International Journal of Sciences

Research Article Volume 8 – November 2019 (11)

Enzymatic Production of *Sarotherodon galillaeus* Muscle Protein Hydrolysates and Assessment of its Alpha-Amylase Inhibitory and Antioxidant Potential

Oludele Olyemi Odekanyin[1], Abideen Oyinlola Ayangbemi[1], Aanuoluwapo Joy Jerome[1]

[1]Biochemistry and Molecular Biology Department, Obafemi Awolowo University, Ile-Ife, Nigeria

Abstract: The study was carried out to evaluate the antioxidant activity and α-amylase activity inhibitory potential of *Sarotherodon galillaeus* muscle protein hydrolysates. *Sarotherodon galillaeus* muscle protein isolate was hydrolysed with three digestive proteases namely trypsin, chymotrypsin and pepsin. Degree of hydrolysis was determined. The antioxidative potential of the hydrolysates was investigated using DPPH radical scavenging, ferric reducing power, hydrogen peroxide scavenging and metal chelating activity. The ability of the hydrolysates to inhibit the activity of sugar-hydrolysing enzyme was also evaluated. Highest degree of hydrolysis was obtained with pepsin (55.86%) followed by trypsin (47.11%) and chymotrypsin (42.36%) after 6 hrs of hydrolysis. The half maximal inhibitory concentration (IC_{50}) of hydrolysates produced by trypsin, chymotrypsin and pepsin for DPPH radical scavenging activity were 1.26 ± 0.95, 0.98 ± 0.07 and 1.18 ± 0.34 mg/ml respectively. Trypsin-produced hydrolysates displayed highest metal chelating activity and ferric reducing power while hydrolysates obtained with pepsin showed highest hydrogen peroxide scavenging activity. Amylase activity inhibitory potential of all the hydrolysates was low with pepsin-produced hydrolysates shown highest inhibitory effect of 18.46 ± 1.51%. The hydrolysates showed antioxidative potential that can be used in prevention of food oxidation.

Keywords: *Sarotherodon galillaeus*, Hydrolysate, Amylase, Antioxidant, Degree of Hydrolysis

1. Introduction

Among freshwater fishes that belong to the family Cichlidae is Tilapias. Tilapia group consists of three important genera namely *Sarotherodon*, *Oreochromis*, and *Tillapia*, which are most economically and commercially important fishes of the Tropical Africa (Toniato *et al.*, 2010). *Sarotherodon galillaeus*, known as Mango Tilapia, Galilae Tilapia, or St Peter's fish, is a species of genus *Sarotherodon*. It is widely and commonly cultured in Nigeria, though it can be found in lakes, rivers and other fresh and brackish habitats in Northern and Central Africa, the Congo River Basin and Ethiopia (Froese *et al*., 2014). Tilapia culture, still developing in Nigeria, has contributed to the reduction of poverty level through increase in income generation for fish farmer, improved food security and also create more employment opportunities for Nigerian. Scientifically, tilapias have been receiving more attention because of the number of species being cultured and also they are important source of animal protein for humans (Toniato *et al*., 2010).

Currently, researchers are focussing on reduction of waste generated by fish processing industry as by-products by utilizing the wastes to generate health promoting ingredients or food additive that could inhibit lipid peroxidation in food. Generation of bioactive peptides from fish proteins have also received a great interest from researcher worldwide (Chalamaiah *et al*., 2012). Bioactive peptides are sequence of amino acids that are not active within the precursor protein sequence but when released have been found to have various biological functions such as antidiabetic (Ramadhan *et al*., 2017; Xia *et al*., 2017), antithrombotic (Erdmann *et al*., 2008), antihypertensive (Elavarasan *et al*., 2016; Korczek *et al*., 2018), antimicrobial (Bernardini *et al*., 2011), antioxidant (Nasri, 2017; Korczek *et al*., 2018), antiproliferative (Picot *et al*., 2006)and different applications in pharmaceutical, cosmetics and food processing industries (Chalamaiah *et al*., 2012; Nasri, 2017).

Oxidative stress is a phenomenon that reflects an imbalance between the occurrence of reactive oxygen species and capacity of organisms to counteract their

Published at: http://www.ijsciences.com/pub/issue/2019-11/
DOI: 10.18483/ijSci.2174; Online ISSN: 2305-3925; Print ISSN: 2410-4477

Oludele Olyemi Odekanyin (Correspondence)
+ 234806-287-9457

action by the antioxidative protection system (Persson *et al*., 2014). Increase in oxidative stress is linked to diabetes and other chronic diseases such as cancer, hypertension (Rahimi *et al*., 2005). Treatment of oxidative stress along with the diabetes clinical treatment may result in better management of diabetes and its associated complication. Among the approaches of managing diabetes is inhibition of α-amylase and α-glucosidase enzymes that responsible for increase in blood glucose level by releasing monosaccharides from carbohydrates degradation into blood stream (Kehinde and Sharma, 2018). Search for naturally occurring antioxidant agents that have potency for diabetes treatment is on the increase. Apart from the antioxidant peptides with antidiabetic effects that are of plant origin (Harnedy and FitzGerald, 2013; Wang *et al*., 2018), bioactive peptides obtained by enzymatic hydrolysis of animal proteins have also shown to possess potent antioxidant ability as well as α-amylase and α-glucosidase inhibitory activities (Ramadhan *et al*., 2017; Zambrowicz *et al*., 2015; Huang and Wu, 2010) and more additional effects which includes anticoagulant, antihypertensive, antimicrobial and antiproliferative (Ngo *et al*., 2012; Xia *et al*., 2017). Reports of antidiabetic peptides from fish are limited.

