Journal of

Fisheries and Aquatic Science

ISSN 1816-4927

Academic Journals Inc.

www.academicjournals.com

Journal of Fisheries and Aquatic Science 7 (6): 485-493, 2012
ISSN 1816-4927 / DOI: 10.3923/jfas.2012.485.493

Protein Quality of Stingray (*Himantura gerrardi*) Fish Flakes

Ainul Mardiah, Nurul Huda and Ruzita Ahmad
Fish and Meat Processing Laboratory, Food Technology Programme, School of Industrial Technology, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia

Corresponding Author: Nurul Huda, Fish and Meat Processing Laboratory, Food Technology Programme, School of Industrial Technology, Universiti Sains Malaysia, Minden 11800, Malaysia Tel: +60-4-6532112 Fax: +60-4-6573678

ABSTRACT

The objectives of this research were to determine the protein quality of fish flakes made from stingray meat (*Himantura gerrardi*) and to evaluate the changes of the protein quality; Amino Acid Composition (AAC), Chemical Score (CS), Amino Acid Score (AAS) and Essential Amino Acid Index (EAAI) during the processing stages. Fish flakes was made from stingray meat which is added with tapioca starch and spices (garlic, onion, tamarind, palm sugar, ginger, coriander and root of galangal). All ingredients were mixed well and then spread into layers of 3 mm thickness and then steamed at 100°C for 1 h. Samples were cut into pieces measuring 12×4 cm rectangles and dried them in an oven dryer at 60°C until the moisture content was reduced to 25%. The results showed that the CS, AAS and EAAI of fish flakes before drying (dough) were 68.47, 116.51 and 94.06 g/100 g protein, respectively. Meanwhile, fish flakes after drying were 63.20, 99.21 and 88.00 g/100 g protein, respectively. The results indicates that chemical score, amino acid score and essential amino acid index of stingray fish flakes was not greatly decrease during processing stages, indicating that protein quality of stingray meat did not deteriorate significantly.

Key words: Stingray meat, drying, amino acid composition, intermediate moisture (IM) product, fish flakes

INTRODUCTION

Fish flakes is an Intermediate Moisture (IM) fish product which is made from stingray meat, Tapioca Starch (TS) and curing ingredients. In their preparation, TS was used as a binder in order to achieve a good texture product. TS contributes to texture enhancement, binding properties and improved mouth feel to meat products. It is relatively simple to process, it has own characteristic taste and stable without refrigerator (Mardiah *et al.*, 2010). Fish flakes has brown color with ±25% of moisture content. This product is similar with bak kua in Malaysia which is known as dried pork/chicken meat product among Chinese diet. Bak kua is sweet meat product with optional chilies or black pepper and it is served with rice or bread as a sandwich. It is sold in ready-to-eat form by grilling over charcoal fire until the brownish color was reached (Narasimha Rao, 1997). This product is also similar to dendeng in Indonesia which is processed from beef and prepared with salt and sun drying as preservation method. Prior to eat, dendeng was fried in palm oil at 170°C for ±45 sec. This product is largely consumed in West Sumatera region as snack food or eaten together with rice.

In South America, the product that is quite similar to fish flakes is called jerky. Jerky has been a favorite snack food for many years (Carr *et al.*, 1997). It is prepared from sliced whole muscle of large animal which has been marinated and dried (Choi *et al*., 2008). The term jerky is derived from the Spanish word "charqui", meaning dried meat strips (Carr *et al*., 1997). Today, Jerky is more convenient snack food that has safe preservation, flavor and texture. Those characteristics are important value in food products. Jerky can be made from almost various lean meat, including beef, pork, poultry, or game (Nummer *et al*., 2004). The manufacture of jerky which is made from stingray meat is a new product and it is not found yet in the market. The lack of product from stingray meat was due to the urea odor in the fish flesh. By utilizing the simple method of fish flakes processing, the urea odor could be reduced and the product is more acceptable and has a specific characteristic taste. According to sensory analysis, fish flakes with addition 15% of TS is more preferred by the panelists (Mardiah *et al*., 2010). It means that a small amount of TS added into formulation can increase the taste of fish flakes. In terms of nutritional values, each processing stages did not effect significantly to the protein quality of fish flakes.

