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Nutritional evaluation of Fish Protein Hydrolysate from Tuna, *Thunnus albacares* (Bonnaterre, 1788)

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Abstract: **The present study is to explore the nutritional aspects of protein hydrolysate of Tuna (*Thunnus albacores*, Bonnaterre, 1788) emphasize on biochemical composition, and minerals. Protein hydrolysate was prepared from Dark and White meat of Tuna by enzymatic hydrolysis. Enzyme digestion was carried out with the enzyme Papain. Nutritional evaluation of fish includes moisture TDMH 9.4% and TWMH 10%, Protein TDMH 72% and TWMH 70%, Fat TDMH 3.32% and TWMH 3.9%, and Ash TDMH 12.95% and TWMH 13.42% respectively. Minerals were found in a significant level in both the fish Phosphorous TDMH 1483mg/100g and TWMH 1657mg/100g, Potassium TDMH 26.89mg/100g and TWMH 31.92mg/100g Calcium TDMH 593.84mg/100g and TWMH 626.34mg/100g, Sodium TDMH 27.04mg/100g and TWMH 35.21mg/100g and Iron TDMH 88.2mg/100g and TWMH 196.8mg/100g. The heavy metals illustrated were Copper, Manganese, Zinc, Magnesium, Mercury and Chromium present in both the protein hydrolysate. The knowledge about the biochemical composition of the fish enhances the utility of the fish as good source of quality protein.**

Keywords: **Heavy metals, Mineral analysis and Proximate composition.**

1. INTRODUCTION

Fish are profoundly nutritious nourishment products. Fish are one of the major sources of animal protein and of important nutrients in most nations for health maintenance and protection against various illnesses, including blood pressure, cancer and cardiac stroke (Fawole *et al* 2007). Sea foods decrease their vulnerability to famine by offering a supplementary source of nutrition when there is a seasonal low in other sources of food, like agriculture (Wisuthiphaet *et al* 2016). According to Foran *et al* (2005), fish are a major intake of protein sources in a larger percentage of the world's population, due to its reliability and nutritional value. Nowadays, knowledge about healthy food is growing and fish receive more acceptances because of its special qualities in terms of biochemical and nutritional (Wisuthiphaet N, Kongruang S 2015).

The commercial manufacturing of powders based on fishery protein is intended to strengthen the water binding and stability of frozen fish (Urch 2001). For the last five to six decades, researchers have focused on fish protein hydrolysate (FPH) preparing for better fish use using fish and fish waste. Protein hydrolysates were usually used to modify functional features and the use of high quality raw material protein in low costs. In addition, their elevated solubility properties add to the characteristics of the fragile populations of developing and developed nations as an optimum protein complement (Sathivel *et al*., 2005).

The biochemical composition of the fish indicates their nutritional properties. There for, proximate, biochemical composition of species helps to assess its nutritional and edible qualities. The most prevalent issues of nutrition assessment of diets and populations are nutritional adequacy and potential adverse effects of low or inadequate nutrient consumption. However, many nutrients in the normal diet are not only abundant in the advanced country, but they can also be taken as nutritional supplements for a percentage of the population so that general consumptions can be far above the demands and the recommended intakes (Nesse *et al*., 2014). The present research thus provides a pioneering analysis of the nutrient properties of the essential components of the species in the Tunas protein hydrolysates that encompasses several nutritional parameters and health indicators.

2. MATERIALS AND METHODS

2.1 Sample collection

The fish Tank goby was selected to the study and collected from Munambam fishing harbour, Cochin, Kerala, India and brought into lab under iced condition (ratio1:1) in an insulated box. The tissue was separated by meat bone separator and observed that 55.63% of yield obtained and stored in -20^{0}C for future purposes.