Therefore, current study was designed to evaluate the degree of hydrolysis of Mango tilapia (*S. galilaeus*) muscle protein with digestive proteases and determine whether the hydrolysates have antioxidant activity and can also inhibit α-amylase activity.

2. Materials and Methods

2.1. Collection of Sample

Sarotherodon galillaeus was obtained from the Opa reservoir, Eleyele, Ile-Ife, Osun State, Nigeria. The fish was identified and authenticated at the Fishery Research Unit, Zoology Department, Obafemi Awolowo University, Ile- Ife.

2.2. Preparation of Crude Fish Muscle Protein

The *Sarotherodon galillaeus* muscle (fillet) was excised and rinsed in chilled distilled water. The fillet was pounded to increase surface area, then immediately frozen. The frozen muscle was lyophilized. The freeze-dried fish muscle was blended to powdery form, using warring blender. Soluble proteins were extracted from the powder by homogenizing in distilled water at a ratio 1:10 (fillet : distilled water). The mixture was stirred on a magnetic stirrer for 4 hrs, centrifuged at 10,000 xg in a cold centrifuge for 20 min at 4^{o}C and the homogenates, which is the fish muscle protein concentrate, was freeze-dried.

2.3. Preparation of Fish Muscle Protein Hydrolysate (FPH)

The fish muscle protein hydrolysate was prepared by incubating the fish protein concentrate with pepsin, trypsin and chymotrypsin separately. Optimum hydrolysis conditions reported by Fan *et al*., (2012) were used. Glycine-HCl buffer (0.1M, pH 2.0) was used for pepsin at 37 ^{o}C and enzyme to substrate ratio of 1:100 w/w. The same condition was used for trypsin and chymotrypsin (0.1 M phosphate buffer, pH 7.5 and at 45^{o}C; enzyme to substrate ratio of 1 : 100 w/w). Each mixture was incubated in a waterbath for 6 hours with continuous stirring. The enzyme was inactivated by incubating in a boiling waterbath for 10 minutes. The content was rapidly cooled in ice-cold water and centrifuged at 10,000 xg for 20 minutes to obtain the supernatant which is the fish protein hydrolysates (FPH). The supernatant was freeze-dried and stored in the refrigerator until use.

2.4. Degree of Hydrolysis Determination

Degree of hydrolysis (DH) of protein hydrolysates was determined according to the percentage soluble protein in 10% of trichloroacetic acid (TCA) in relation to the total protein content of the sample as described by Hoyle and Merritt (1994) but modified by Morais *et al*. (2013). Aliquots of the protein hydrolysates were removed at interval of 30 minutes and treated with the same volume of 20% TCA to make 10% TCA-soluble protein. The mixture was centrifuged after 30 minutes at 3500 xg for 20 minutes and supernatants were analysed for soluble protein content by Lowry *et al*. (1951). Degree of hydrolysis (DH) was calculated by the expression below.

$$\% \text{ DH} = \frac{Soluble\ protein\ content\ in\ 10\%TCA\ (mg)}{Total\ protein\ content\ (mg)}\ X\ 100$$

2.5. Determination of Protein Concentration

Protein concentration of the crude and protein hydrolysates was determined by the method of Lowry *et al*. (1951) using Bovine Serum Albumin (BSA) as standard.

2.6. Antioxidant Assays

2.6.1. 1,1-diphenyl-2-picrylhydrazyl (DPPH) Free Radical Scavenging Assay

Radical scavenging properties of protein hydrolysates from the muscle of *S. galillaeus* was determined by the stable radical DPPH method described by Cao *et al*. (2013) with slight modification. Fresh DPPH solution containing 0.1 mM DPPH in 95% methanol was prepared daily. 100 µL of varying concentration of protein hydrolysates was mixed with 100 µL of the DPPH solution in a 96 wells plate. The mixture was incubated for 30 minutes in the dark at room temperature and the absorbance taken at a wavelength

of 517 nm. Distilled water was used as blank and GSH as the positive control.

$$\text{DPPH Free radical Scavenging}(\%) = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100\%$$

2.6.2. Metal Chelating Activity Assay

The metal chelating properties of protein hydrolysates from the muscle of *S. galillaeus* was determined by iron chelating method described by Cristina *et al*. (2012) with slight modification. 100 µL of varying concentration of protein hydrolysates was pipetted and reacted with 100 µL of 2 mM $FeCl_2$ and 100 µL of 5 mM ferrozine in a 96 well microplate. Ferrozine (5 mM) was diluted 20 times. The mixture was left in a dark cupboard for 20 minutes at room temperature. The absorbance was measured at a wavelength of 560 nm. The control was prepared in the same way as the sample except distilled water was used instead of the sample. EDTA was used as positive control.