Protein is an important material to form a new network system that always happens in the body. Protein is also used as fuel when the needs on body's energy are not fulfilled by carbohydrate and fat (Winarno, 1984). Protein is the major nutrients in fish and their levels can help define the nutritional status of the particular organism (Oduor-Odote and Kazungu, 2008). The protein contains essential amino acids that are required for human body. The essential amino acid composition is one of the most important nutritional qualities of protein (Sudhakar *et al*., 2011). Amino acid plays an important role for the metabolic system and maintaining the health and vitality. There are 20 kinds of amino acids can be found in the human body, 18 kinds of them are very important in human nutrition (Usydus *et al*., 2009). Some of these amino acids may not be synthesized in human body and must be supplied from the diet. These amino acids are called essential amino acids and include isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (FAO/WHO, 1973). The essential amino acids are required for maintenance of life, growth, synthesis of vitamins and reproduction (Sudhakar *et al*., 2011). The deficiencies of these nutrients will affect to the degradation of protein muscle in our body (Usydus *et al*., 2009) and interfering growth for children (Winarno, 1984). The world health organization recommended leucine and isoleucine requirements for adult of 14 and 10 mg amino acid kg^{-1} b.wt. day^{-1} (FAO/WHO/UNU, 1985). Because of the importance of protein for human body and the lack of research about the protein quality of stingray meat become the reasons of this study. This research aims to determine the effect of processing stages to protein quality of stingray meat and compare the result found by Sikorski and FAO/WHO committee (FAO/WHO, 1973; FAO/WHO/UNU, 1985; Sikorski *et al*., 1990). This study determined the protein quality of fish flakes and to evaluate the change of the protein quality.

MATERIALS AND METHODS

Sample preparation: The fresh Stingray (*Himantura gerrardi*) was purchased from a local market, Bayan Baru, Penang, Northern part of Malaysia. Then, it was transported in a cold ice box to the Fish and Meat Processing Laboratory, Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia. On arrival at the laboratory, the fish flesh was washed immediately. Next, the bones and the skin were separated from the flesh. The fish flesh was then washed until it was free from blood. Then, it was placed in a plastic bag, sealed and kept in a freezer at ±-20°C before further processing. Hundred gram of stingray meat was mixed with the ingredients. The ingredients used were garlic (1%), tamarind (5%), salt (1%), palm sugar (20%), ginger (0.5%), onion (1.5%), coriander (1.5%) and galangal root (2.5%). All those ingredients were purchased from a local traditional market. Preparation of fish flakes as shown in Fig. 1. The raw

