https://dx.doi.org/10.4314/ijs.v21i2.16

COMPARATIVE STUDIES ON THE ANTIOXIDANT POTENTIAL OF HYDROLYSATES OF *MORMYRUS RUME* MUSCLE PROTEIN

Jerome, J. A. and *Odekanyin, O. O.
Biochemistry and Molecular Biology Department, Obafemi Awolowo University, Ile-Ife, Nigeria.
E-mail Addresses-JJA: joymagdalene@gmail.com; OOO: dekanyin@oauife.edu.ng
*Correspondence Author's Tel.: +234806-287-9457
(Received: 17th March, 2019; Accepted: 29th June, 2019)

ABSTRACT

This study determined the degree of hydrolysis of *Mormyrus rume* muscle proteins by digestive proteases and the antioxidant potential of the hydrolysates produced was evaluated. The proteins were extracted with distilled water adjusted to neutral pH. The proteases used include trypsin, chymotrypsin and pepsin and the degree of hydrolysis was determined using trichloroacetic acid precipitation method. The antioxidative activities of the hydrolysates were evaluated using different *in-vitro* antioxidant assay methods such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, hydrogen peroxide scavenging, metal chelating capacity and reductive potentials. Degree of hydrolysis was generally low and ranges between 17.68% and 32.20% with pepsin given the highest degree of hydrolysis. The protein hydrolysates obtained with trypsin exhibited the highest antioxidant activities when assessed with metal chelating activity assay with half maximal inhibitory concentration (IC_{50}) of 0.32 ± 0.01 mg/ml; hydrogen peroxide scavenging assay with IC_{50} of 0.18 ± 0.01 mg/ml and reductive potential of 0.41 ± 0.002 units at the highest concentration tested (1.0 mg/ml). Pepsin-produced hydrolysates showed the highest DPPH radical scavenging potential at all concentrations with IC_{50} of 0.35 ± 0.003 mg/ml. The study concluded that the hydrolysates produced contain bioactive peptides which possessed significant amount of antioxidant activities, which may be a promising health promoting agent if isolated.

Keywords: Hydrolysates, Pepsin, Trypsin, Chymotrypsin, Antioxidant, Enzyme, *Mormyrus rume*.

INTRODUCTION

Fish contributes immensely to good health status of consumers in many developing countries. It is the main source of animal protein apart from supplying minerals, vitamins and polyunsaturated fatty acids. Recently, much attention has been directed towards obtaining more biological functions for the fish or fish by-products such as proteins (Korczek *et al*., 2018). This is with a view to reducing the wastage recorded annually in the fish production and also to utilize some undervalued fish species which are good nutritional source. Better utilization of fish and fish by-products is possible by hydrolysing the proteins (Chalamaiah *et al*., 2012). The hydrolysis yields protein hydrolysates which are bioactive peptides concentrate. Bioactive peptides are specific protein fragments that are inactive within the sequence of the parent protein (Sarmadi and Ismail, 2010). They exert various physiological functions after they are released from their parent protein by hydrolysis. It has been reported that bioactive peptides have improved immunomodulatory, antimicrobial, antihypertensive, antioxidative, antithrombotic, anticancer and anticoagulant functions (Najafian and Babji, 2012; Nasri *et al*., 2012; Daud *et al*., 2013; Nasri, 2017; Korczek *et al*., 2018).

Currently, bioactive peptides are being produced mainly by enzymatic hydrolysis though microbial fermentation, autolysis and chemical hydrolysis are also in use. However, enzymatic hydrolysis of fish protein is preferred because it requires mild reaction conditions, produces low undesirable by-product and high-quality products of better functionality and higher nutritional value (Roslan *et al*., 2014; Korczek *et al*., 2018). Peptides derived from fish proteins by enzymatic hydrolysis have shown significant antioxidative activities in different oxidative systems (Lassoued *et al*., 2015). Chalamaiah *et al*. (2012) and Korczek *et al*. (2018) reviewed the production of antioxidative protein hydrolysates of various fish species.