$$\text{Metal Chelating Activity}(\%) = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100\%$$

2.6.3. Hydrogen peroxide (H_2O_2) scavenging activity assay

The hydrogen peroxide scavenging activity of protein hydrolysates from the muscles of *S. galillaeus* was determined according to the method described by Sun *et al*. (2012) with slight modification. A solution of 20 mM H_2O_2 was prepared in 0.1 M PBS (pH 7.4). Volumes of 100 µL of protein hydrolysates were mixed with 200 µL of the H_2O_2 solution in a 96 well microplate. Absorbance was measured at a wavelength of 230 nm after 10 minutes incubation. Blank solution was PBS with H_2O_2 and distilled water instead of sample. GSH was used as positive control.

$$\text{Scavenged H2O2}(\%) = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100\%$$

2.6.4. Ferric reducing potential activity assay

Method described by Babu *et al*., (2013) was adopted with modification to evaluate the ferric reducing potential of the *S. galillaeus* muscle protein hydrolysates. Phosphate buffer (0.2 M, pH 6.6, 0.5 ml) was pipetted into various test tubes and 0.2 ml of varying concentration of muscle protein hydrolysates was added. The mixture was vortexed after which 0.5 ml of 1% (w/v) potassium ferriccyanide solution was added to each tube. This was followed by incubation at 50^{o}C for 20 minutes in a waterbath. 10% (w/v) TCA (0.5 ml) was added and the tubes content was centrifuged at 3,000 rpm for 10 minutes. Equal volume (100 µl) of the supernatant and distilled water were mixed and followed by addition of 20 µl of 0.1% (w/v) ferric chloride solution in a 96-well microtitre plate. Absorbance was taken at a wavelength of 700 nm using microtitre plate spectrophotometer reader. Ascorbic acid was used as a positive control.

2.7. Statistical analysis

The experiments were performed in triplicate and results expressed as mean ± SEM. ANOVA was used to analyse the data using Graphpad prism statistical software. Significant differences were determined by using Duncan's multiple range tests at $p<0.05$.

3. Results and Discussion

Based on the different specificity possessed by digestive enzymes, protein hydrolysates, containing peptides with different number and sequence of amino acids, were prepared from *S. galillaeus* muscle using various digestive proteases: chymotrypsin, trypsin and pepsin. The degree of hydrolysis of the *S. galillaeus* muscle protein displayed by various enzymes varies with the highest hydrolysis degree of 55.86% shown by pepsin. Degree of hydrolysis obtained with chymotrypsin and trypsin after 6 hours of hydrolysis were 42.36% and 47.11% respectively. However, the hydrolysis curves showed the same pattern (Figure 1).The degree of hydrolysis was slow and could not reach the maximum even at 6 hours of hydrolysis, because hydrolysis was observed to be increasing at that time. The proteins present in the *S. galillaeus* muscle may be complex proteins which are hydrolysed more slowly (Benjakul and Morrissey, 1997). Also, the reduced rate of hydrolysis may be as a result of low enzyme – substrate ratio used in this study coupled with reduced accessibility of peptide bonds to each protease (Daud *et al*., 2013). The hydrolysis curves followed the trend reported for Smoothhound fish (Bougatef *et al*., 2009), Cuttlefish (Balti *et al*., 2010), Gobyfish (Nasri *et al*., 2012), Sardinelle (Ben-Khaled *et al*., 2012) and Trunkfish (Jerome and Odekanyin, 2019).

