Thesis for the Degree of Master of Fisheries Science

Effect of Cooking Conditions on Protein Quality of Nile Tilapia *(Oreochromis niloticus)* Meat

by

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KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

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틸라피아 육단백질 품질에 미치는 조리조건의 영향

Advisor: Professor Hong-Soo Ryu

by

Didas Clemence

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Abstract

To suggest the effective method of protein source utilization for third world people, optimum cooking time and temperature for protein quality of Nile tilapia (*Oreochromis niloticus*) meat were studied. A significant change in proximate composition between raw and cooked samples was observed in fried samples. Protein content of tilapia meat was ranged from 71.13±0.24 to 94.23±0.34 % on dry weight base. Lipid content was increasing in fried and grilled samples while decreasing in boiled and steamed samples. In nutritional bases, these results prove that tilapia (*Oreochromis niloticus*) meat is a comparable protein source with other fish species.

Protein digestibility was decreased with the increase of cooking time and temperature. When tilapia meat was boiled for 0.5 minute, digestibility was reached to 88.41±0.33% while it showed 87.66±0.22% in boiled sample for 10 minutes. Similar trend was observed in the rest of cooked samples. Though this difference is not statistically significant but nutritionally it has the meaning that tilapia meats have to be cooked for short time.

Trypsin indigestible substrate (TIS) increased with the decrease of digestibility. When digestibility was 86.96±0.22% in fried samples, TIS was 48.18±1.23mg/g solid but in case of samples showing 84.39±0.83% digestibility, the TIS was 56.46±0.87mg/g solid. This phenomenon entails protein in tilapia is available for digestion by digestive proteolytic enzymes in the digestive tracts.

Higher computed protein efficiency ratio (C-PER) and discriminant computed protein efficiency ratio (DC-PER) than standard ANRC casein could imply that protein found in tilapia is an excellent protein source especialy in essential amino acids (EAA). C-PER was 2.62 for boiled sample and 2.60 for fried sample which showed maximum protein digestibility. DC-PER was 2.70 for boiled and 2.67 for fried sample. Presence of EAA in different levels also supports this winding up. Total Amino acids for boiled samples were around 100 g.a.a/16g N and that of fried samples were 98 g.a.a/g N.

Experimental results suggest that best cooking condition is boiling trailed by steaming and grilling. Although fried samples showed digestibility within the necessary range, it is not nutritionally advised due to its high fat content and low protein digestibility due to severe protein-oxidized lipid interaction.

1. INTRODUCTION

1.1. Importance of fish

Fish is greatly perishable but very important foodstuff, especially in third world countries. The significance is due to its high protein content and nutritional value of unsaturated fatty acids and affordability by the masses when compared with beef (Adeyemi et al., 2013). Approximately 14% of the animal protein consumed by humans comes from marine fisheries and fresh water though there is variation between countries.

Fish is known to be a source of protein rich in essential amino acids (lysine, methionine, cysteine, threonine, and tryptophan). Fish muscle also contains micro- and macro - elements and fat-soluble vitamins (Larsen et al., 2007). The high protein levels with good digestibility and also low fat content are advantages of seafood (Pigott and Tucker, 1990). Fish is reported to contain omega-3 fatty acids which are important in preventing cardiovascular diseases.

1.2. Fish in Tanzania

In Tanzania, fish contributes to about 30 percent of total national animal protein consumption. The per capita fish consumption is about 8 kg (National Economic Survey, 2012) while post harvest loss is approximated 15~20%. In Nigeria, fish constitutes 40% of the animal protein intake of the people but 40% of the total fish catch in Nigeria are lost annually due to inadequate or poor preservation, processing and handling (Daramola et al., 2007).

Unlike Europeans and Asians countries who are able to consume raw seafoods (Whoe in Korea and Sashimi in Japan); many Tanzanians mainly consume ready processed (smoked, sun-dried, and salted-sun dried) and well cooked (grilling, boiling, steaming and frying) fish dishes.

It is considered that cooking of fish is an excellent reliable processing method for fish preservation in the processing chain and can improve quality attributes.

In cooking, value addition to fish and fishery products can be achieved including protein increase due to dehydration. According to Hall (1992) fish

is an excellent source of high quality protein, compared with those found in meat and poultry. Most raw fish is 16~24% protein which can give rise to as much as 35% in cooked fish. However, cooking processes can play same role as smoking and became more valuable in terms of quality and marketability.

Historically and surely recently - it is well known that, the smoking and drying processes are the affordable and most widely used method for fish preservation in developing countries. It also aimed at preventing or reducing post-harvest losses (Govindan and Velankal 1958).

1.3. Tilapia in Tanzania

Nile tilapia *(Oreochromis niloticus)* is the third commercial important species in Lake Victoria fish with Nile perch (*Lates niloticus*) and dagaa (*Rastrineobola argentea*). Tilapia is also found in other small lakes (Nyasa and Rukwa), as well as in local dams (Mtera and Nyumba ya Mungu). Fishing of *Oreochromis spp* is besides made in other minor waters. Furthermore, tilapia is leading among farmed and consumed fish species in

the United Republic of Tanzania. This is probably due to its white flesh muscles and low fat content compared to other species like Nile perch and other marine species. Nonetheless, due to its protein properties, it is considered that Nile tilapia could be suitable for manufacturing of surimi and other fishery products.

On the other hand, tilapia is the fourth lean protein rich fish producing of about 17g; the richest being tuna, salmon, halibut, snapper (all 22g), perch, flounder (all 21g) and cod (20g) (USDA National Nutrient Database for Standard, Release 25, 2013). Lean protein has been identified beneficial for myriad health benefits.

Studies have shown lean protein can help whether peoples are looking to lose weight, bulk up, improve heart health or boost your energy. Incorporating lean protein into the diet is a critical component of a healthy eating plan. However, though protein offers heart health benefits, many protein-rich

foods like as livestock meats are high in saturated fat, raising cholesterol and increasing the risk of coronary heart disease.

Furthermore, post harvest losses and low value fish products are increasingly expanding in the fishery industry of Tanzania. This makes fishing activities to be less profitable. Among many causes of this problem is the lack of proper technology in fish processing.

Studying different cooking conditions, identifying and recommending the best one, may not only solve the post harvest (protein) losses problems but also give path to introduction and implementation of recommended processing methods which will give optimum quality attributes, add value to fish products and thus improvement of the quality which will ultimately contribute to fish protein consumption, per capital income and the economy of the country as well.

With understanding, aquaculture is radically getting bigger and bigger in the country as most people are employed in fish farming activities. In a short period to come, these people will have a need to process, add value and preserve their fish before marketing. The best cooking condition recommended will give farmers confidence to progress with fish farming activities and others to join, as it will assure them long shelf-life and value addition for better marketing and income.