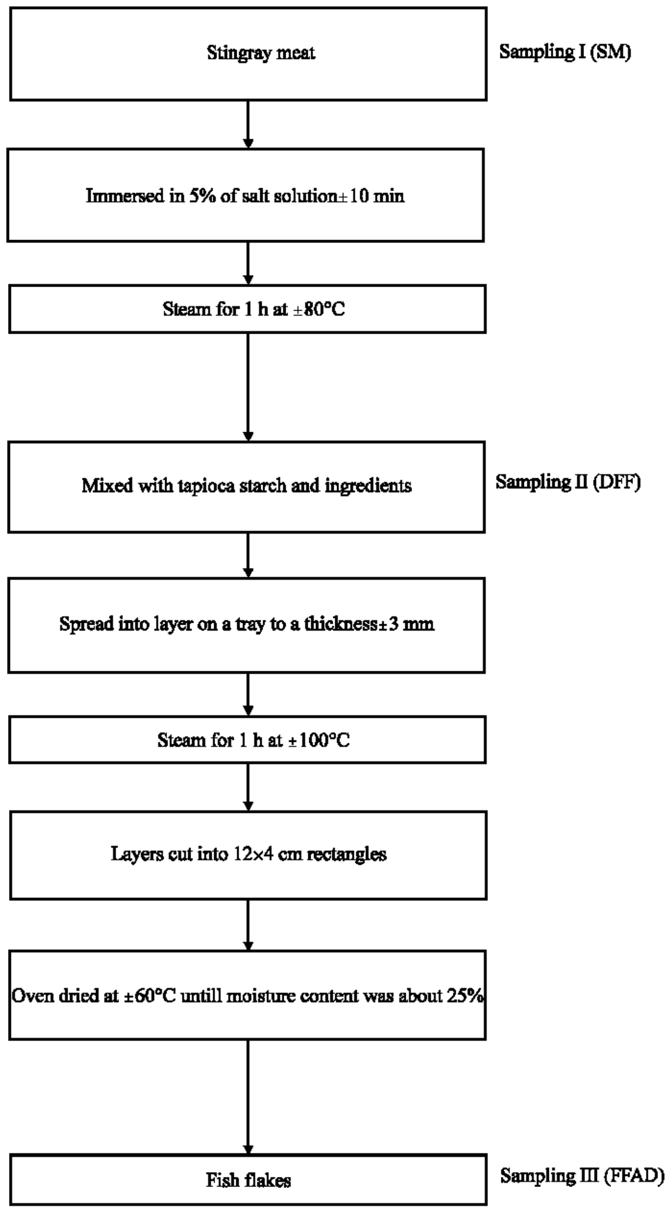

Fig. 1: The preparation of fish flakes

samples were described as SM; the dough fish flakes samples were described as DFF; the fish flakes after drying samples were described as FFAD.

Amino acid analysis and amino acid profile: The method for amino acid analysis according to Cohen (2003). Amino acid analysis was determined by using High Performance Liquid Chromatography (HPLC) system which consisted of Waters 1525 Binary HPLC Pump, 717 Plus AutoSampler (Water®) and Water 2475 Multi λ Fluorescence Detector optics (the wavelength; 250 nm for excitation and 395 nm for emission). The chromatographic separation was performed using An AccQ Tag™ reversed phase 3.9×150 mm analytical column (Waters®). Altogether, seventeen components of amino acid were obtained in this experiment. Methionine and cysteine were analysed after cold performic acid oxidation for 16 h before acid hydrolysis was done. However, tryptophan was not determined in this study. Duplicate analysis was performed per each sample.

Samples were freeze-dried using vacuum-freeze dryer (LABCONCO, US) prior to analysis. Acid hydrolysis was performed by weighing approximately 0.1 g sample in stoppered tubes and added with 5 mL of 6 N HCl. The tubes were placed in an electric oven at a temperature of 110°C for 24 h. The 400 µL of AABA (Alpha-Amino Butyric Acid) solution was added to the hydrolysate sample and filled up to 100 mL with distilled water in a volumetric flask. The aliquot was filtered using Whatman filter paper No. 1 followed with the use of a syringe filter. The solution was kept in a freezer for further derivatizations.

Performic acids oxidation was performed to analysed the cysteine and methionine components in the samples. Approximately 0.1 g of sample was weighed and placed into a stoppered tube. It was then added with 2 mL of fresh chilled performic acid and kept in refrigeration for 16 h. Then, 0.4 mL of chilled bromic acid (HBr) was added and then it was kept for 30 min in a refrigerator. Furthermore, the sample was dried using a rotary evaporator at 80°C. Finally, the sample was processed into acid hydrolysis.

Derivatization was performed by mixing the 10 µL of aliquot samples, 70 µL of borate buffer and 20 µL of AQC reagent. Derivatization was completed after one minute. Then, the 10 µL of aliquot were injected into HPLC system using eluent A (AccQ Taq TM concentrate, Waters) and eluent B (Acetonitrile 60%, Sigma). The eluent was flowed at a rate of 1 mL min^{-1}. The resulting peaks were analysed using a breeze software package.