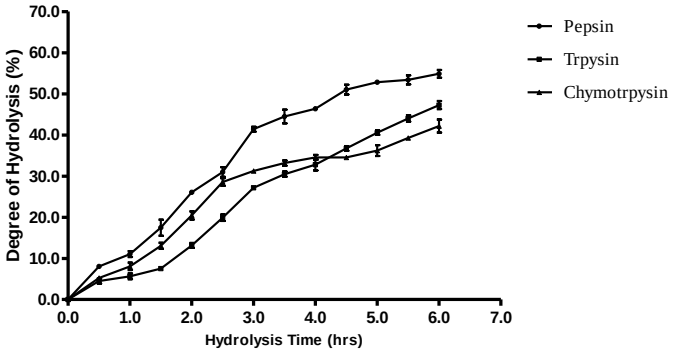

Figure 1: Degree of Hydrolysis of *Sarotherodon galillaeus* Muscle Protein

The current increasing interest of researchers in natural antioxidants had led to more studies investigating the antioxidant capacity of biologically active peptides obtainable from protein hydrolysates of fish. Nasri (2017) stated that biological and functional properties of protein hydrolysates depend on the amino acids sequence and peptide length which is determined by the specificity of the enzyme, nature of protein substrate and hydrolysis conditions. The effect of the extent of enzymatic hydrolysis on the antioxidant potential of *S. galillaeus* muscle protein was studied by using various antioxidant assays methods because antioxidant activity of protein hydrolysates may not be attributed to a single mechanism. DPPH radical scavenging assay is simple and most widely used to determine the ability of natural compounds to donate electron or hydrogen radical. DPPH is a stable free radical that shows maximum absorbance at 517nm but the absorbance reduces when it scavenged radical. The decrease in absorbance is taken as a measure for radical scavenging ability. The percentage inhibition of DPPH radicals by varying concentration of protein hydrolysate of *S. galillaeus* muscle was obtained and the results shown in Table 1. The result shows that the DPPH radical scavenging ability of all the hydrolysates was significantly different from that of the glutathione used as standard antioxidant. The activity was concentration dependent. However, trypsin, chymotrypsin and pepsin obtained hydrolysates gave radical scavenging ability of 24.87 ± 0.11, 30.92 ± 0.90 and 25.37 ± 0.15 mg/ml respectively at 0.5 mg/ml of the hydrolysate. The half maximal inhibitory concentration (IC_{50}) of hydrolysates produced by trypsin, chymotrypsin and pepsin were 1.26 ± 0.95, 0.98 ± 0.07 and 1.18 ± 0.34 mg/ml respectively while glutathione gave IC_{50} of 0.02 ± 0.004 mg/ml. This was in agreement with Jia *et al.*, (2010) who reported that the DPPH radical scavenging ability of the hydrolysate from Alaska Pollack skin was lower than that of glutathione. The half maximal inhibitory concentration (IC_{50}) showed that the hydrolysates obtained in the present study have a better radical scavenging potential than Alaska Pollock skin hydrolysate with IC_{50} of 2.5 mg/ml. Also, results of this study are within the IC_{50} range (0.6 mg/ml – 1.2 mg/ml) reported by Bougatef *et al.*, (2009) for hydrolysates obtained by treatment of different proteases with Smoothhound muscle protein.

Table 1: DPPH Radical Scavenging Activity of Varying Concentration of *Sarotherodon galillaeus* Muscle Protein Hydrolysates

Concentration (mg/ml)	**DPPH Free Radical Scavenging (%)**		
	Trypsin	**Chymotrypsin**	**Pepsin**
0.05	5.02 ± 0.15	8.55 ± 0.04	7.46 ± 0.16
0.1	8.60 ± 0.72	9.54 ± 0.78	10.45 ± 0.45
0.2	10.75 ± 0.17	13.82 ± 0.16	12.69 ± 0.90
0.3	13.26 ± 0.46	15.79 ± 0.16	15.67 ± 0.72
0.4	14.81 ± 0.17	21.05 ± 0.61	20.52 ± 0.45
0.5	24.87 ± 0.11	30.92 ± 0.90	25.37 ± 0.15
IC_{50} (mg/ml)	1.26 ± 0.92	0.98 ± 0.07	1.18± 0.34

Ferrous ion (Fe^{2+}) is a metallic ion that promotes the generation of reactive oxygen species such as hydroxyl radical and superoxide anion (Ajibola *et al.*, 2011; Farvin *et al.*, 2014). These radicals can accelerate several chain reactions that may lead to lipid peroxidation and allied medical disorders and diseases. Therefore, the ability of any biologically active compound to chelate the transition metal ion could ultimately reduce the susceptibility of lipids to oxidative peroxidation. The percentage metal chelating power of varying concentration of protein hydrolysate of *S. galillaeus* muscle was obtained and found to increase with increase in concentration. The results are presented in Figure 2. Hydrolysate obtained with trypsin displayed the highest metal chelating power of 69.47 ± 0.54 % at the highest concentration of 0.33 mg/ml but still lower than the metal chelating power displayed by EDTA as standard at the same concentration (93.91 ± 0.90 %). Tremendous increase was noticed in the metal chelating potential of all the hydrolysates when compared with the fish protein concentrate

((unhydrolysed protein) potential. This is in agreement with previous observation made by Farvin *et al*. (2014). The crude protein of Cod (*Gadus morhua*) has lower metal chelating activity (Farvin *et al*., 2014). There was significant different between the ability of the hydrolysates to chelate metal and that of the standard (EDTA) at $p<0.05$. All the hydrolysates showed metal chelating power higher than 60% at the highest concentration tested. These hydrolysates could serve as preservative antioxidant in the food industry preventing food lipids oxidation caused by metal ions. Presence of acidic and basic amino acids in the hydrolysate peptides may have contributed to the metal chelating ability of the hydrolysates. Saiga *et al*. (2003) reported the crucial roles played by acidic and/or basic amino acids in ability of peptides fraction to chelate metal ion.