2.2 Preparation of Fish Protein Hydrolysate

Enzymatic hydrolysis of Tuna (dark and white) was carried out according to the procedure described by Liceaga-Gesualdo and Li-chan, (1999). Hydrolysed fish protein was produced by mixing fish, usually ground or mixed with water and proteolytic enzymes. A 20gm portion of dark meat and white meat were thawed. They were finely chopped and boiled for 15 minutes with an equal volume of water and immediately cooled. The mixture was stirred, and the p^H was adjusted using p^H meter (Cyberscan 500p^H) to the desired level (p^H 6.5 for Papain). The enzyme Papain (commercial grade activity 10 units/mg enzyme) was added separately at 0.5% (wt/wt) of protein in mince. The mixture was continuously stirred for 240 minutes, maintaining the temperature at 55^{0}C by keeping in a thermostatically controlled water bath. The enzyme activity was terminated by heating the solution at 90^{0}C for 30 minutes for Papain (Benjakul and Morrissey, 1997). The soluble fraction was sieved through a muslin cloth, and the resultant solutions were kept in a petri plate for freezing and lyophilized using Lyolab-Techs-HINHDIVAC lyophilizer. Then the lyophilized hydrolysate powder was stored in airtight plastic container and used for further analysis.

2.3 Degree of Hydrolysis (%)

Degree of hydrolysis (DH %) was evaluated as percentage of a-amino nitrogen in the hydrolysates with respect to the total nitrogen content in the wet meat. Total nitrogen content was determined by Kjeldahl method (AOAC, 2000) and a-amino nitrogen was determined by formol titration method (Taylor, 1957).

$$\text{Degree of hydrolysis} = \frac{\alpha -\ \text{amino Nitrogen}}{\text{Total Nitrogen}} \times 100$$

2.4 Mineral and Trace metal analysis

Analyses of iron (Fe), copper (Cu), manganese (Mn), cadmium (Cd), nickel (Ni), magnesium (Mg), sodium (Na), phosphorus (P), potassium (K), and calcium (Ca) contents in the sample were carried out in Flame Atomic absorption spectrophotometer (Perkin Elmer,AAnalyst200, Version 8.0, 2013), according to the method of AOAC (1999). Sample (4 g) was mixed well with Conc.HNO_3 and $HClO_4$in the ratio 1:4 using a heating digestion furnace at 700^{0}C until all organic matter had been destroyed. After evaporation of HNO_3, the residual solution was transferred to a volumetric flask and the volume was made up to 25ml with deionised water and stored in plastic bottles.The solution was then subjected to analysis. All samples were analysed in triplicate as per standard conditions. The operating parameter for working elements was set as recommended by manufacturer. The blank and calibration standard solution were also analysed in the same way as for the samples. The concentration of minerals and trace metal were calculated and expressed as ppm.

