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Original Article

Determination of proximate composition and amino acid profile of Budu from Setiu, Terengganu and Tumpat, Kelantan

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Abstract

This study was conducted to determine the proximate composition and amino acid profile of anchovy sauce (Budu) produce in Terengganu and Kelantan, since there were only a few studies on this fermented product from Malaysia. Six samples of Budu which is three samples from Setiu (Terengganu) and three samples from Tumpat (Kelantan) respectively were collected and analyzed. Results showed that Budu contained high amount of protein with average between 9.69% - 15.02 %. Present data also indicated that Budu contained low amount of fat and carbohydrate, where fat content was less than 1%, and carbohydrate was between 0.07% and 6.51%. However, salt content were recorded high at around 38 to 51% in all samples. The data also showed that ash and moisture content in Budu were not significantly different. It contained about 15.75% - 18.81% of ash, and 64.27% - 69.46% of moisture. For the amino acids profile, glutamic acid showed the highest concentration in all of the samples from both states. The content of essential amino acids (lysine and leucine) was found to be dominant in Budu. Therefore, these results suggest that Budu can be an important protein supplement in the diet.

Keywords: Fermented, Proximate analysis, Acid amino profile, Anchovy sauce

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Introduction

Fish sauce is a popular fermented liquid seasoning seafood food product that have unique characteristics of flavour. It is a brownish to greyish in colour that is produced in Asian countries and limited areas of Europe. Fermentation of fish is an ancient technology that has already been employed by our ancestors a long time ago. The processing is traditionally used to overcome the perishable nature of fish. These products have unique characteristics, especially in terms of aroma, flavour, and texture developing during fermentation process. Only some types of fermented fish products have been widely known, such as fish

sauce and shrimp paste (Irianto, 2012). The types of fish and shellfish and fermentation process used in its production depend on the region from which it derives. Variations in ingredients and fermentation procedures yield end products with different smells, tastes and colours. It is obtained by mixing fish material with salt, which is subsequently fermented under natural conditions (Nakano et al., 2017).

Budu is a traditional food spearheaded by communities around the east coast of peninsular Malaysia, Kelantan and Terengganu. Not only that, it has generally been a popular food of the Malaysian society, including in major cities like Kuala Lumpur and Johor Bahru. Among its specialties is this Budu has different taste compared to other fish sauce and has long been used in some local food recipes such as '*nasi kerabu'*, '*nasi ulam'* and '*gulai kawah*'. In addition, Budu stocks in the market are also readily available especially in the states of Terengganu and Kelantan. As a result, Budu processing and packaging has begun to change to ensure greater market penetration is achieved by producers. Budu packaging in plastic tubes has begun to replace glass bottles for export purposed since the last few years (Umar, 2014). Additionally, Bernama (2018) had reported research and development work that allowed Budu to be processed into powder without altering the original flavour of the product. These efforts are important because the product can be introduced and penetrate oversea market for the benefit of the national fisheries industry.

According to Ahmad (2018), the fisheries segment in Malaysia had produced 2.0 million MT/year in 2015 and valued at US$ 3.3 billion. It was also estimated average consumption of fish in Malaysia at 56.8 kg/person/year with fish trade was valued at US$ 1.7 billion. This value had been surpassed target set by Department of Fisheries Malaysia (DoFM) which had reported that the fish supply per capita was 46 kg in 2010 and only projected increase to 55 kg by 2020 (DoFM, 2015). Meanwhile, the consumption of fish sauces in our neighbouring ASEAN countries likes Vietnam also significantly high as people are confidence that the sauce market (Transparency Market Research, 2017).The market value of fish sauce in Vietnam is already estimated about USD 502 million with 70,000 tons production of fish sauces (Intelligence, 2018). The biggest fish sauce producer in Southeast Asia is Thailand with the annual production of more than 400 million litres. The interest for this fermented fish item has been expanded for more than couple of years (Zaman et al., 2009). Thus, it is estimated a larger market value being shared between ASEAN countries for this segment in the market.

Nutritionally the fish sauce is more than a mere palatable condiment. The liquid is biochemically a concentrated mixture of various free amino acids, oligopeptides, nucleosides and their respective bases. Short-chain organic acids, aldehydes, and esters together with vitamins and minerals are contained in the sauce (Park et al., 2001). Food chemists have been interested in characterizing their chemical composition for a very long time. There have been many reports focusing on the chemical compositions of fish sauces. However, these reports only deal with sauces from individual countries and not many reported on Budu from Malaysia. Furthermore, it have been reported that the chemical composition of fish sauce will be difference among factories and among countries. Therefore, objective of this study were to determine proximate composition and amino acid profile of Budu from three difference producer each from Setiu and Tumpat.