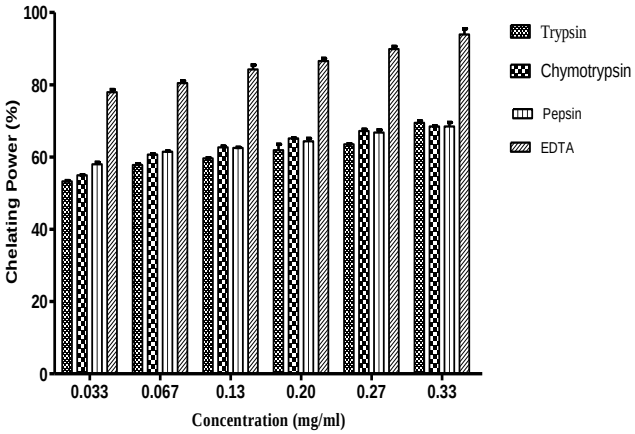

Figure 2: Metal Chelating Activities of Protein Hydrolysates of *Sarotherodon galillaeus* Muscle

It is well documented in the literatures that there is correlation between the reducing power activity of biological active compound and its antioxidant activity. The ability of the hydrolysates to cause the reduction of ferric/ferricyanide complex to ferrous form was monitored by measuring Prussian blue at 700nm. Increase in absorbance indicates better reducing power and also correlate with high antioxidant activity. Figure 4 shows the reducing power activity of protein hydrolysates of *S. galillaeus* muscle compared with ascorbic acid. Hydrolysate obtained using trypsin displayed the highest ferric reducing power activity at a concentration of 1.0 mg/ml with a reductive potential of 0.39 ± 0.01 which was lower than the reducing power activity of ascorbic acid as standard with a reductive potential of 0.69 ± 0.01 at the same concentration. It was observed that the reducing power of the hydrolysates and standard ascorbic acid were concentration dependent though a significant difference ($p<0.05$) was noticed between the hydrolysates reducing power and that of the standard. At all concentrations tested, no significant difference in the reducing power of all the hydrolysates was observed. The present results are similar to the reducing power activity observed in Smoothhound fish muscle and Cod (*Gadus morhua*) protein hydrolysates (Bougatef *et al*., 2009; Farvin *et al*., 2014) within the concentration tested while the hydrolysates produced in the present study have better reducing power than hydrolysate of Alaska Pollack skin (Jia *et al*., 2009).

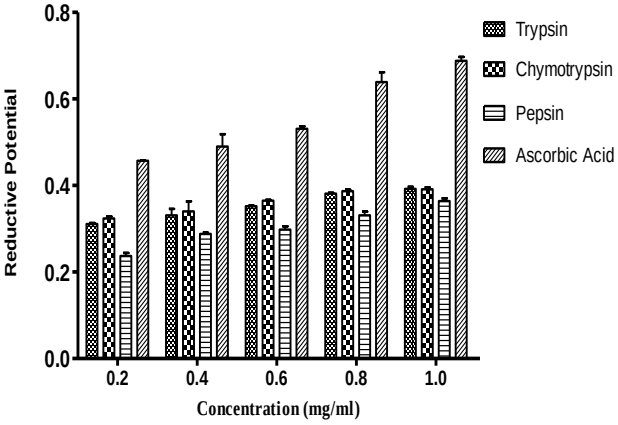

Figure 4: Reductive Potential of Protein Hydrolysates of *Sarotherodon galillaeus* Muscle

Non-reactive hydrogen peroxide (H_2O_2) could become toxic in the presence of reduced transition metal ions. Hydrogen peroxide, when reacts with Fe^{2+}, is converted to hydroxyl radical by the Fenton reaction in the cells and the product can mediate damages that may lead to premature aging, cancer and several diseases (Ajibola *et al*., 2011).Therefore, to defend living cells against various diseases, it is biologically important to remove the hydroxyl radicals from the cells. The ability of protein hydrolysates of *S. galillaeus* muscle to scavenge hydrogen peroxide was assessed and the result presented in Figure 3. Hydrolysate obtained with pepsin displayed the highest scavenging activity of 88.89 ± 1.77 % at the highest concentration of 0.33 mg/ml which was lower than glutathione with a scavenging activity of 91.67 ± 0.43 % at the same concentration. Generally, the peroxide scavenging ability of the hydrolysates was dependent on concentration. There was no significant difference between the standard (glutathione) and hydrolysates scavenging ability except at higher concentration where trypsin-produced hydrolysate scavenging activity was found to be significantly different from pepsin-produced hydrolysate and glutathione at $p<0.05$. The report of Zhuang *et al*., (2010) on Jellyfish gelatine hydrolysates ability to scavenge

hydrogen peroxide is comparable to the result of this study. Three fractions obtained by Zhuang *et al*., (2010) were able to scavenge hydrogen peroxide in a concentration dependent manner and up to 70% of the hydrogen peroxide was scavenged by fraction SCP2.