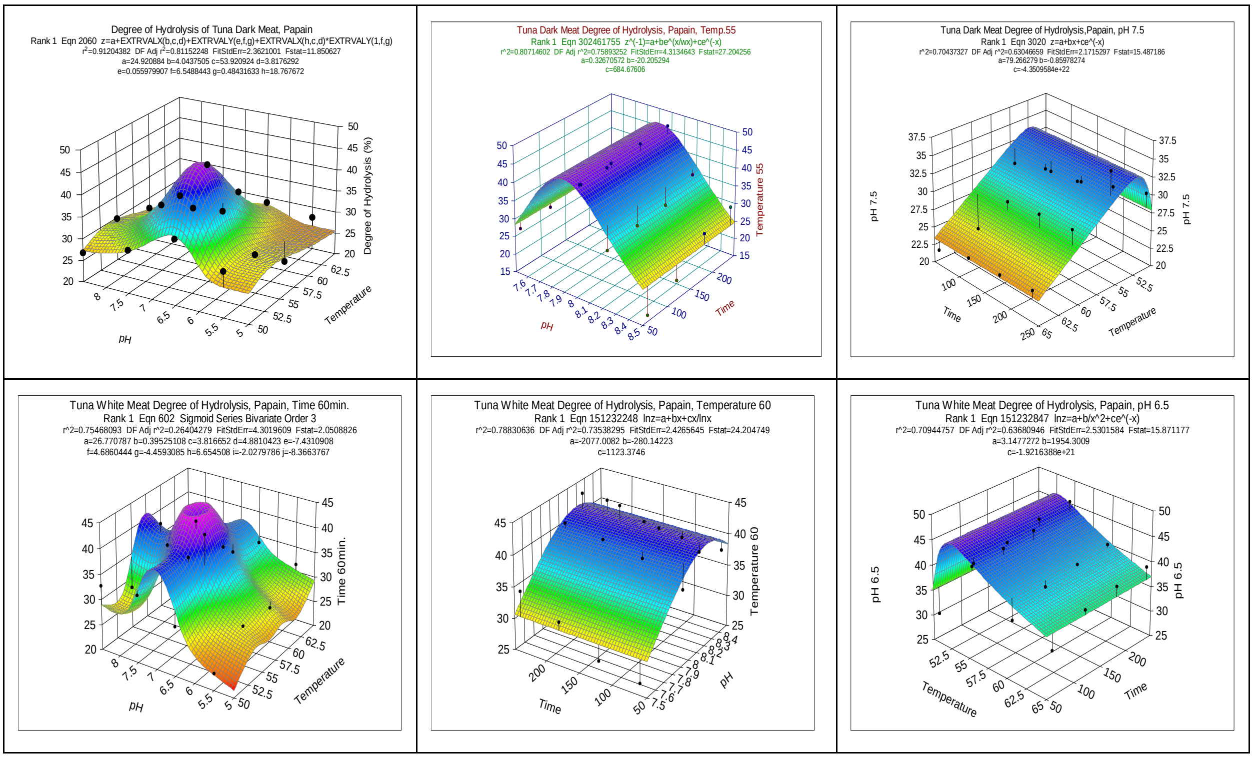
2.5 Statistical analysis

All the tests were done in triplicate and data were averaged. Standard deviation was also calculated. The data were analysed using Systat 3D plot software.

3. RESULT AND DISCUSSION

Degree of Hydrolysis (%)

Degree of hydrolysis of tuna dark meat and white meat determined at various temperature and p^H on incubation for 240 minutes are presented in the graph respectively. The results show that both dark meat and white meat show relative changes in their hydrolysis level. The maximum degree of hydrolysis (Dark 46.7%, White 46.126%) occurred at 55^{0}C and p^H 6.5 in both the dark meat and white at the fourth hour of hydrolysis. The degree of hydrolysis diminished when the p^H was brought down or expanded since the optimum activity happened at near to the neutral, p^H 6.5 for enzyme Papain. The degree of hydrolysis showed slightly higher value in the study of Slizyte *et al*., (2005), (26 to 32 %) on cod. Balaswamy (2011) reported that the degree of hydrolysis of Catla hydrolysate after 90min was 60.8% to 68.5% respectively.



Proximate composition

The hydrolysate prepared from Tuna dark and white meat had comparably high moisture content (9.4% and 10%). The protein content was 72.09% in dark meat hydrolysate and 70.37% in the white, and it was noticed that Dark meat shows higher protein content. The total fat content of hydrolysate is comparably less than the raw material (6-9%). Decreasing lipid content in hydrolysate contributes significantly to the stability of material towards lipid oxidation (Diniz and Martin, 1997; Nilsang *et al*., 2005). Ash content of the two hydrolysates was observed to be high (12.95 % and 13.42 %), and this was because of the addition of surplus alkali into the sample to regulate the pH at the desired level (Liceaga-Gesualdo and Li-chan, 1999). Thankappan *et al*., (1998), reported approximately near the chemical composition of hydrolysate except in the case of fat and moisture. The protein content of different hydrolysates was within the range reported by others of 63.4% to 90.8% (Bhaskar *et al*., 2008) Moisture content within the range of 3.5% - 10% (Ovissipour *et al*., 2009; Chalamiah *et al*., 2010) ash 5%-25% (Nilsang *et al*., 2005; Ovissipour *et al*., 2009) respectively.