Chemical Scores (CS) and Amino Acid Score (AAS) are a method for evaluating protein quality by comparing the amino acid composition in the samples with the reference pattern of amino acid (whole egg protein); each ratio is multiplied by 100 (FAO/WHO, 1973). The amino acid score was calculated using the following formula:

$$\text{Amino acid score (AAS)} = \frac{\text{Amino acid sample in 1 g of test protein (mg)}}{\text{Amino acid in reference pattern (mg)}} \times 100$$

The Chemical Score (CS) of whole egg protein, according to FAO/WHO (1973) standards are; Lysine 6.98; Methionine+Cystine 5.79; Threonine 5.12; Isoleucine 6.29; Leucine 8.82; Valine 6.85; Phenylalanine+Tyrosine 9.89 and Tryptophan 1.49 g/100 g.

Amino acid scores were calculated by comparing the EAAI of sample with the EAAI suggested for an adult (Sarwar and McDonough, 1990). Amino Acid Score (AAS) refers to FAO/WHO/UNU standards 1985 which are Histidine 1.9; Lysine 5.8; Methionine+Cysteine 2.5; Threonine 3.4; Isoleucine 2.8; Leucine 6.6; Valine 3.5; Phenylalanine+ Tyrosine 6.3 and Tryptophan 1.1 g/100 g (FAO/WHO/UNU, 1985). The lowest score of amino acid and chemical ratio (%) were termed as amino acid scores and chemical scores (Acton and Rudd, 1987).

The Essential Amino Acid Index (EAAI) was calculated by the same way as the Chemical Score (CS) method. The value obtained was converted into log10. The EAAI was obtained from the antilog of the average of essential amino acid in the sample. The result was compared with EAAI reported by Sikorski *et al.* (1990).

RESULTS AND DISCUSSION

The Amino Acid Composition (AAC) of Stingray Meat (SM), Dough Fish Flakes (DFF) and Fish Flakes After Drying (FFAD) are presented in Table 1. The results display did not in homogeneous trend, levels are fluctuated compare to raw material. The main amino acids were found in SM samples to be arginine, glutamic acid, glycine, leucine and the less abundant ones was cysteine. Drying treatment cause changes in several amino acids. From raw to dry samples, have significant changes were found for arginine, glycine, histidine and tyrosine.

Generally, amino acid composition of FFAD did not show significant decrease after processing stage. Therefore, cooking method did not significant effect to the amino acid composition of fish flakes. The same results was found by Wu and Mao (2008), explained that hot drying has less influence to amino acid composition of dry grass carp (*Ctenopharyngodon idellus*) fillets. However, Steiner-Asiedu *et al.* (1991) reported there is no effect of processing method (cooking, frying and smoking) on the amino acid composition of *Sardinella* sp., *Dentex* sp. and *Tilapia* sp., as compare with the fresh fish. In this experiment isoleucine, phenylalanine and threonine of FFAD was slightly decreased when compared with SM and DFF samples. It occurs because the different of amino acid, so it also has different effects to the processing and heating (Wu and Mao, 2008). Compared to result found by Sikorski *et al.* (1990), phenylalanine and threonine had the higher score meanwhile isoleucine was decrease.

The major of amino acid of FFAD samples were arginine, aspartic acid, glutamic acid, leucine, lysine and proline. Arginine (7.41 g/100 g) is an essential amino acid for children growth (Aremu and Ekunode, 2008) and it was high in the sample. Similar result was observed by Ozden (2005) for anchovy and trout fish. According to Erkan *et al.* (2009), aspartic acid, glutamic acid and lysine are greatly found in sea food products and necessary for enzyme activity, maintaining solubility and ionic character of protein. However, valine, phenylalanine and tryptophan tended to be lower in dehydrated fish (Hoffman *et al.*, 1977). The amino acids tyrosine,

Table 1: Amino acid composition (AAC) of stingray meat (SM), dough fish flakes (DFF) and fish flakes after drying (FFAD) (g/100 g protein)