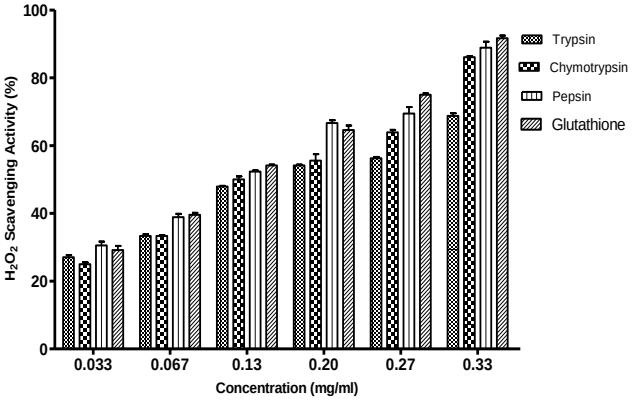

Figure 3: Hydrogen Peroxide Scavenging Activities of Protein Hydrolysates of *Sarotherodon galillaeus* Muscle

Among the mechanism of controlling diabetes mellitus is the inhibition of enzymes that are involved in the digestion of carbohydrates such as α-amylase and α-glucosidase. Inhibition of these enzymes leads to overall reduction in blood glucose (Chonlatid *et al*., 2018). Natural inhibitors of these enzymes especially from food sources have been characterized. Nasri *et al*., (2015) reported decrease in serum glucose level, α-amylase activities and hepatics glycogenesis *in-vivo* when Goby fish protein hydrolysates were administered to diabetic rats. The *in-vitro* inhibition of α-amylase activities carried out in this study revealed that *S. galillaeus* muscle protein hydrolysates possessed inhibitory activity against α-amylase which is concentration dependent(figure 5). Hydrolysate obtained using pepsin displayed the highest inhibitory effect with an inhibition of 18.46 ± 1.51 % which was significantly lower than the inhibitory effect displayed by acarbose which was the control with an inhibition of 69.45 ± 0.86%. Jan *et al*., (2016) reported higher percentage of in-vitro α-amylase inhibition for hydrolysates obtained from sheep milk casein using trypsin, chymotrypsin and pepsin and the inhibition was also concentration dependent.

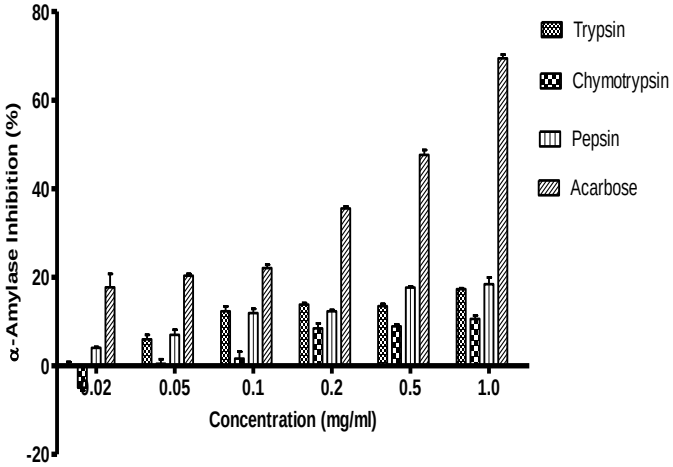

Figure 5: α-Amylase Inhibitory Activity of Protein Hydrolysates of *Sarotherodon galillaeus* Muscle

4. Conclusion

The present study produced hydrolysates that possessed strong antioxidant activity determined by DPPH and hydrogen peroxide scavenging, metal chelating activities and ferric reducing potential. It was also found that *S. galillaeus* muscle protein hydrolysates possessed inhibitory activity against α-amylase which confers antidiabetic potential on the hydrolysates. Further studies on isolation and purification of the specific peptides that is responsible for the biological activities needs to be carried out. Notwithstanding, *S. galillaeus* muscle protein hydrolysates possess potential that can be used to increase food antioxidative stability or that can serve as antidiabetic agent.

Conflicts of Interest

There are no conflicts of interest among the authors.

References

1. Ajibola, C.F., Fashakin, J.B., Fagbemi, T.N., Aluko, R.E. (2011). Effect of peptide size on antioxidant properties of African yam bean seed (*Sphenostylis stenocarpa*) protein hydrolysate fractions. Int. J. Mol. Sci., 12: 6685–6702.
2. Babu, D., Gurumurthy, P., Borra, S. K., and Cherian, K. M. (2013). Antioxidant and free radical scavenging activity of triphala determined by using different in vitro models. Journal of Medicinal Plants Research, 7 (39): 2898-2905.
3. Balti, R., Nedjar-Arroume, N., Bougatef, A., Guillochon, D. and Nasri, M. (2010). Three novel angiotensin I-converting enzyme (ACE) inhibitory peptides from cuttlefish (*Sepia officinalis*) using digestive proteases. Food Research International 43:1136–1143.
4. Benjakul, S. and Morrissey, M.T. (1997). Protein hydrolysates from Pacific whiting solid wastes. J Agric Food Chem. 45(9):3423–3430. doi: 10.1021/jf970294g.
5. Ben-Khaled, H., Ghlissi, Z., Chtourou, Y., Hakim, A., Ktari, N., Fatma, M. A., Barkia, A., Sahnoun Z., and Nasri, M. (2012). Effect of protein hydrolysates from sardinelle (*Sardinella aurita*) on the oxidative status and blood lipid