Additionally, evidence suggests that smoked foods may contain carcinogens. The smoking process contaminates food with polycyclic aromatic hydrocarbons (PAHs) and nitrosamines, which are known carcinogens. Therefore, consuming smoked food could result in increasing the risk of gastrointestinal cancer.

1.4. Processing methods

Cooking methods (boiling, baking, roasting, frying, grilling, steaming, or microwaving) aim at not only enhancing the taste, flavor, increasing the shelf life of the product but also quality addition of the product in terms of protein.

Bognar (1998) reported that; heating (boiling, grilling, baking and frying) is applied in food to enhance its flavour and taste, inactivate pathogenic microorganisms and increase shelf life. On the course and effects of heat, related concept was also reported by Garcia - Arias et al, (2003) that, application of heat is mostly achieved by boiling, baking, roasting, frying, grilling, steaming, or microwaving. Each of these involves the application of heat at different levels.

Heating intends at serving and enhancing the taste and flavor as well as increase the shelf life of the product. However, it should be noted that; the main purpose of fish processing is not only to increase the aroma, specific taste but also value addition; and these methods still differ from one place to another depending on the amount of additives, percentages of salts, vinegar and temperatures applied.

In addition, boiling, frying, grilling and steaming are the oldest but efficient foods preserving methods, with certain temperature and humidity. Boiling,

grilling, steaming and frying not only increase resistance of food but changes appearance taste and smell of food. Various pretreatments like as salting and drying have been applied prior to cooking (boiling, grilling, steaming and frying), and marinating has been applied after cooking in the food industry.

However, boiling, grilling, steaming and frying are not absolute preserving method. For this reason, the quality of raw material, the concentration of salt, water activity of the fish, way of packaging, hygienic circumstances and heat storage have important effects in reducing the risk of deterioration (Kaya et al., 2006).

1.5. Effects of cooking conditions on protein quality

While cooking improves both digestibility and flavor, care must be taken to retain nutritional benefits. Protein represents one of the most valuable compounds of our diet and should be retained during processing (Ramos et al., 2012). Nonetheless, protein quality of fish is affected during processing

as a result of the application of heat which results in protein denaturation (Oduro et al., 2011).

Some benefits of processing are destruction of antinutritional factors, such as amylase and trypsin inhibitors and increased digestibility of starch and protein (Piggot and Tucker 1990). Also, it should be noted that, the variation of levels of nutrients in raw materials is due to more than just processing.

Most nutrients loss occurs during preparation, but some losses (post harvest) occur during harvest, processing, storage and distribution. Stability of nutrients varies with pH, oxygen, heat and light. When food is processed, the tissues are damaged and nutrients interact with other components. Hence, the choice must be made between the risk of nutrient loss and benefit of food availability (Ramos et al., 2012).

The extent of protein denaturation depends on the duration of heat, the temperature as well as processing facility (Sikorski, 2001). It should be noted however that, the nutritive value of protein is determined not only by their

quantitative and qualitative composition of amino acids, but also by their availability to digestive tract proteolytic enzymes (Lee and Ryu, 1986). Therefore, the rate of digestibility of a protein is indicative of its availability to digestive enzymes. Piggot and Tucker (1990) reported that, important chemical reactions that decrease the nutritional value of seafood are lipid oxidation and non enzymatic browning.

On the other hand, an important and frequently observed effect of food processing is the reduction of protein nutritive quality. These changes may depend on the denaturation of the protein and reduction in amino acid availability by cross – linking, racemization, degradation and formation of complexes with sugar and may result in loss of digestibility (Gauthier et al., 1982) as cited by (Ramos et al., 2012). It is hypothesized that processing by heat increase food digestibility because it breaks protein and carbohydrates which are less digestible.

Despite this advantage however, vitamins, minerals, some essential amino acids and other beneficial nutrients are lost (Mirnezami et al., 2002). Therefore, when attempting to estimate protein quality, one of the first factors that must be evaluated is its digestibility. Because the nutritional quality of a protein is related both to its amino acid content and the capacity of digestive enzymes to liberate them, method using digestion enzymes have been tried (Gauthier et al., 1982).

Lipid oxidation is one factor that contributes to loss of protein quality. Fish oils are converted to ketones, aldehydes, and hydroxyacids. These reactions are enhanced by iron and copper ions, so red muscle readily becomes rancid, especially in scombroid fishes as tuna, swordfish, bluefish, and mackerel. This appears as a thin brownish-gray layer next to the larger portion of edible flesh (Oduro et al., 2011).

Chemical reactions of oxidized lipids with amines, amino acids, and proteins have received considerable attention because they are associated with changes in functional properties, nutritive value, flavor, and color of foods (Xiong, 2000).

Reduced digestibility, as well as limiting the amount and degree of amino acid availability, is mediated partially by the formation of amino acid bonds with lipid oxidation products (Lee and Ryu, 1987). These protein-lipid complexes contribute to the quantity of indigestible substances in foods that are not available to proteolytic enzymes.

In vitro protein digestibility assay is an inexpensive way of determining the protein quality of seafood. This method uses a multi-enzyme assay to imitate human and animal digestive systems. Due to the time-consuming and expensive *in vivo* method of determining protein digestibility, researchers have tried to correlate *in vivo* and *in vitro* methods to develop reliable methods for determining the protein efficiency ratio (PER) measurement. Two such methods are the computed protein efficiency ratio (C-PER) (Satterlee et al., 1982) and the discriminant computed protein efficiency ratio (DC-PER) (Jewell et al., 1980). The C-PER is a PER prediction calculated from essential amino acid information and *in vitro* protein digestibility,

whereas the DC-PER is solely dependent on amino acid compositional data. These two methods are known to have a high correlation with *in vivo* assays (Phimphilai et al., 2006). Lee and Ryu (1987) used this model to evaluate the protein quality of seafood.

Protein qualities of seafood have been determined by different studies in different ways. According to Pigott and Tucker (1990) cooking practices could cause modified proximate composition, fatty acids, and amino acids, as well as changes in nutritional quality.

Ryu et al., (1992) observed an increase in *in vitro* protein digestibility, a decrease in trypsin indigestible substrates (TIS), a reduction in some essential amino acids, and an increase in fat oxidation of seasoned and smoked squid. There was also observation of an increase in *in vitro* protein digestibility and nutritional indices in the Persian sturgeon (*Acipenser persicus*) after grilling and frying by Jannat et al., (2010).

1.6. Objective of this study

In spite of many studies being carried out on protein quality of tilapia, most of these dealt only with proximate composition. Little focus has been given for investigation on the *in vitro* protein digestibility, quality of protein and trypsin inhibitor substrate (TIS). It is thus the objective of this study to investigate the effect of cooking conditions on the *in vitro* protein, quality of protein and TIS content of Nile tilapia (*Oreochromis niloticus)*.

The study further seeks to recognize suitable cooking temperature and time. Last, the study intends to identify and advice on the paramount cooking condition (s).

