salt content can increase up to 30.0% if the large amount of salt was used during the Rusip processing. The differences amount in salt content in Budu and Rusip may occurred because of different ratio between salt and fish used by the manufacturer respectively. The used of high concentration of salt was an important factor during the fermentation process. These could retard the pathogenic bacteria and spoilage microorganisms and at the same time will act as natural preservative (Rosma et al., 2009).

The water activity (A_w) tested in this study is ranged from 0.819 – 0.840 and 0.856 – 0.877 for Budu and Rusip, respectively. There are significant different ( p < 0.05 ) between this two samples. Water activity is important to determine the quality and the safety of the food because it will affect the shelf life of the product. This value also can be used to predict the microbial growth of bacteria, yeasts and moulds. From this result, the water activity for Budu is lowers than 0.85. This will prevent the growth and toxin production of pathogenic bacteria. Besides that, the water activity for Rusip was greater than 0.86. This value is potential tend to cause microbial attack and also can cause to the risk of food poisoning.

The pH value of the Budu samples was found in the range from 5.15 - 5.20 while pH values range for Rusip samples was slightly lower which is ranged from 4.90 - 5.09. The total average value for Budu and Rusip represent 5.17 and 4.98, respectively and shows significantly different (p < 0.05). Owens and Mendoza, (1985) found that mostly pH of fermented food product ranged from 4.50 to 5.00 to inhibit the effect of spoilage and pathogenic bacteria. Nevertheless, according to Mohamed et al., (2012), pH values of commercial Budu ranged from 4.50 to 4.92. The pH obtained for Budu in this study was higher than previous work. Besides, the results obtained for Budu and Rusip were lower compared to the average pH of fish sauce from Thailand, Vietnam, Malaysia, Burma, China, Japan and Philippines was between 5.30-6.70 (Kim et al., 1999).

The minimum and maximum for degree of hydrolysis in Budu samples tested were 47.78% - 67.15%. For Rusip samples, the degree of hydrolysis varied between 37.40% - 47.64%. All the samples have significant differences between Budu and Rusip (p < 0.05). According to Sikorski et al., (1995), the hydrolysis process of fish protein was slower due to the decrease in enzyme activity during the storage period. Gildberg, (2001) stated that the degree of hydrolysis indicate to the protease activity at the beginning of fermentation process. Meanwhile, Orejana & Liston (1982) stated that during the manufacturing process of fish sauce period, the endogenous enzymes particularly were acted on muscle protein degradation. For that reason, it can be concluded that the endogenous enzymes plays a key role for protein digestion. The lower in degree of hydrolysis value of Rusip compared to Budu probably due to the incomplete fermentation process. This is because based on gross observations in the samples; anchovies in Rusip were not fully destroyed. The higher degree of hydrolysis probably increases the value of amino acid compositions.

The three parameters (L*, a*, b*) are indicators colour of the lightness (L*), the redness (a*) and the yellowness (b*) of the samples. The results showed that lightness values of Rusip samples ranged from 42.36 - 45.89 whereas the values of Budu lightness ranged from 32.50 - 34.63. The total average of lightness (L*) obtained in this study indicate that the lightness of Rusip was higher than Budu and there were significant difference (p < 0.05). At the same time, there were also showed significantly differences (p < 0.05) between Budu and Rusip for redness (a*) and yellowness (b*). Budu showed higher value of redness (a*) and yellowness (b*) than Rusip. Redness and yellowness of Budu samples ranged from 18.58 - 20.71 and 24.21-27.37, respectively. For Rusip samples, the redness and yellowness values were ranged from 4.04 - 4.95 and 13.81 - 16.45, respectively.

The results indicated the lower value of lightness (L*) and increases value in redness (a*) of Budu samples.
were associated with brown colour development. This is due to the Maillard reaction between free amino acid and sugar. The Maillard reaction induces to the browning of fish sauce and will lower the lightness value (BeMiller & Whisler, 1995). Besides that, the higher value of redness in Budu might be due to the addition of artificial food colouring. This is because from visual observation, Budu was look more red than Rusip. According to Kim et al., (2004), the colour parameters (L*, a*, b*) of fish sauce are decreased during storage.

### Amino acid compositions and protein quality

The amino acid compositions of the samples tested in this study are presented in Table 2. In this study, Rusip obtained the higher total amino acid than Budu. The amount of total amino acid was 111.60 mg/g and 88.80 mg/g, respectively. In the total amino acid compositions, there was significant difference (p < 0.05) in lysine, methionine, tryptophan, arginine, aspartic acid, glutamic acid and tyrosine concentration between Budu and Rusip. Besides, the amino acid alanine, isoleucine, glutamic acid and lycine in Rusip showed higher concentrations than Budu and it was fulfill the statement reported by Ijong & Ohta (1996) for Indonesian fish sauce.