profile of cholesterol-fed rats. Food Research International 45(1): 60-68. doi.org/10.1016/j.foodres.2011.10.003
6. Bernardini, R. D., Harnedy, P., Bolton, D., Kerry, J., O'Neill, E., Mullen, A. M., and Hayes, M. (2011). Antioxidant and antimicrobial peptidic hydrolysates from muscle protein sources and by-products-Review. Food Chemistry 124: 1296-1307
7. Bougatef A., Hajji M., Balti R., Lassoued I., Triki-Ellouz Y. and Nasri M. (2009).Antioxidant and free radical-scavenging activities of smooth hound (*Mustelus mustelus*) muscle protein hydrolysates obtained by gastrointenstinal proteases. Food Chemistry**,** 114 (4)**:**1198-1205.
8. Cao W., He X., Zhao Z. and Zhaung C. (2013). Analysis of protein composition and antioxidant activity of hydrolysates from *Paphia undulate*. Journal of Food and Nutrition Research 1(3): 30-36.
9. Chalamaiah, M., Kumar, B. D., Hemalatha, R., and Jyothirmayi, T. (2012). Fish Protein Hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. Food Chemistry, 135: 3020-3038.
10. Chonlatid, S., Opeyemi, J. O. and Chitchamai, O. (2018). Evaluation of in-vitro α-amylase and α-glucosidase inhibitory potentials of 14 medicinal plants constituted in Thai folk antidiabetic formularies. Chemistry and Biodiversity 15(4):e1800025. http://doi.org/10.1002/cbdv.201800025
11. Cristina, T. F., Manuel, A. and Vioque, J. (2012). Iron-chelating activity of chickpea protein hydrolysate peptides. Food Chemistry, 134:1585-1588.
12. Daud, N. A., Babji, A. S. and Mohamad Y. S. (2013). "Antioxidant activities of Red Tilapia (*Oreochromis niloticus*) protein hydrolysates as influenced by thermolysin and alcalase". In AIP Conference Proceedings 1571: 687-691. https://doi.org/10.1063/1.4858734
13. Elavarasan, K., Shamasundar, B. A., Badii, F. and Howell, N. (2016). Angiotensin I-converting enzyme (ACE) inhibitory activity and structural properties of oven- and freeze-dried protein hydrolysate from fresh water fish (*Cirrhinus mrigala*). Food Chemistry, 206: 210–216.
14. Erdmann, K., Cheung, B.W.Y. and Schroder, H. (2008). The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. Journal of Nutritional Biochemistry, 19(10):643-654.
15. Fan, J., He, J., Zhuang, Y., and Sun, L. (2012). Purification and identification of antioxidant peptides from enzymatic hydrolysates of tilapia (*Oreochromis niloticus*) frame protein. Molecules. 17(11): 12836-12850.
16. Farvin, K.H.S., Andersen, L.L., Nielsen, H.H., Jacobsen, C., Jakobsen, G., Johansson, I., and Jessen. F. (2014) Antioxidant activity of Cod (*Gadus morhua*) protein hydrolysates: in-vitro assays and evaluation in 5% fish oil-in-water emulsion. Food Chemistry 149: 326-334.
17. Froese I., Rainer K. and Pauly D. (2014). *Sarotherodon galillaeus*. Fish Base**,** 23**:**3-10.
18. Harnedy, P. and FitzGerald, R. J. (2013). Extraction of protein from the macroalga *Palmaria palmate*. LWT- Food Science and Technology 51(1):375–382DOI: 10.1016/j.lwt.2012.09.023
19. Hoyle, N. T. and Merritt, J. H. (1994). Quality of fish protein hydrolysates from herring (*Clupea harengas*). Journal of Food Science, 59: 76-79.
20. Huang, F. J. and Wu, T. (2010). Purification and Characterization of a New Peptide (S-8300) from Shark Liver. Journal of Food Biochemistry. 34: 962–970. DOI: 10.1111/j.1745-4514.2010.00336.x.
21. Jan, F., Kumar, S. and Jha, R. (2016). Effect of bPP-IV inhibition on the antidiabetic property of enzyme treated sheep milk casein. Veterinary World 9(10):1152–1156. http://doi.org/10.14202/vetworld.2016.1152-1156
22. Jerome, J. A. and Odekanyin, O. O. (2019). Comparative studies on the antioxidant potential of hydrolysates of *Mormyrus rume* muscle protein. Ife Journal of Science 21(2): 431-440
23. Jia J., Zhou Y., Lu J., Chen A., Li Y. and Zheng G. (2010). Enzymatic hydrolysis of Alaska pollack (*Theragra chalcogramma*) skin and antioxidant activity of the resulting hydrolysate. Journal of the Science of Food and Agriculture. 90(4)**:**635-640
24. Kehinde, B. A., and Sharma, P. (2018). Recently isolated antidiabetic hydrolysates and peptides from multiple food sources: a review. Critical Reviews in Food Science and Nutrition, 21:1-19
25. Korczek, K., Tkaczewska J. and Migdal, W. (2018). Antioxidant and Antihypertensive Protein Hydrolysates in Fish Products- a Review. Czech Journal of Food Sciences 36(3): 01-25.
26. Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. (1951). Protein measurement with the folin phenol reagent. Journal of Biological Chemistry. 193**:** 265-275.
27. Morais, H. A., Silvestre, M. P. C., Silva, V. D. M., Silva, M. R., Cristina, A., Silva, S. E. and Silveira, J. N. (2013). Correlation between the degree of hydrolysis and the peptide profile of whey protein concentrate hydrolysates: Effect of the enzyme type and reaction time. American Journal of Food Technology, 8(1): 1-16.
28. Nasri, M. (2017). Protein hydrolysates and biopeptides: Production, biological activities and applications in foods and health benefits. A review. Advances in Food and Nutrition Research, 81: 109-159.
29. Nasri, R., Ben Khaled H., Nedjar-Arroume N., Chaâbouni M. K., Dhulster P. and Nasri M. (2012). Antioxidant and Free Radical-Scavenging Activities of Goby (*Zosterisessor ophiocephalus*) Muscle Protein Hydrolysates Obtained by Enzymatic Treatment. Journal of Food Biotechnology 26(3): 266-279.
30. Nasri, R., O. Abdelhedi, I. Jemil, I. Daoued, K. Hamden, C. Kallel, A.Elfeki, M. Lamri-Senhadji, A. Boualga, M. Nasri, and M. Karra-Chaabouni. (2015). Ameliorating effects of goby fish protein hydrolysates on high-fat-high-fructose diet-induced hyperglycemia; oxidative stress and deterioration of kidney function in rats. Chemico-Biological Interactions 242:71–280. https://doi.org/10.1016/j.cbi.2015.08.003
31. Ngo, D.H., Vo, T.S., Ngo, D.N. and Kim, S.K. (2012).Biological activities and potential health benefits of bioactive peptides derived from marine organisms. International Journal of Biological Macromolecules. 51(4)**:**378-383.
32. Persson, T., Popescu, B. O. and Cedazo-Minguez, A. (2014).Oxidative Stress in Alzheimer's Disease: Why Did Antioxidant Therapy Fail?. Oxidative Medicine and Cellular Longevity, vol. 2014, Article ID 427318. https://doi.org/10.1155/2014/427318.
33. Picot, L., Bordenave, S., Didelot, S., Fruitierarnaudin, I., Sannier, F., Thorkelsson, G., Berge, J., Guerard, F., Chabeaud, A. and Picot, J. (2006). Antiproliferative activity of fish protein hydrolysates on human breast cancer cell lines. Process Biochem. 41:1217–1222.
34. Rahimi, R., Nikfar, S., Larijani, B., and Abdollahi, M. (2005). A review on the role of antioxidants in the management of diabetes and its complications. Biomed Pharmacother. 59:365–373.
35. Ramadhan, H. A, Nawas, T. Zhang, X., Pembe, W. M., Xia W. andXu, Y. (2017). Purification and identification of a novel antidiabetic peptide from Chinese giant salamander (*Andrias davidianus*) protein hydrolysate against α-amylase and α-glucosidase. International Journal of Food Properties, 20: S3360-S3372. DOI: 10.1080/10942912.2017.1354885
36. Saiga, A.; Tanabe, S.; Nishimura, T. Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *J. Agric. Food Chem.* 2003, *51*, 3661–3667.