1.7. Parameters for investigation

In this research experiment, we investigated proximate composition (crude protein and lipid) of the raw fish samples. Other parameters researched are; *in vitro* protein digestibility, trypsin indigestible substrate(TIS), PER (computed protein efficiency ratio, C-PER and discriminant computed protein efficiency ratio, DC – PER) and amino acid profiles for all cooked samples.

2. MATERIALS AND METHODS

2.1. Sample collection and preparation

On 17th March, 2014 ten live Nile tilapia each with one thousand grams were caught from Pukyong National University (PKNU) aquaculture farm and sent to sashimi restaurant near PKNU for being processed (de-heading, removal of viscera, scaling, filleting, skinning and deboning) before being brought to Food and Nutrition Laboratory.

In the laboratory, fillets of fish were semi-dressed into two pieces and sliced. Average lengths of the sliced samples were 10cm in length and weighing 70~80grams per piece. These measured and weighed samples were further divided into five subunits. One unit was kept raw and stored to be used as the control. The remaining four units were again divided into eighteen units and started to be cooked by frying, grilling, steaming and boiling.

Ten samples were boiled and steamed for from 0.5 to 10 minutes categorically; for these two cooking conditions, temperature was maintained at 100^0C. Hand transparent thermometer was used to measure the temperature during boiling and steaming. However, frying and grilling were performed at a temperature of 180^0C from 0.5~5 minutes. While frying was done in the frying pan using soybean oil (Ottogi, Korea), grilling was achieved using an oven (Convotherm, OAS6.10 Germany). All eighteen samples were refrigerated for three days then freeze dried for four days.

2.2. Experimental Procedure

2.2.1. Proximate Composition

Analysis in foods and food products is performed by extracting components such as fiber, minerals, crude ash, moisture content, crude protein, crude fat and soluble carbohydrate. Proximate analysis was carried out for the purpose of determining all components present in Nile tilapia. However, it should be noted that the nutritional labels on the side of food containers are produced because of proximate analysis achieved on the food.

Table 1. Cooking conditions of tilapia meats.

Methods	Time (min.)					Temperature (°C)
Boiling	0.5	1	2.5	5	10	98±2
Steaming	0.5	1	2.5	5	10	98±2
Frying	0.5	1	2.5	5	NA	178±2
Grilling	NA	1	2.5	5	10	178±2

In this experiment, cooked tilapia meat were ground using a dry kitchen blender and meshed in less than 100mm. The raw sample was also ground but not meshed. Crude fat was determined by the method described by (AOAC, 1990) using Soxhlet solvent extractor (Model VELP SCIENTIFICA SER 148). Crude protein (%) was determined by the semi–micro Kjeldahl procedure (Gerhardt Vapodest 30) using conversion factor of 6.25 (AOAC, 1990) after acid digestion (Digestor Model Gerhardt Turbotherm, distillatory model Gerhardt Vapodest). The remaining samples were freeze dried for other tests. The percentage content of crude protein and crude fat were determined in relation to the dry basis of the samples analyzed, dry basis being expressed in g/100g solid. All determinations were made in triplicate for each run.

2.2.2. *In vitro* protein digestibility

The *in vitro* digestibility values of all samples were determined by the method of Oduro et al., (2011) with modification by the AOAC procedure (AOAC, 1982), which used four enzymes method for protein digestibility

using proteolytic enzyme. Oduro et al. (2011) in trying the three enzyme method, determined the correlation coefficient between two assays and it showed high correlation (R^2=0.9955).

The *α-chymotrypsin* (Sigma 38 units/mg solid, trypsin (Sigma 13,390 BAEE units/mg solid), protease (*Streptomyces griceus*, Sigma 46units/mg solid) was used in the three enzyme method. The reference protein used is ANRC casein and digestibility was calculated as follows:

% digestibility (three enzymes) = 234.84 – 22.56x; where x is the pH of sample at 10 minutes.

% digestibility (four enzymes) = 1.03x (three enzymes digestibility) - 0.34

2.2.3. Trypsin indigestible substrate (TIS)

These are chemicals that reduce the availability of biologically active trypsin; an enzyme essential to nutrition of many animals including humans. These can be found in soy bean, lima bean, ovomucoid, serum just to mention a few.

In tilapia, the TIS concentration of all samples was determined using the procedure of Ryu and Lee (1985), which is a modification of Rhinehart method (1975). Results of TIS are expressed in trypsin inhibitor equivalents which equals the mg of purified soybean trypsin inhibitor per gram sample. The correlation coefficient between pH and TI content was 0.987 calculated using the following equation;

Y = 1.504x –10.33; where y = purified soybean trypsin inhibitor (mg) and x is pH at 10 minutes incubation.