Table 2: Proximate composition of Tuna Dark and White meat Protein Hydrolysate using Papain

ENZYME	PAPAIN			
SAMPLE	MOISTURE (%)	PROTEIN (%)	FAT (%)	ASH (%)
TDMH	9.4±0.46	72.09±0.93	3.32±1.8	12.95±2.52
TWMH	10±0.24	70.37±1.6	3.9±0.2	13.42±1.46

Mineral composition

Minerals contents of Tuna white and dark meat had been studied Sodium, Potassium, Calcium, Phosphorus, and Iron showed in Table: 3. Sodium is a very vital mineral that is needed with the aid of the body to alter blood quantity and blood pressure. The sodium content of TDMH and TWMH was found to be 27.04±2.21mg/100g and 35.21±1.9mg/100g, which was lower than Mola 39 mg g^{-1}, *H. ilisha* 44 mg g^{-1}, *C. catla* 74 mg g^{-1}, (Bogard *et al*; 2015), *S. longiceps* 289.6mg g^{-1} (Sinduja *et al*; 2013).

Potassium is an essential mineral for regular body functions. Potassium intake has an indispensable role in regulating blood pressure, high potassium intake helps to reduce the risk of stroke, preventing the development of renal vascular, glomerular, and tubular damage, decreasing urinary calcium excretion, reducing the formation of kidney stones, and reducing demineralisation of bone (osteoporosis). (He and Mac Gregor, 2001). Potassium content in TDMH and TWMH was observed to be 26.89±0.67mg/100g, and 31.92±0.01mg/100g which was higher than *S. seenghala* (13780.01 ppm) (Mohanty *et al*., 2012), and lower than *R. kanagurta* 2397 mg g^{-1}, *S. longiceps* 268.9 mg g^{-1}, *Lates calcarifer* 1086.3mg g^{-1}, *M.cephalus* 878.3mg g^{-1}, *P. viridis* 1810.2 mg g^{-1} (Mohanty *et al*., 2016).

Phosphorus is an essential mineral required for cell structure, signaling, energy transfer, and other important functions. The latest research recommends that excessive phosphorus consumption ought to have damaging outcomes on the skeletal, renal, and cardiovascular systems (Chang and Anderson, 2017). The amount of Phosphorous was highest in TDMH and TWMH (1483.2±0.54mg/100g and 1657.68±0.4 mg/100g) which was higher than those reported for K. pelamis 698mg g^{-1}, *Leiognathus splendens* 1249mg g^{-1}, *S. longiceps* 1389 mg g^{-1}, *C. catla* 146.8mg g^{-1}, *L. calcarifer* 920.9mg g^{-1} (Mohanty *et al*., 2016).

Calcium as a nutrient provides rigidity to the skeleton and calcium ions associated many if not most metabolic processes. Rigidity is usually given by calcium carbonate in a primitive exoskeleton and shells, but in a vertebrate skeleton it is provided by a shape, approximating hydroxyapatite [Ca10(OH)2(PO4)6] and is integrated into collagen fibrils (FAO / WHO, 2001). TDMH 593.84±0.91 mg/100g, and TWMH 626.34±0.67 mg/100g, which was higher than Epinephelus spp 162.1 mg g^{-1}, *S.longiceps* 523.9 mg g^{-1}, *C. catla* 161.1 mg g^{-1} (Mohanty *et al*., 2016).

The ability of Iron to function as an electron donor and an electron receiver is crucial for life. Most body iron is located inside protein complexes and the main component of a hema molecule is formed. Thus, iron plays a key role in the transportation and storage of oxygen in the muscle through the body. Iron has a significant role for the transport, breathing and hormone synthesis of electronic products (Harvey et al, 2013). Iron concentration was reported 88.2±6.075mg/100g and 196.8±2.61mg/100g which was higher than *R. kanagurta* 5.0 mg g-1, *S. longiceps* 7.8 mg g-1, *T. albacares* 7.05mg g-1, *C. catla* 1.6 mg g-1, *L. rohita* 2.2 mg g-1, *L.calcarifer* 11.8 mg g-1, *M. cephalus* 12.8 mg g-1 (Mohanty 2016).