Amino acid	SM	DFF	FFAD	Mean values of ten species of fish**
Alanine	4.56	5.26	5.28	7.91
Arginine	10.45	8.16	7.41	5.95
Aspartic acid	6.01	7.58	7.90	10.34
Cysteine	1.51	2.02	1.68	1.04
Glutamic acid	10.55	13.80	14.15	14.91
Glycine	10.50	6.38	5.62	4.60
Histidine	3.84	2.99	2.95	2.01
Isoleucine	4.68	4.88	4.61	6.03
Leucine	7.68	8.35	8.05	8.41
Lysine	5.01	7.72	6.96	8.81
Methionine	4.41	5.91	6.03	2.97
Phenylalanine	5.89	4.95	4.64	3.92
Proline	4.12	4.18	8.34	3.52
Serine	4.80	4.83	4.61	5.14
Threonine	6.49	5.90	5.58	4.62
Tyrosine	5.05	2.39	1.61	3.27
Valine	4.45	4.69	4.58	5.95

**Cod, coalfish, haddock, redfish, catfish, plaice, halibut, ling, torsk and mackerel (Sikorski *et al.*, 1990)

Table 2: Chemical scores (CS) of stingray meat (SM), dough fish flakes (DFF) and fish flakes after drying (FFAD)

Amino acid	SM	DFF	FFAD	Mean values of nine species of fish**
Phenylalanine+tyrosine	110.62	74.22	63.20	
Isoleucine	74.40	77.58	73.29	
Leucine	87.07	94.67	91.27	
Lysine	71.78	110.60	99.71	
Methionine+cysteine	102.25	136.96	133.16	
Threonine	126.76	115.23	108.98	
Valine	64.96	68.47	66.86	
Chemical score	64.96	68.47	63.20	67.00

**Seafood group: Clupeiformes (Clupeoidei include pilchard, anchovy, herring), Salmonoidei include trout, salmon, white fish, smelt, Gadiformes include cod, hake, haddock, ling, torsk, Galiformes include shark, Perciformes (scombroidei include tuna, mackerel, swordfish, skipjack, albacore), Other include goby, mullet, meagre, dorado, perch, Pleuronectiformes include sole, flounder, turbot, Crustaceans, Mollusks (Sikorski *et al*., 1990)

arginine and lysine are very important during fish spoilage, since these amino acids can produce biogenic amines (Ozden, 2005). Amino acids such as aspartic acid, glycine and glutamic acid are known to play an important role in the process of wound healing (Zuraini *et al*., 2006). The most important amino acids from quantitative point of view are proline, arginine, glycine, alanine, histidine and glutamic acid. Some of amino acids such as, alanine, glutamic acid and glycine are responsible for flavor and taste in sea food products (Erkan *et al*., 2009). In cooked fish, amino acids are directly responsible for flavor and taste and can be precursors of aromatic component (Ozden, 2005).

The chemical score of SM, DFF and FFAD samples was observed in Table 2. The result was explained that DFF obtained the highest value when compared to SM and FFAD. During the processing stage, chemical score of the sample was slightly decreased; however the range of chemical scores in the samples is similar to that reported by Sikorski *et al*. (1990) which ranged from 61-75. The decreasing of this amino acid may be due to some heat treatment given during the processing stage. However, fish flakes after drying still have high chemical scores. Valine was used for determining the chemical score for SM and DFF samples. Meanwhile phenylalanine+tyrosine were used for determining chemical score for Fish Flakes After Drying (FFAD). High chemical scores indicate a high protein quality of the samples.