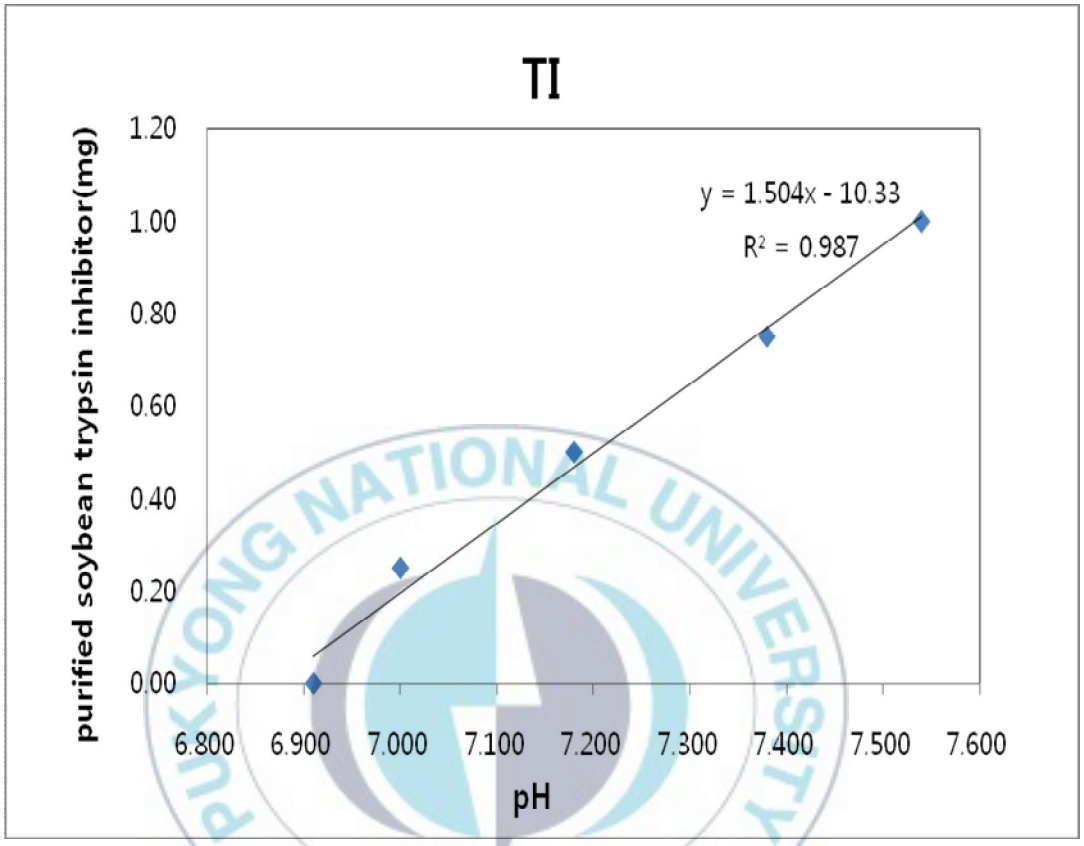

Fig. 1. Relationship of pH at 10 minutes with purified soyabean trypsin inhibitor concentration

2.2.4. Amino acid profiles

Samples were taken to the feeds and foods Nutrition Centre, PKNU of which amino acid composition of each cooking condition with its time was carried out. They were determined using amino acid analyzer (S433; Eresing, Germany). Samples were hydrolyzed with 6N HCl in vacuo at 110^0C for 25hours. The method of Hugli and Moore (1972) was used to determine tryptophan using hydrolyzates of 5N NaOH.

2.2.5. Computed *in vitro* protein quality

C-PER were calculated in software, using the data obtained from the *in vitro* protein digestibility (four enzymes) and amino acid profiles. The calculation based on the procedure of AOAC (1982), to obtain the protein quality data compared to casein as standard protein.

2.3. Statistical analysis

Mean values and standard deviations (SD) from the 3 separate experiments or replicate analysis was reported. The statistical significance of observed differences among treatment means was evaluated by analysis of variance (ANOVA). The significance of results was at 5% and the confidence level of analyzed data was at 95%.

3. RESULTS AND DISCUSSION

3.1. Proximate composition

3.1.1. Crude protein content

Variation in protein content was observed among different cooking conditions as expressed in Table 2. All boiled samples showed decreasing trend (90.39±0.94%) at ten minutes being the lowest. Though, this had no significant difference ($P<0.05$) indicating that a severe protein extraction was not occurred during those short boiling time. Interestingly, crude protein decreased in cooked crab's brown meat, which might be due to protein loss occurred during boiling and steaming, since brown meat has a much softer and liquid texture compared to muscle (Maulvaulat et al., 2012).

Protein content for the raw samples was 91.79±0.34% dry base. The tendency was different in grilled samples which showed significant increase of protein content and the sample boiled at ten minutes giving high protein

content (94.23±0.34% dry base) except the sample which was grilled at one minute (89.2±10.04% dry base). It was thought that absorption of oils resulted those decreased protein content in fried tilapia meats during frying time. Significant decrease was shown by all fried samples; 71.13±024% was the lowest decrease. Steamed samples at all time except at 0.5 minutes showed slight increase mainly to all samples steamed at ten minutes. Protein decrease in boiled samples could be due extraction and loss of nutrients in water, also because of the increase of lipid content and moisture content.

Experiments were carried out after freeze drying and expression on dry basis. Jucier et al., (2008) observed the decrease of protein content on catfish when protein and other parameters were expressed in dry basis. However, it is the hypothesis that protein increase with the decrease of moisture content. (Gokoglu et al., 2004) reported that the decrease in moisture content has been described as the most prominent change that makes the protein content increase significantly in cooked fish.

Grilling produces more water loss and more dehydration though not more than frying. This is the reason as to why there is high protein content for grilled samples of tilapia. Also, these results are related to those reported by Gokoglu et al,. (2004) in the rainbow trout, Jucier et al., (2007) and Garcı´a-Arias et al., (2003) in sardines.

During cooking of meat, the thermal denaturation of different muscle proteins such as myosin, sarcoplasmic protein, collagen, and actin occurs at different temperatures. The physical properties and quality of cooked meat are strongly affected by the degree of protein denaturation resulting from different heat treatment conditions, such as temperature and time (Ishiwatari et al., 2013). Slight increase of protein content to steamed samples was due to low water loss.

Statistically, the decrease and increase of crude protein content in different cooking conditions had no significant difference ($P<0.05$) and showed that there were no significant protein loss except for fried samples at 2.5 and 5

minutes. Furthermore; in her paper to the UNU fisheries training programme Besharati (2004) observed percentage increase of protein from 22% to 27.2% for hot smoking and from 20.9% to 23.5% for cold smoking.

3.1.2 Crude lipid content

Fat content for raw samples was 1.47±0.11% (dry base) (Table 2). Fat content decreased in nearly all cooked samples only being highest in fried samples at 2.5 minutes (18.73±1.92% dry base) under 180^{0}C in oil. This increase of fat content in fried samples implies that there was absorption of oil in tilapia fillets during frying.

Similar results were found for sardines fried in sunflower oil (Candela et al., 1998). Fat increase can be due to the oil penetration on the food after water is partially lost by evaporation (Saguy and Dana, 2003). Either, oil absorption in fried foods may range from 10% to 40%, depending on the conditions of frying and the nature and size of the food (de Man, 1999).

Table 2. Various crude protein and crude lipid contents of Nile tilapia meat according to cooking time (min.) (g/100g solid)

Sample**	Crude protein	Crude lipid
Raw	91.79±0.34^{b}	1.47±0.11^{a}
BS 0.5	91.73±0.57^{c}	1.33±0.50^{c}
BS 1	91.12±0.62^{ca}	1.53±0.11^{a}
BS 2.5	90.93±0.99^{dc}	1.26±0.11^{a}
BS 5	90.87±0.34^{b}	1.40±0.20^{b}
BS 10	90.39±0.94^{dc}	1.33±0.11^{a}
SS 0.5	89.41±0.33^{b}	1.26±0.11^{a}
SS 1	92.40±0.31$_{b}$	0.60±0.00^{a}
SS 2.5	93.24±0.07^{a}	0.86±0.41^{c}
SS 5	93.10±0.52^{c}	0.46±0.11^{a}
SS 10	94.16±0.29^{b}	0.73±0.11^{a}
FS 0.5	81.95±0.17^{a}	9.7±0.42^{c}
FS 1	80.07±0.66^{ca}	10.7±30.30^{dc}
FS 2.5	74.32±0.47^{b}	18.73±1.92^{cd}
FS 5	71.13±0.24^{a}	21.13±1.67^{de}
GS 1	89.21±0.04^{a}	2.20±0.34^{b}
GS 2.5	91.35±0.33^{b}	3.20±0.20^{a}
GS 5	91.56±0.18^{a}	2.07±0.11^{a}
GS 10	94.23±0.34^{b}	1.27±0.23^{a}

- *Mean ±SD of three triplicates
- **Sample categories: BS (boiled), SS (steamed), FS (fried), GS (grilled) and Raw (control)
- 0.5,1, 2.5, 5, 10 min.: (cooking time)
- $^{a-e}$ Different letters in column of each sample category show significant differences ($P<0.05$)

It is however the observation of the research that, fat content increase increased with time of fillets remaining in oil. The more time fillet stay in oil, the high the fat content increase. Moreover, fat content decrease in boiled and steamed samples could be due to the fact that – some fats were lost in water during boiling and steaming. There was extreme loss of lipid in steaming smoke. Related trend was reported by (Gokoglu et al., 2004) in rainbow trout proximate composition experiments. Also de Castro et al., (2007) reported 0.48% lipids in skinless tilapia.