Table 3: Mineral compositions of Tuna Dark and White meat Protein Hydrolysate

Minerals	TDMH	TWMH
Pottassium	26.89±0.67	31.92±0.01
Sodium	27.04±2.21	35.21±1.9
Calcium	593.84±0.91	626.34±0.67
Phosphorus	1483.2±0.54	1657.68±0.4
Iron	88.2±6.075	196.8±2.61

Heavy metals

The heavy metals studied were Copper, Manganese, Zinc, Magnesium, Mercury, and Chromium. Copper is a heavy metal, that plays an important role as an important cofactor in various metabolisms including oxidation, gene regulation, and free radical homeostasis. But when it exceeds the required level affects biologic process requirements and can cause anaemia, liver and kidney damage, it becomes harmful and plays a major role among pollutants (Singer *et al*, 2005). The copper showed TDMH 0.030±0.0065 ppm and TWMH 0.048±0.006ppm, which is relatively lower than *R. kanagurta* 1.0 mg g^{-1}, *L. calcarifer* 0.5 mg g^{-1}, *T. ilisha* 2.78 mg kg^{-1} (Ganguly *et al*., 2017 and Mohanty 2016).

The synthesis and activation of several enzymes is based on manganese (MN); the increased rates of protein synthesis, glucose and lipid metabolism; vitamin C and vitamin B; hematopoiesis catalysis, endocrine regulation and immune function improved (Aschner and Aschner, 2005). Mn content in TDMH 6.182±0.93ppm and TWMH 5.978±0.55 ppm which is lower than that of *S. argus* 35.8 mg kg^{-1} (Vijayan *et al*., 2016), *S. longiceps* 0.4 mg g^{-1}, *L. calcarifer* 0.2 mg g^{-1}, *M. cephalus* 0.04 mg g^{-1} (Mohanty 2016).

Zinc deficiency in humans currently renowned for being a critical dietary deficiency disease downside world-wide. Zinc is especially important during periods of rapid growth, both pre and postnatally, and for tissues with fast cell separation and turnover, for instance, the defense mechanisms and the gastrointestinal tract. Critical functions that are influenced by zinc nutriture incorporate maternity results, physical development, susceptibility to infection, and neurobehavioral development, among others (Brown *et al.*, 2001; Roohani *et al.*, 2013). The Zn content of TDMH and TWMH was 42.5±2.81ppm and 33.86±2.69ppm which was lower than *S. argus* 39.1 mg kg^{-1} (Vijayan *et al.*, 2016), *R. kanagurta* 13.0mg g^{-1}, *S. longiceps* 4.0mg g^{-1}, *L. calcarifer* 5.56mg g^{-1}, *Crassostrea madrasensis* 0.43mg g^{-1}, Japanese leatherjacket fish 6.63 ppm (Mohanty 2016; Ajeeshkumar *et al.*, 2015).

Chromium (III) is relatively nontoxic and identified as a novel micronutrient for its beneficial role in human nourishment by serving as a vital cofactor in the action of insulin as well as nutritional enhancement to energy, glucose, and lipid metabolism. (Zafra-Stone 2007). TDMH had a Cr level of 18±3.215ppm, and TWMH had 25.81±3.41 ppm the obtained results are higher than *H. fossilis* 1.32 µg/g, *L. rohita* 1.40 µg/g, *C. marulius* 0.71 µg/g (Maurya and Malik, 2018).

Magnesium is critically linked to the cell viability and physiological regulation of all systems and bodies. It modulates the mechanical, electrical and structural functions of cardiac and vascular cells in the cardio-vascular system, and there can have deep impacts on heart excitability, contractility and reactivity in the extracellular concentrations of magnesium and in addition free intracellular concentration of Magnesium (Rios 2017). In TDMH the Mg concentration found to be (114.6±0.2 ppm) and TWMH (114.5±0.33 ppm) lower than other species, Indian mackerel (710.99 mg/100g), Malabar red snapper (898.40 mg/100g), Cuttle fish (1317.30 mg/100g) (Maurya and Malik, 2018).

Table 4: Heavy metals concentration of Tuna Dark and White meat Protein Hydrolysate

Metal	TDMH	TWMH
Copper	0.030±0.0065	0.048±0.006
Manganese	6.182±0.93	5.978±0.55
Zinc	42.5±2.81	33.86± 2.69
Chromium	18.99±3.215	25.81±3.41
Magnesium	114.6±0.20	114.5±0.33
Mercury	ND	ND

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