The lowest amino acid ratio is termed amino acid score. The score above 100 would be considered as 100 (Sarwar and McDonough, 1990). Amino acid scores of FFAD is 99.21 (Table 3). The result was higher compared to the amino acid scores found by Sikorski *et al*. (1990) and Huda (2001) for a group of nine species of fish and surimi powder, respectively. Processing led to an increase in the amino acid scores in the samples. However, El and Kavas (1996) reported that amino acid score of raw, broiled and smoked rainbow trout was similar to the amino acid score of FFAD. Results also showed that the amino acid score in SM was determined by lysine while that for DFF and FFAD was determined by phenylalanine and tyrosine. Fish proteins are one of the best sources of lysine for human nutrition (Erkan *et al*., 2009). Therefore, the rate of lysine loss is significantly influenced by water activity (a_w) and drying process (Malec *et al*., 2002). Moreover, the high amount of lysine may provide good protein supplementation for food. In addition, protein with high amino acid score indicated that has a high biological values and high net protein utilization. It was encouraging that processing stages did not induce much loss of amino acid quality in the fish flakes. Foh *et al*. (2011) also reported that the processing stages of Nile Tilapia into hydrolysates still have a good nutritional quality and could be used as protein ingredient in the food industries.

Table 3: Amino acid scores (AAS) of stingray meat (SM), dough fish flakes (DFF) and fish flakes after drying (FFAD)

Amino acid	SM	DFF	FFAD	Mean values of nine species of fish**
Phenylalanine+tyrosine	173.65	116.51	99.21	
Histidine	202.11	157.37	154.74	
Isoleucine	167.14	174.29	164.64	
Leucine	116.36	126.52	121.97	
Lysine	86.38	133.10	120.00	
Methionine+cysteine	236.80	317.20	308.40	
Threonine	190.88	173.24	164.12	
Valine	127.14	134.00	130.86	
Amino acid score	86.38	116.51	99.21	98.00

**Seafood group: Clupeiformes (Clupeoidei include pilchard, anchovy, herring), (Salmonoidei include trout, salmon, white fish, smelt), Gadiformes (include cod, hake, haddock, ling, torsk), Galiformes (include shark), Perciformes (scombroidei include tuna, mackerel, swordfish, skipjack, albacore), (other include goby, mullet, meagre, dorado, perch), Pleuronectiformes (include sole, flounder, turbot), Crustaceans, Mollusks (Sikorski *et al*., 1990)

Table 4: Essential amino acid index (EAAI) of stingray meat (SM), dough fish flakes (DFF) and fish flakes after drying (FFAD)

Amino acid	SM	DFF	FFAD	Mean values of nine species of fish**
Phenylalanine+tyrosine	2.0438	1.8705	1.8007	
Isoleucine	1.8716	1.8897	1.8650	
Leucine	1.9399	1.9762	1.9603	
Lysine	1.8560	2.0438	1.9987	
Methionine+cysteine	2.0097	2.1366	2.1244	
Threonine	2.1029	2.0616	2.0373	
Valine	1.8126	1.8355	1.8252	
	(Σ/7= 1.9481)	(Σ/7= 1.9734)	(Σ/7= 1.9445)	
Index amino acid	88.74	94.06	88.00	85.00

**Seafood group: Clupeiformes (Clupeoidei include pilchard, anchovy, herring), Salmonoidei include trout, salmon, white fish, smelt, Gadiformes include cod, hake, haddock, ling, torsk, Galiformes include shark, Perciformes (scombroidei include tuna, mackerel, swordfish, skipjack, albacore), Other include goby, mullet, meagre, dorado, perch, Pleuronectiformes include sole, flounder, turbot, Crustaceans, Mollusks (Sikorski *et al*., 1990)

Essential Amino Acid Index (EAAI) of fish flakes are shown in Table 4. The results indicated that EAAI of FFAD sample was slightly decrease compared to SM and DFF. The DFF sample was obtained the highest amount of EAAI. The FFAD sample showed a higher of EAAI compared to that reported by Sikorski *et al*. (1990) which the ranged from 83-90. EAAI values generally show a better measurement to the quality of the protein compare to chemical score (Acton and Rudd, 1987).

CONCLUSION

The chemical score, amino acid score and Essential Amino Acid Index (EAAI) decreased by only 7.7, 14.8 and 6.4%, respectively during the processing stage and the fish flakes still retained the high protein quality.

ACKNOWLEDGMENT

This study was supported by the short term research grant Universiti Sains Malaysia 305/PTEKIND/636055.

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