3.2. *In vitro* protein digestibility

Maximum *in vitro* digestibility (88.41±0.33%) was observed in boiled samples at 0.5 minute at 98±2^{0}C; the lower being grilled fillets for 2.5 minutes at 178±2^{0}C which its digestibility was 85.86±0.27% (Table 3).

On their evaluation of seafood protein quality, Lee and Ryu (1986) instituted that *in vitro* digestibility for fresh and live fishes between species is ranging from 78.5 to 88.7 percent. The white-fleshed finfish as tilapia had higher *in*

vitro digestibility than dark-fleshed finfish. Protein digestibility is influenced by the presence of antinutritive factors the levels of which are affected by different processing and cooking methods.

Moreover, utmost digestibility for 0.5 minute fried and steamed skinless fillets were 86.96±0.22% and 88.18±0.68% respectively (Table 3). Even so, *in vitro* digestibility increases with fat level decrease. This relationship is due to oxidized fats formed during processing.

Fat content of skinless tilapia immersed in oil may be an important factor which affects the digestion of protein. Oduro et al., (2011) observed the increase of protein digestibility in fried anchovy from 79.97±0.01 raw to 82.51±0.09 fried and said that the reason could be due to samples being treated with brine (NaCl) 10% concentration.

Table 3. *In vitro* protein digestibility of tilapia meat for different cooking conditions

Sample	Digestibility (%)	Sample	Digestibility (%)
Raw	87.66±0.29^{b}	FS 0.5	86.96±0.22^{b}
BS 0.5	88.41±0.33^{c}	FS 1	86.84±0.12^{a}
BS 1	88.41±0.19^{a}	FS 2.5	84.98±0.48^{ab}
BS 2.5	87.77±0.12^{a}	FS 5	84.39±0.83^{cd}
BS 5	87.71±0.19^{a}	GS 1	85.57±0.22^{b}
BS 10	87.66±0.22^{b}	GS 2.5	85.86±0.27^{b}
SS 0.5	88.18±0.68^{ac}	GS 5	85.86±0.19^{a}
SS 1	87.83±0.13^{a}	GS 10	85.04±0.13^{a}
SS 2.5	87.71±0.19^{a}		
SS 5	87.77±0.12^{a}		
SS 10	87.48±0.19^{b}		

- *Mean ±SD of three triplicates; DW = dry weight
- **Sample categories: BS (boiled), SS (steamed), FS (fried), GS (grilled) and Raw (control)
- 0.5,1, 2.5, 5, 10 min.: (cooking time)
- $^{a-d}$ Different letters in column of each sample category show significant differences (P<0.05).

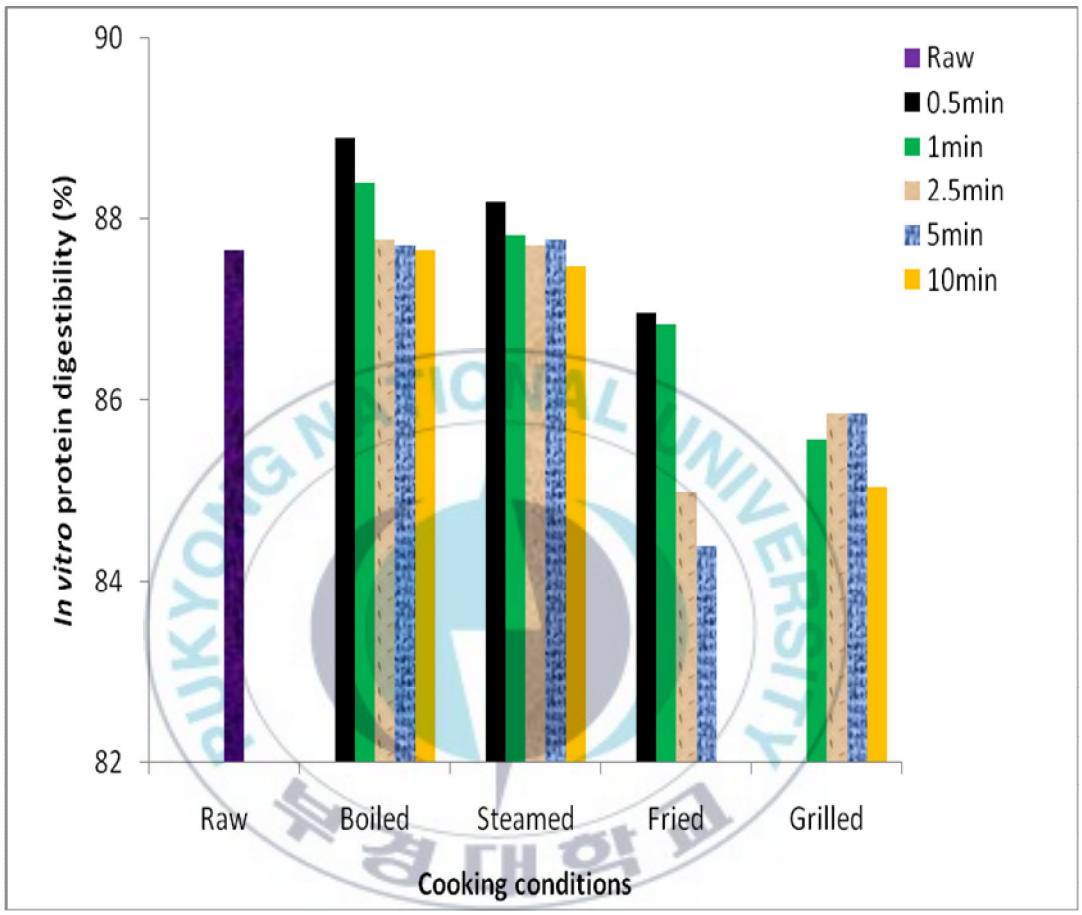

Fig. 2. Comparison of *in vitro* protein digestibility of tilapia meat according to cooking methods and time

Statistically these different results in protein digestibility for different cooking conditions imply that protein in tilapia is available for being digested by digestible proteolytic enzymes predominantly if cooked under controlled temperature and time.