Material and Methods

Sample collection of Budu

Budu samples were purchased from six local manufacturers, three from Tumpat, Kelantan, (Sample A; Sample B; Sample C) and another three from Setiu, Terengganu, (Sample D; Sample E; and Sample F). All the samples were stored in the refrigerator prior to proximate analysis, chemical analysis of salt and determination of amino acid profile. The proximate analysis were consist of determination of crude ash, moisture content, crude fat, total nitrogen (crude protein), and total carbohydrate.

Proximate analysis

Moisture, total ash, crude protein, fat and total carbohydrate were determined using standard method (AOAC, 1995). Moisture was determined by drying 100 g samples in a vacuum oven at 100 °C for 24 h to a constant weight. Crude protein was determined using Tecator Digestion System (Foss, USA). Ash was determined by ignition at 550 °C in an electric furnace (Carbolyte, United Kingdom). Fat was determined using Soxtec system HT6 1043 (Foss, USA) and total carbohydrate was calculated using difference of the value of each nutrient, moisture, total ash, crude protein, fat and total carbohydrate were

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Moisture, crude ash, crude protein, fat and total carbohydrate were determined using standard method (AOAC, 2000). Moisture was determined by drying 2 g samples in a vacuum oven at 100 °C for 24 hr to a constant weight. Crude protein was determined using

Kjeldahl Method, (AOAC, 2000) with System Kjeltec® (2100 Distillation Unit and 2006 Digestion Unit Foss Tecator, Sweeden). Crude ash was determined using dry ashing method (AOAC, 2000). Crude fat was determined using Soxhlet method system (AOAC, 2000) and the carbohydrates content was estimated as the weight of food material remaining after subtracting the weights for ash, moisture, crude protein, and total fat.

Determination of salt content (NaCl)

The salinity/salt content (NaCl) was determined using Auto titrator (799GPT Titrino, Metrohm, Malaysia) and the probe used was 6.0430.100 Ag Titrode (Anon, 2006). For the sample preparation, 0.05 g sample was weighed and put into a beaker. Then, 40 ml of distilled water and 10 drops of 2 M HNO_3 were added. HNO_3 was prepared by diluting 13.85 ml of HNO_3 with 100 ml of distilled water. The HNO_3 solution was added to give the acidic medium for the reaction between $AgNO_3$ and NaCl. A magnetic stirrer was added into the beaker to stir the sample while analysing. After a few seconds, the result in % displayed on the screen was recorded.

Determination of amino acids profile

Total amino acids profile content in Budu was determined using HPLC (Perkin Elmer, Model Series 200 Autosampler, USA) by HCl hydrolysate (6 N HCl) and Performic acid hydrolysate (AOAC, 2000).

Statistical analysis

Data was expressed as mean and standard deviation from four replicate analysis. ANOVA was conducted using Statistical Analysis System (SAS) (released 6.12., SAS Institute Inc., USA) and Duncan's multiple range tests were used to estimate significant differences among the mean values. Mean values and standard error of the means were reported and significance was defined at $p<0.05$.

Results and Discussion

Proximate analysis

Moisture content of all the samples showed more than 50% of the moisture content, ranging from 62.89% to 69.46%. The highest moisture content was sample C (69.46%), and the lowest was sample E (62.89%). Sample E was significantly different ($p<0.05$) among others. The other samples were contained not so much different of moisture content among others. Since Budu is a liquid product, so the moisture content was expectedly be high in all the samples. This result was very close to the moisture content in commercial fish sauce which is about 68% in Thailand and Korea (Lopetcharat et al., 2001). The differences of the value among the samples might be due to the more solids matters in the liquids resulting from protein hydrolysis. The moisture or the liquid yielded in the sauce production also possibly caused by the migration of salt and re-equilibrium of soluble component (Hjalmarsson et al., 2007).