37. Sun L., Zhaung Y., He J. and Fan J. (2012). Purification and identification of antioxidant peptides from enzymatic hydrolysis of tilapia *(Oreochromis niloticus)* frame protein.Molecules.17**:**12836-12850.
38. Toniato, J., Penman, D. J. and Martins, C. (2010). Discrimination of tilapia species of the genera Oreochromis, Tilapia and Sarotherodon by PCR-RFLP of 5S rDNA. Aquaculture Research 41(6): 934–938DOI: 10.1111/j.1365-2109.2009.02366.x
39. Wang, J., K. Du, L. Fang, C. Liu, W. Min, and J. Liu. (2018). Evaluation of the antidiabetic activity of hydrolyzed peptides derived from *Juglans mandshurica* maxim. fruits in insulin-resistant HepG2 cells and type 2 diabetic mice. Journal of Food Biochemistry 42(3):e12518. http://doi.org/10.1111/jfbc.12518
40. Xia, E. Q., Zhu, S. S., He, M. J., Luo, F., Fu, C. Z. and Zou, T. B. (2017). Marine peptides as potential agents for the management of Type-2 Diabetes Mellitus-A Prospect. Marine Drugs 15(4): 88-104.
41. Zambrowicz, A., Eckert, E., Pokora, M., Bobak, Ł., Da˛browska, A., Szołtysik, M., Trziszka, T. and Chrzanowska, J. (2015). Antioxidant and antidiabetic activities of peptides isolated from a hydrolysate of an egg-yolk protein by-product prepared with a proteinase from Asian pumpkin (*Cucurbita ficifolia*). RSC Advances 5(14):10460–10467. http:// doi.org/10.1039/C4RA12943A.
42. Zhuang, Y. L., Sun, L. P., Zhao, X., Hou, H., Li, B. F. (2010) Investigation of Gelatin polypeptides of Jellyfish (*Rhopilema esculentum*) for their antioxidant activity in vitro. Food Technol. Biotechnol. 48(2): 222-228.