In general, heating improves digestibility of protein by inactivating enzyme inhibitors and mild denaturing the protein, which exposes new sites to digestive enzyme action (Sikorski, 2001). Lee and Ryu (1986) stated that the nutritive value of protein is determined not only by their quantitative and qualitative composition of amino acids, but also by their availability to digestive tract proteolytic enzymes.

3.3. Trypsin indigestible substrate (TIS)

Trypsin indigestible substrate (TIS) content in tilapia meats were compared to those in fried samples (Table 4, Fig. 3, 4 and 5). The reason for selecting

these samples was that - boiled samples showed highest digestibility nearly similar to steamed samples while fried samples showed lowest digestibility almost similar to grilled samples.

As shown in Table 3, TIS level increased with decreased protein digestibility. Increase of TIS was probably caused by fat oxidation, interaction between oxidized fat and protein denaturation due to prolonged boiling and frying time and temperature that inactivated available active trypsin. However, there are two types of TI, one being found in the original sample and that one caused by TIS induction of fat and protein.

There was significant difference between samples boiled for 10 minutes (54.20±1.23mg/g solid) and samples boiled for 0.5 minutes (45.92±1.94mg/g solid). The same trend was observed in fried tilapia meat where samples fried for short time had lower TIS (48.18±1.23mg/g/solid) and that fried for long time had higher TIS (56.46±0.87mg/g solid).

In other words, it can be stated that – TIS was low to all samples with highest digestibility. Similar trend was reported by Ramos et al. (2013) in raw, minced and surimi of fried anchovy kamaboko.

Table 4. Protein digestibility and trypsin indigestible substrate (TIS) for boiled and fried tilapia meat

Sample	Protein digestibility (%)*	TIS (mg/g solid)*
Raw	87.66 ±0.29 ^{b}	45.95±0.87^{de}
BS 0.5	88.41 ±0.33 ^{c}	45.92±1.94^{ef}
BS 1	88.41 ±0.19 ^{a}	46.30±1.44^{f}
BS 2.5	87.77 ±0.12 ^{a}	51.56±0.75^{d}
BS 5	87.71 ±0.19 ^{a}	51.94±1.50^{f}
BS 10	87.66 ±0.22 ^{b}	54.20±1.23^{e}
FS 0.5	86.96±0.22^{b}	48.18±1.23^{bd}
FS 1	86.84±0.12^{a}	48.56±1.44^{df}
FS 2.5	84.98±0.48^{b}	53.07±1.44$_{f}$
FS 5	84.39±0.83^{d}	56.46±0.87^{cd}

- *Mean ±SD of three triplicates
- $^{a-f}$ Different letters in column of each sample category show significant differences (P<0.05).
- Abbreviations of samples are same as in Table 2

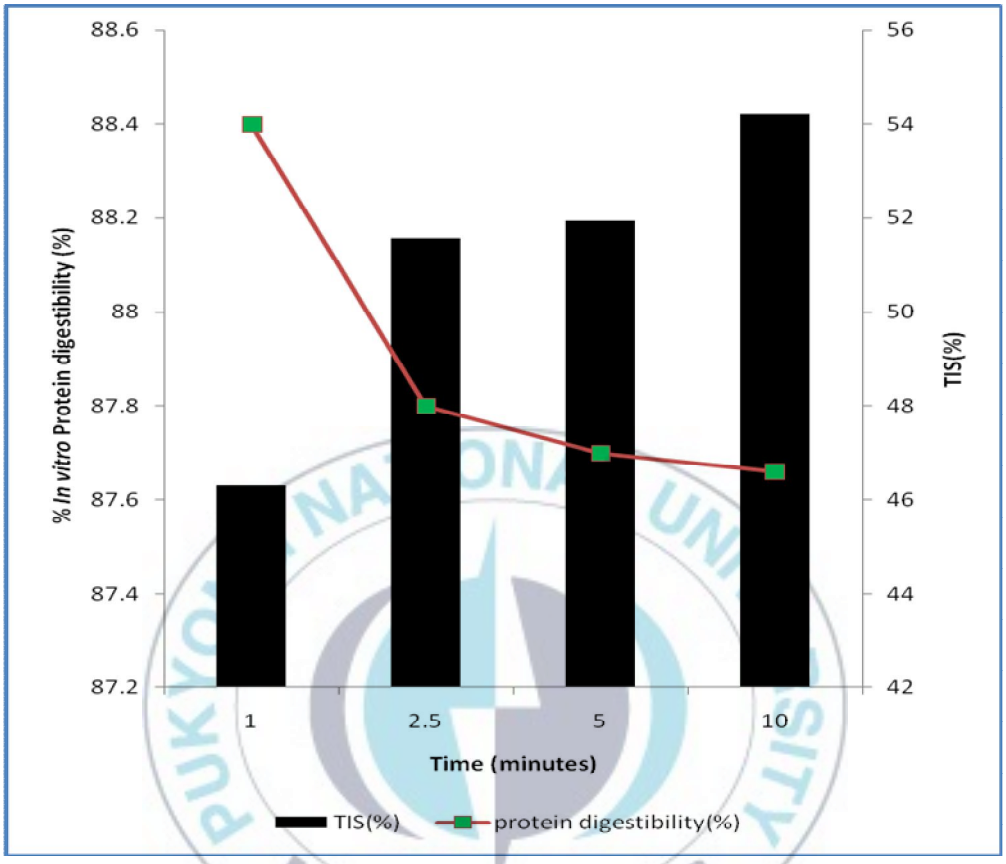

Fig. 3. Changes in protein digestibility and trypsin indigestible substrate (TIS) content of tilapia meat during boiling period

Nevertheless, it should be noted that, oxidation took place in all boiled and fried samples but it is more extreme to high temperature and duration of samples in boiling and frying pan. Lee and Ryu (1986) reported that in several instances, it was thought that the boiling and salting processes resulted in lipid extraction, which diminished formation of enzymes-indigestible substrate.

Oduro et al., (2011) also stated that TI include typical proteinaceous inhibitory materials contained in raw sources and indigestible materials such as trypsin indigestible substrate (TIS) induced from the results of interaction between protein and other components such as lipid oxidation.