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Budu (mg/g)</th>
<th>Rusip (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>3.10 ± 0.03^a</td>
<td>4.00 ± 0.05^a</td>
</tr>
<tr>
<td>Isoleusine</td>
<td>4.60 ± 0.04^a</td>
<td>5.60 ± 0.06^a</td>
</tr>
<tr>
<td>Leucine</td>
<td>7.10 ± 0.06^b</td>
<td>8.80 ± 0.08^b</td>
</tr>
<tr>
<td>Lysine</td>
<td>7.50 ± 0.03^a</td>
<td>10.4 ± 0.09^b</td>
</tr>
<tr>
<td>Methionine</td>
<td>3.00 ± 0.02^b</td>
<td>3.60 ± 0.03^b</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.80 ± 0.03^b</td>
<td>4.60 ± 0.04^b</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.60 ± 0.04^b</td>
<td>5.40 ± 0.04^b</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.00 ± 0.01^b</td>
<td>0.70 ± 0.01^a</td>
</tr>
<tr>
<td>Valine</td>
<td>5.50 ± 0.05^a</td>
<td>6.60 ± 0.06^a</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.60 ± 0.00^a</td>
<td>1.00 ± 0.02^a</td>
</tr>
<tr>
<td>Non essential</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>5.70 ± 0.06^a</td>
<td>6.70 ± 0.05^a</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.70 ± 0.02^b</td>
<td>3.50 ± 0.04^b</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>8.20 ± 0.06^b</td>
<td>10.4 ± 0.07^b</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>17.4 ± 0.13^b</td>
<td>23.0 ± 0.19^b</td>
</tr>
<tr>
<td>Glycine</td>
<td>5.10 ± 0.04^b</td>
<td>5.80 ± 0.03^b</td>
</tr>
<tr>
<td>Proline</td>
<td>3.50 ± 0.03^b</td>
<td>4.00 ± 0.04^b</td>
</tr>
<tr>
<td>Serine</td>
<td>3.20 ± 0.02^a</td>
<td>3.60 ± 0.03^a</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>3.20 ± 0.02^b</td>
<td>3.90 ± 0.02^b</td>
</tr>
<tr>
<td>ΣAA</td>
<td>88.80</td>
<td>111.60</td>
</tr>
<tr>
<td>ΣEAA</td>
<td>40.80</td>
<td>50.70</td>
</tr>
<tr>
<td>ΣNAAA</td>
<td>48.00</td>
<td>60.90</td>
</tr>
<tr>
<td>Chemical score</td>
<td>5.88</td>
<td>4.12</td>
</tr>
<tr>
<td>Amino acid score</td>
<td>9.09</td>
<td>6.36</td>
</tr>
<tr>
<td>EAA Index</td>
<td>8.56</td>
<td>7.72</td>
</tr>
</tbody>
</table>

Data are Mean ± SD. Means with the same letter(s) within the same row are significantly different (p<0.05).

Amino acid lysine gave the higher amount of essential amino acid for both samples. On the other hand, amino acid tryptophan and amino acid cysteine have the lowest values in Budu and Rusip. The predominant amino acids amongst the non essential amino acid were glutamic acid and aspartic acid whereas amino acid arginine was not dominant. The total non essential amino acid showed that Rusip has highest value than Budu. The values were 60.90 mg/g and 48.00 mg/g, respectively.

From the amino acid composition data, we calculate the chemical score, amino acid score and essential amino acid index in order to determine the quality of the fish sauce. The chemical score data was obtained from calculating the limiting amino acid by using the 1985 FAO/WHO/UNU (FAO/WHO, 1991) standard pattern requirements of whole egg protein (Mitchell & Block, 1946). The whole egg was use because it was estimated as a complete protein requirement. Chemical score values between 0-100.

The chemical score was important to explain the protein quality and the nutritional value. When compared with the standard pattern requirements of whole egg, chemical score obtained in Budu and Rusip was respectively 5.88 and 4.12. Generally, the value of fish protein chemical score is range 57-75 (Acton & Rudd, 1987). The chemical score in this study was lower compared to that ranged because the protein content values also lower. The results revealed in the table shows that the limiting chemical score in this study was amino acid tryptophan. The tryptophan amino acid chemical score value is the lowest compared to the average of other essential amino acids.