The study showed that the crude protein was between 9.69% and 15.02%. The highest content of protein was in sample B, and D with 14.83%, and 15.02% respectively, and the lowest protein content was in sample C, 9.69%. Sample C was significantly different among the other samples. The protein content reported was varied for different samples and may be due to the raw materials used, fish and salt ratio, and fermentation time that lead to differences in total protein content. Nadiah et al., (2014) proposed the different level of salt added during preparation of the fermented products produce differences in protein content. Processed foods also have been certified to have a different effect on the physico-chemical of the product (Abraha et al., 2018). Above all, this study showed that all sample purchased were complied with Malaysian Food Act (1983) which stated that Budu should contain not less than 5% of protein.

Fat content in all samples were small but varied in the entire purchased sample. Sample B contained the highest fat content (0.82%) and showed a significant different ($p<0.05$) with the other samples. Meanwhile, the lowest fat content was recorded in sample D, at about 0.20% which was not significantly different from sample A, B, C, E and F. The difference of the crude fat content in the samples might be due to the different of raw materials used especially fish. Factors of fish species, sex, season and sites of collection were reflected for the dissimilarities in fat content.

Ash sample A and D showed higher ash content among all other samples, with 18.81% and 18.71% respectively. Then followed by sample E (17.65%), F (17.53%), B (16.68%), and lastly sample C (15.75%). Both sample B and C were significantly different ($p<0.05$) among others. The ash content is represented the mineral content in the samples (Pomeranz and Meloan, 1994), so it was suggested that the mineral content in the samples were also high.

Table-1: **Percentage of moisture content, crude protein, crude fat, crude ash, and total carbohydrate and salt content in all Budu samples**.

	Tumpat (%)			Setiu (%)		
	Sample A	**Sample B**	**Sample C**	**Sample D**	**Sample E**	**Sample F**
Moisture content	68.50±0.43^{a}	65.48±1.37^{b}	69.46±0.14^{a}	65.40±0.14^{b}	62.89±0.76^{c}	64.27±0.34^{b}
Crude protein	12.21±0.45^{b}	14.83±0.34^{a}	9.69± 0.19^{c}	15.02±0.19^{a}	12.17±0.42^{b}	11.94±0.44^{b}
Crude fat	0.41± 0.16^{b}	0.82±0.1^{a}	0.30±0.15^{b}	0.20±0.16^{b}	0.29±0.15^{b}	0.48±0.04^{b}
Crude ash	18.81±0.07^{a}	16.68±0.02^{c}	15.50±0.01^{d}	18.71±0.1^{a}	17.65±0.03^{b}	17.53±0.02^{b}
Total carbohydrate	0.07±0.02^{e}	2.19±1.62^{c}	4.80±0.25^{b}	0.38±0.39^{d}	6.51±1.08^{a}	6.51±0.56$^{a.b}$
Salt(NaCl)	46.46±0.11^{b}	40.70±0.57^{c}	38.37±0.86^{c}	51.23±5.18^{a}	44.16±2.56^{b}	45.82±2.18^{b}

$^{a-e}$ values with different superscript is significant different at $p<0.05$

However, some minerals like iodine and fluoride are only necessary in very small quantities to help our body function (NIH, 2019).

From Table 1, it also shows a significant different ($p<0.05$) of the total carbohydrates content in the Budu samples. The highest total carbohydrates content was in sample E (6.51%), while the lowest carbohydrate content was in sample A (0.07%). The carbohydrates content in the samples analyzed was not differ from the content in the reference, 0-7.4%. Even though the carbohydrates content in all of the samples were small, but it provides great attributes to the sauce especially on its flavours and aromas (Nielsen, 1998).

The results of salt shows sample D contained the highest salt content (51.23%) compared to the other samples, and it was significantly different ($p<0.05$) with others. The second highest salt content was sample A (46.46%), and then followed by sample F (45.82%), E (44.16%), B (40.7%), and C (38.3%). This result shows higher salt content compare to previous recorded in Budu (Nadiah et al., 2014), Nampla and Fish Sauces (Puat et al., 2015), and Rusip (Koesoemawardani et al., 2018). However, these results also showed that all the Budu samples produced in Setiu and Tumpat were complied with the regulatory requirement in Malaysia, which stated that, budu should contain not less than 15% of salt (Food Regulations 1985, 2005). The differences of the salt content in different samples suggested that the salt and fish ratio used to produce the sauce potentially different in different factory. The high use of salt was to control protein hydrolysis that prevents putrefaction and development of food poisoning such as botulism, and yield meaty, savoury sauces (Steinkraus, 2002).