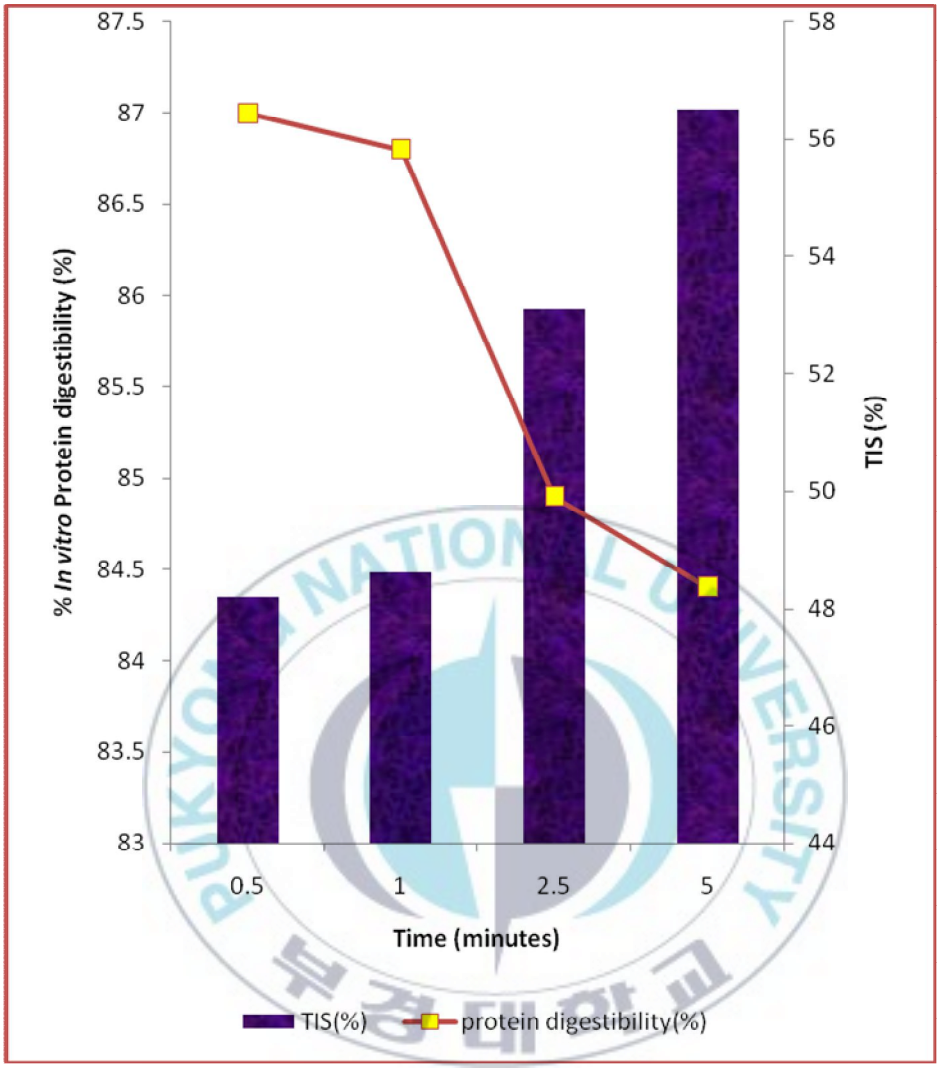

Fig. 4. Effect of frying on the protein digestibility and formation of trypsin indigestible substrate (TIS) in tilapia meat

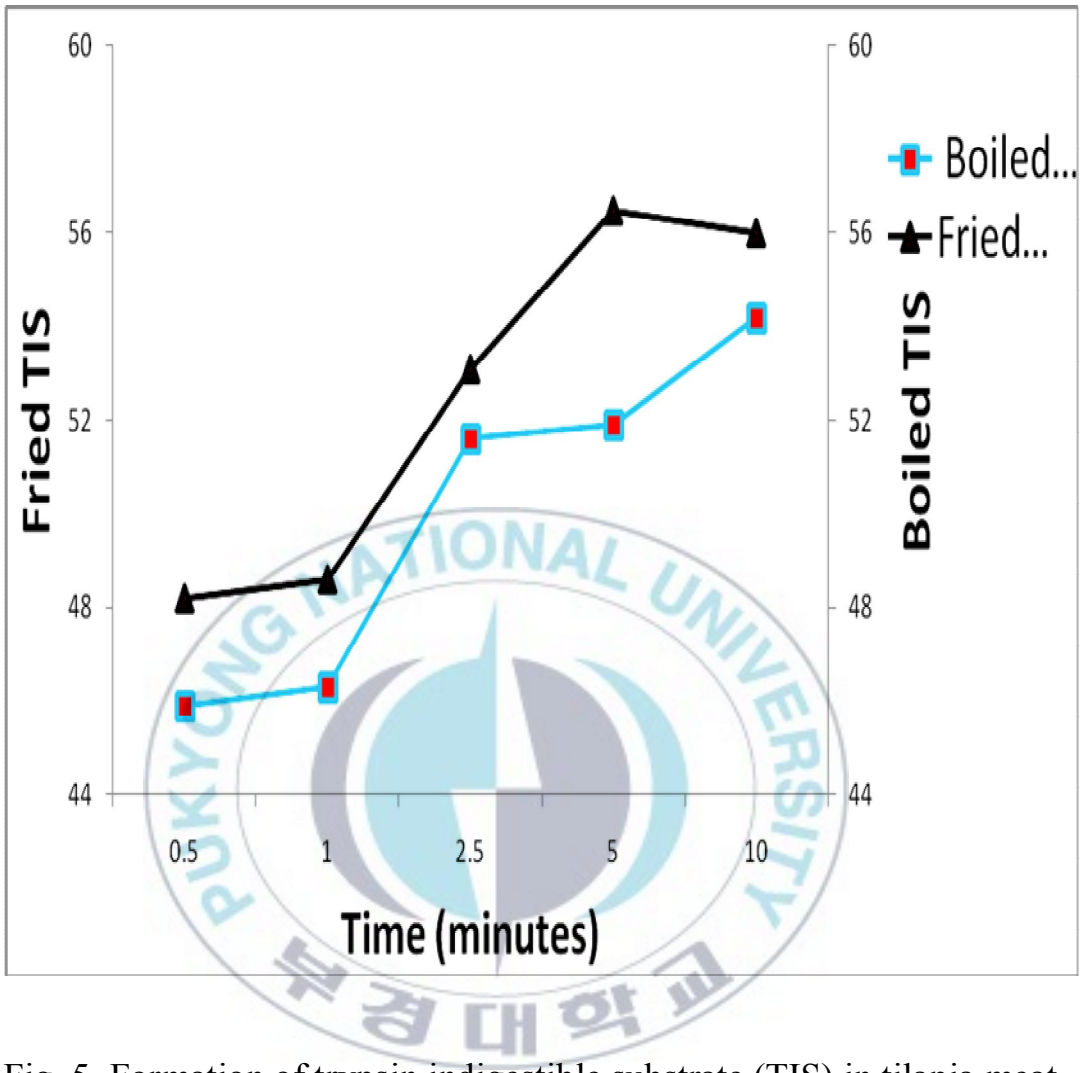

Fig. 5. Formation of trypsin indigestible substrate (TIS) in tilapia meat during frying and boiling.