Next, 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 using 1985 FAO/WHO/UNU (FAO/WHO, 1991).

Amino acid score was important to find out how far the contribution of essential amino acids in a protein source for human needs. Amino acid score value is between 0-100. The amino acid score data for Budu and Rusip were expressed in Table 2. The amino acid tryptophan obtained from this study remains as the limiting amino acid compared to others. However, the lack of amino acids from fish protein can be overcome by amino acid content than other protein sources (Acton & Rudd 1987). The study conducted shows a method of mixing two or more sources of protein in the diet more effective than methods of direct addition of the limiting amino acid in the diet (Sawar & McDonough, 1990). Budu and Rusip can be eaten with other source of food which contains higher tryptophan value to fulfill the lacking amino acid tryptophan.
The essential amino acid index was the amino acid compositions that cannot be synthesized by human. However, this index does not represent how essential amino acids are preserved and used by the body (Crisan & Sands, 1978). The essential amino acid index (EAAI) was a standard to obtain the quality of protein in food. It also was important to overcome the lack of chemical and amino acid scores (Huda et al., 2011). The average value of each essential amino acid used as the final value in the determination of this index. From the results obtained, the EAAI for Budu was 8.56 and EAAI for Rusip was 7.72. This means Budu higher potential of amino acid compositions that cannot be synthesized. According to Acton and Rudd (1987), the essential amino acid index is a value that can specify the quality of protein in an in vivo more accurate compared to traditional chemical score or a score of amino acids. However, this method cannot determine the essential amino acids become limiting.

Protein digestibility and PDCAAS

In this study, in vitro method has been carried out to estimate protein digestibility using a four-enzyme method. Protein digestibility is an important parameter in the determination of protein quality. The protein digestibility and the protein digestibility-corrected amino acid scores (PDCAAS) score for Budu and Rusip samples are shown in Table 3.

Table 3: Protein digestibility values and PDCAAS score of Budu and Rusip

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Budu</th>
<th>Rusip</th>
</tr>
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<tbody>
<tr>
<td>Protein digestibility (%)</td>
<td>67.29 ± 1.68a</td>
<td>67.82 ± 1.71a</td>
</tr>
<tr>
<td>PDCAAS (%)</td>
<td>9.80</td>
<td>12.24</td>
</tr>
</tbody>
</table>

* Control casein = 89.33 %. Values in the same row with different letter is significantly different (p<0.05)

For Budu samples, the protein digestibility varied from 65.15% - 71.21% whereas Rusip samples ranged between 64.39% - 71.21%. The average values of protein digestibility in Budu when compared to the protein digestibility in Rusip were found to be similar. In order to calculate the relative digestibility, the control used was casein with value 89.33%.

The value of protein digestibility obtained from the analysis described the levels of the protein that can be absorbed by the body. Foods with higher protein content and protein digestion showed that the food was good to provide a source of protein for human. This was due to the high protein digestibility value that makes fish easily digested by human digestive system. According to Nettleton, (1985), the community from childhood to old citizens can obtained the nutrition of fish protein by its high percentage of protein digestion of fish. Otherwise, if the rate of digestibility was lower in high protein food indicates that the food was not suitable to provide the protein nutrition in the diet. According to Neisheim, (1977), in the food industry, the determining of protein digestibility was important to control the quality of protein in food labels and product ingredients.

By using protein digestibility data, PDCAAS was calculated., Budu sample has a PDCAAS of 9.80 - 51.60 while Rusip sample has a PDCAAS of 12.24 - 67.13. The limited acid is leucine which obtained 9.80% and 12.24% for Budu and Rusip, respectively. According to El & Kavas, (1996), PDCAAS, the base amount of limiting amino acid, was recommended as the most appropriate method for measuring the protein quality of food with based to the requirements of essential amino acid for preschool-children and adjusted to digestibility.

CONCLUSIONS

Budu was higher in moisture, salt, pH and degree of hydrolysis than Rusip. Meanwhile, the protein content and water activity of Rusip was higher Budu. The colour of Rusip was lighter than Budu, meaning that Rusip has less brown colour compared to Budu because the lightness (L*) of Budu was lower than Rusip. The protein digestibility shows that Budu and Rusip obtained the similarly results. The total amino acid composition of Rusip was higher than Budu as well as total essential amino acid and non-essential amino acid. The chemical score, amino acid score and PDCAAS score showed the higher values obtained for Rusip sample compared to Budu.

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