Amino acid profile

Quantitative determination of amino acids concentration in Budu was conducted by HPLC. In overall, 17 amino acids were able to be detected and the separations of the amino acids in the samples were reasonable good. Classification of amino acids according to group of EAA or NEAA is as per Shaheen et al. (2016). The composition of amino acids in Budu samples were shown in the Table 2.

According to Table 2, ten nutritionally essential amino acids, namely histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, tyrosine and cysteine were present in Budu samples. Only tryptophan was not available in this EAA group because it is not included in the study at this time. Furthermore, sample A contained the highest amount of essential amino acids, with 70.29 mg/g. Then, followed by sample B (69.53 mg/g), D (67.56 mg/g), E (64.19 mg/g), F (56.05 mg/g), and lastly sample C (51.41 mg/g). This analysis indicates that Budu was a good source of EAAs as showed by high amount of lysine, leucine, isoleucine, phenylalanine, threonine and valine, which suggested that Budu will contribute to the supply of essential amino acids in the diet. On the other hand, cysteine has the lowest values of EAAs and it was in according with finding reported by Nadiah et al. (2014) in Budu and Rusip. Amid highly present of lysine and leucine in all samples, it also appeared few differences on the amino acids concentration among the samples. As reported by Ijong and Ohta (1995), the differences might be associated with the effects of dynamic balance of free amino acids by autolysis and microbial action. This is common because there will be a slight difference in the way of Budu processing between different manufacturers in Setiu and Tumpat.

Table-2: Amino acids composition in Budu samples obtained from different manufacturer in Tumpat and Setiu.

Amount (mg/g)						
Essential AA	**A**	**B**	**C**	**D**	**E**	**F**
Lysine[b]	12.75±0.11	13.24±2.57	9.08±1.07	12.67±0.62	11.46±1.94	10.90±0.23
Leucine[b]	11.52±0.16	13.05±1.22	9.08±1.00	10.45±0.08	10.27±0.07	8.19±0.46
Isoleucine[b]	7.23±0.07	8.17±0.70	5.67±0.57	7.35±0.05	6.82±0.09	5.53±0.29
Valine[b]	8.33±0.09	9.45±0.48	6.53±0.56	9.22±0.03	8.53±0.13	7.67±0.33
Histidine[b]	5.54±0.12	5.97±0.12	4.74±0.16	3.49±0.24	5.60±0.11	4.32±0.24
Methionine[b]	5.13±2.85	4.93±2.38	3.94±0.14	5.19±0.29	4.17±3.33	4.25±0.78
Threonine[s]	7.17±0.02	7.13±0.64	5.78±0.59	8.02±0.28	7.39±0.06	6.59±0.18
Phenylalanine[b]	6.09±0.10	5.11±0.65	5.15±0.42	6.26±0.42	6.09±0.05	5.44±0.12
Cysteine[b]	4.83±0.10	1.02±0.51	0.38±0.02	1.86±0.12	0.78±3.19	0.06±0.97
Tyrosine[b]	1.70±0.11	1.46±0.62	4.66±0.66	3.05±0.65	3.08±0.14	3.10±0.13
∑ EAA	70.29	69.53	51.41	67.56	64.19	56.05
Non-essential AA						
Glutamic acid[c,u]	22.30±0.01	30.53±2.23	16.65±1.81	23.87±0.84	20.74±0.49	25.40±0.12
Aspartic acid[u]	13.57±0.01	14.06±0.64	9.69±0.23	13.88±0.14	12.76±0.12	12.38±0.01
Alanine[s]	10.01±0.11	10.93±1.02	7.61±0.76	11.28±0.31	9.48±0.18	9.36±0.02
Glycine[c,s]	9.27±2.92	10.24±0.12	7.49±0.37	10.29±0.27	9.11±0.13	8.28±0.13
Proline[s]	5.58±0.17	6.39±0.21	4.66±0.50	6.02±0.06	5.84±0.05	5.31±0.11
Serine[s]	2.23±1.19	4.54±0.54	4.69±0.35	3.11±0.15	5.80±0.07	4.68±0.09
Arginine[b]	3.63±0.10	3.64±0.82	3.40±0.77	4.44±0.79	3.16±0.16	4.02±0.04
∑ NEAA	66.59	80.33	54.19	72.89	66.89	69.42
∑AA	136.88	149.86	105.60	140.45	131.08	125.47
∑EAA/ ∑NEAA	1.06	0.86	0.94	0.93	0.96	0.81
∑EAA/ ∑AA	0.51	0.46	0.49	0.48	0.49	0.45

Classification of AA as essential/indispensable amino acid or nonessential/dispensable amino acid is as per Shaheen et al. (2016).