3.4. Amino acid profiles

Table 5 shows the amino acid (AA) profiles for each boiled and fried samples at 0.5 and 1 minutes respectively. Tryptophan and cysteine had the lowest concentration among the amino acids. Carpenter et al., (1963) reported that, loss of amino acid availability results mainly from the interaction of proteins with oxidized lipids and their secondary products.

The oxidation of protein leads to both physical and chemical changes, including amino acid destruction, decrease in protein solubility due to polymerization, formation of amino acid derivatives and reactive carbonyls, changes in protein digestibility, and loss of enzyme activity.

Glutamic acid constituted the highest essential amino acid (EAA) concentration while methionine, phenylanine, tyrosine, isoleucine, valine and threonine had medium concentration. The same pattern was reported by Adeyeye, E.I (2009). Also, Zuraini et al., (2006) found that glutamic acid was 21.7±0.9g *in Channa striatus* and 19.4±1.9 (g/16g N) in *Chana micropeltes*.

Table 5. Amino acid profiles of ANRC casein and cooked tilapia meat protein

(g a.a./16g N.)

Amino acid	**ANRC casein**	**BS 1**	**FS 0.5**
Aspartic acid	7.12	9.60	9.60
Threonine*	4.08	4.11	4.08
Serine	5.27	3.54	3.21
Glutamine	22.72	15.57	15.57
Proline	11.00	3.64	3.64
Glycine	1.83	4.93	4.70
Alanine	3.08	6.63	6.63
Valine*	6.60	5.38	4.53
Isoleucine*	5.25	4.24	3.82
Leucine	9.66	8.81	8.81
Tyrosine*	5.66	4.00	4.00
Phenylalanine*	5.21	4.14	4.14
Histidine	2.90	2.45	2.31
Lysine	8.23	8.96	8.96
Arginine	3.87	8.27	8.27
Methionine*	2.84	3.27	3.27
Cystine*	0.58	1.28	1.28
Tryptophan*	1.03	1.18	1.18
Total	**106.93**	**100.00**	**98.00**

- *Essential amino acid; BS – boiled sample at 1 minute (98±2^{0}C); FS – fried sample at 0.5 minutes (178±2^{0}C)
- ANRC – Animal Nutrition Research Council was also quoted by Oduro et al., (2011)

Amino acids are the building block of protein, each protein consisting of a chain made up of amino acids in a unique sequence. Presence of reasonable EAA in tilapia provides the confidence that the species is a good source of protein. Glutamic acid content higher than other amino acids in smoked *Macrone nemurus* and *Cryptopterus micronema* (Huda et al. 2010). Our bodies need essential amino acid for growth, maintenance and reproduction.

In prediction of protein quality for cooked marine and mammalian flesh products, Acton and Rudd (1986) found the same trend of EAA. In this experiment tryptophan and cysteine were lower probably due to their high volatile behavior that they dissolve at lower temperature during heating. This phenomenon has also been reported by Sikorski (2011) also Friedman and Cuq (1988) who in the experiments they conducted for fried marine products found many derivatives of tryptophan.

Main forces involved in the stability of protein structure are hydrogen bonds, electrostatic interactions and covalent disulphide bond formation. Heating of protein solutions may weaken hydrogen bonds and electrostatic interactions but strengthen hydrophobic effects. EAA become biounavailable if the food protein is damaged by heat during cooking.

3.5. *In vitro* protein quality

All computed efficiency ratio (C-PER) and discriminant computed efficiency ratio (DC-PER) for boiled sample at 98±2^{0}C for 1 minute and fried sample in 178±2^{0}C for 0.5 minutes were higher than to standard casein. C-PER values for boiled and fried samples were 2.62 and 2.60 respectively. The DC-PER values read 2.70 in boiled samples and 2.67 for fried samples.

Protein quality can be evaluated using biological methods such as net protein utilization (NPU), protein efficiency ratio (PER), or chemical methods. In this study, computed efficiency ratio (C-PER) and discriminant computed efficiency ratio (DC-PER) was used and compared with standard ANRC casein.

The model (C-PER and DC –PER) that matched with *in vivo* digestibility was developed in computer. They were both calculated from EAA profile of samples of proteins. These models are believed to have correlation with rat bioassay (*in vivo* method). This method was also used successful by Hoyos et al., (2013).

Table 6. *In vitro* protein qualities for cooked tilapia meat

	ANRC Casein	**BS**	**FS**
In vitro protein digestibility (%)	90.00	88.41	86.96
Predicted digestibility (%)	90.00	88.41	86.96
C-PER	2.50	2.62	2.60
DC-PER	2.50	2.70	2.67

- C-PER = Computed Protein Efficiency Ratio
- DC-PER = Discriminant Computed Protein Efficiency Ratio
- ANRC – Animal Nutrition Research Council
- BS – boiled sample at (98±2^{0}C) for 1 minute; FS – fried sample at (178±2^{0}C) for 0.5 minutes

These results however, act in accordance with the statement made by Acton and Rudd (1986) in their research of protein quality for seafood that "A survey of the PERs of seafood materials and products showed that seafood protein quality, with few exceptions, is equivalent to or better than casein, the PER reference protein".

Either, Lilabati and Vishwanath (1995) in the research on nutritional quality of freshwater catfish (*Wallago attu*) available in Manpul- India reported that - The PER value of flesh fish is not significantly different from that of casein, both from flesh fish and reference casein at the 5% level of significance.

4. CONCLUSION

Experimental results suggest that best cooking condition is boiling trailed by steaming and grilling. Although fried samples showed digestibility within the indispensable range, it is not nutritionally advised due to its high fat content in proximate composition analysis. Frying encourage reasonable fat oxidation.

Crude lipid weight under dry basis was increasing in fried and grilled samples; it was decreasing in boiled and steamed samples. Statistically, changes in proximate composition between raw and cooked samples were not significant except in fried samples. On nutritional bases, these results prove that tilapia (*Oreochromis niloticus*) meat is a good source of protein.

In vitro protein digestibility showed that, digestibility decrease with the increase of cooking time and temperature. These differences in digestibility between raw and within cooking conditions themselves were not statistically

significant nutritionally, it has the connotation that tilapia meats should be cooked for short time.

Cooking fish for long time denature protein and become unavailable for being digested. However, it further gives the implications that, tilapia meat can be consumed raw and can be a very good raw materials for manufacturing of other fish and fishery products e.g. surimi.

Low level of trypsin indigestible substrate (TIS) in early times of cooking means protein in tilapia is available for digestion by digestive proteolytic enzymes in the digestive tracts. C-PER and DC-PER being higher than standard casein to bring to a close that protein found in tilapia is of high quality. Either, it implies that tilapia meat amino acids are more excellent than casein especially essential amino acids (EAA) inspite of its digestibility being lower than the standard casein. Presence of essential amino acids in different levels also supports this wrapping up.

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