[c] Conditional essential amino acid, (Wu, 2013).

Taste from EAAs as [b] bitter, [s] sweet, [u] umami and sour (Kawai et al., 2012).

Meanwhile, Figure 1 shows the difference in the profile of the essential amino acids required by humans in general (FAO/WHO, 1991), and EAA values that can be obtained from Budu. The total EAA that can be taken from the Budu is just a little bit off the standard by the FAO. However, Budu which is eaten as a supplement is considered worthwhile to be included in having a good diet to the community.

The glutamic acid was the most abundant amino acid in overall Budu samples with recorded amount about 16.65 mg/g to 30.53 mg/g. Although glutamic acid classified under NEAA, but its possibly giving umami and sour taste as also being reported to be present in aspartic acid (San Gabriel and Uneyama, 2013). High concentration of glutamic acid might be linked to high taste quality of Budu and it has been implied that high concentrations of glutamic acid in the samples might be from the addition of monosodium glutamate (MSG) after the fermentation process to boost the taste. Other than that, anchovies (*Stolephorus sp*.) is the main raw material for budu processing has also been identified to have a high amount of glutamic acid compared to some other species of fish (Mohanty et al., 2014).

This high glutamic acid is seen to coincide with a study by Nadiah et al. (2014), which records the amount of glutamic acid by 17.4 mg/g and 23 mg/g in the Budu and Rusip product respectively. Rusip is a fish sauce product that is almost identical to the Budu in terms of

the type of fish used although there is little difference in the duration of the fermentation process. The comparison between both data available showed some of the samples in present study were higher in the production of glutamic acid than the previous date recorded in previous study (Nadiah et al., 2014).

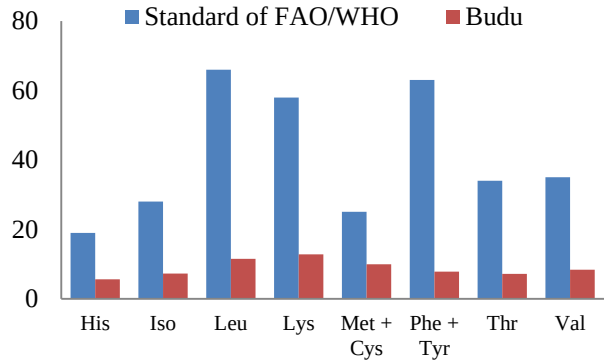

Figure-1: Profiles of essential amino acids (mg/g).

Conclusion

The study conducted here showed that the physical characteristics and the chemical composition of Budu were different between the factories in both district in Kelantan and Terengganu. The results showed that Budu contained high amount of protein, as well as EAAs. The EAAs such as lysine, leucine, valine, isoleucine, threonine, valine and phenylalanine were abundant in overall samples. Glutamic acid which recognized as NEAAs was highly present in all samples of Budu to give nutritional benefit and umami taste for consumer. Therefore, these results suggest that Budu can be an important protein supplement in the diet. However, the relatively high salt content must be considered by all party if manufacturer retain their original salt composition as this could potentially cause health problem like hypertension. Consumer could achieve balanced diet if they consume Budu with other low salt dishes such as salad vegetables. Other than that, the high concentration of glutamic acids, histidine, and proline in Budu may also suggest the presence of potential seasoning agents to give a good taste of Budu with some nutritional properties.

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Contribution of Authors

Ahmad F: Conducting the research and investigation process, specifically data and evidence collection and acquisition of the financial support for the project.
Mahmud MF: Conduct data analysis and assist in technical writing
Ali NSC: Conducted laboratory work.
Ayub MNA: Technical expert for proximate analysis and amino acid analysis
Mohamad SN: Technical expert for amino acid analysis
Ismail N: Mentoring and guiding the research ideas and formulation of research questions
Tuan Zainazor TC: Guiding the research ideas and formulation of research questions
Zamri AI: Guiding the research ideas and formulation of research questions
Khalid MI: Conduct data analysis and assist in technical writing

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