Mormyrus rume Valenciennes, 1847, known as African electric fish lives in freshwaters of tropical Africa. Among the Yorubas, in western Nigeria, it is called "Eja-Osan" or "Lele" because it is used for special ceremonial delicacies due to its special

sweet taste when cooked. They are reported to be bottom dwellers feeding on insect larvae (Fawole, 2002). *Mormyrus rume* are readily available, tasty and relatively cheap (Onimisi and Shittu, 2015). Adedeji *et al.* (2014) also reported that *M. rume* flesh contains the highest amount of crude protein among the fish species studied. In support of this finding, Idowu *et al.* (2015) also recorded that the head region of *M. rume* has higher protein concentration in their study on nutritive value of fishes from Lake Gerio in Yola, Nigeria. Though, *M. rume* is not among species of freshwater fishes listed by Ugoala *et al.* (2009) that are mostly utilized in aquaculture in developing world, Mormyrids are gradually becoming important worldwide in the aquarium business and also in the aquaculture. Therefore, there is need to intensify research in order to explore and exploit the health benefit of the fish species to humans and hence the reason for this study.

MATERIALS AND METHODS

Collection of Sample

Mormyrus rume 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.

Preparation of Crude Fish Muscle Protein

The *Mormyrus rume* 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 x g in a cold centrifuge for 20 min at 4 °C and the homogenates, which is the fish muscle protein concentrate, was freeze-dried.

Enzymatic Hydrolysis of Fish Muscle Protein

The fish muscle protein concentrate was incubated with three different proteolytic enzymes (pepsin, trypsin and chymotrypsin) separately based on the optimum hydrolysis conditions reported by Fan *et al.* (2012). Pepsin was used for the hydrolysis with 0.1 M Glycine-HCl buffer, pH 2.0 and at 37 °C. The enzyme/substrate ratio was 1:100 w/w. The same condition was used for trypsin and chymotrypsin (0.1 M phosphate buffer, pH 7.5 and at 45 °C; enzyme/substrate ratio of 1: 100 w/w). Each mixture was incubated in a waterbath for 4 hours with continuous stirring and then heated in a boiling waterbath for 10 minutes to inactivate the enzyme. The content was rapidly cooled and centrifuged at 10,000 x g for 20 minutes to obtain the supernatant which is the fish protein hydrolysates. The supernatant was freeze-dried and stored in the refrigerator until use.

Determination of the Degree of Hydrolysis

Degree of hydrolysis (DH) of protein hydrolysates was determined according to 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 intervals 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 x g for 20 minutes and supernatants were analysed for soluble protein content by Lowry *et al.* (1951). Degree of hydrolysis (DH) was calculated using the expression below.

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

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.

Antioxidant Assays

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

The hydrogen or radical scavenging properties of protein hydrolysates from the muscle of *M. rume* 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. One hundred microliters (100 µl) of varying concentration of protein hydrolysates was mixed with 100 µl of the DPPH solution in a 96-well 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 glutathione (GSH) as the positive control.

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

Metal Chelating Activity Assay

The metal chelating properties of protein hydrolysates from the muscle of *M. rume* was determined by the method described by Cristina *et al.* (2012) with slight modification. One hundred microlitres 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. The 5 mM ferrozine 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 in a spectrophotometer. The control was prepared in the same way as the sample except that distilled water was used instead of the sample. EDTA was used as positive control.

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

Hydrogen Peroxide (H_2O_2) Scavenging Activity Assay

The hydrogen peroxide scavenging activity of protein hydrolysates from the muscles of *M. rume* 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.

$$\text{Scavenged } H_2O_2\% = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100\%$$

Ferric Reducing Potential Activity Assay

The ferric reducing power activity of protein hydrolysates from the muscle of *M. rume* was determined according to the method described by Babu *et al.* (2013) with slight modification. Exactly 0.2 ml of varying concentrations of protein hydrolysates was mixed with 0.5 ml phosphate buffer, the mixture was vortexed and 0.5 ml of 1% (w/v) solution of potassium ferricyanide was added. The mixture was incubated in a water bath at 50 °C for 20 minutes. Thereafter, 0.5 ml of a 10% (w/v) TCA was added and the mixture was centrifuged at 3000 rpm for 10 minutes and supernatant was collected. The supernatant (100 µl) was mixed with 100 µl of distilled water and 20 µl of a 0.1% (w/v) solution of ferric chloride in a 96-well microplate. Absorbance was measured at a wavelength of 700 nm. Ascorbic acid was used as a positive control.

Statistical Analysis

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

RESULTS

Degree of Hydrolysis

The hydrolysis curves of *M. rume* muscle protein by all the enzymes followed the same pattern (Figure 1). There were high rate of hydrolysis within the first two hours which reduced toward the end of hydrolysis time. The hydrolysis curve produced by trypsin seems to have reached steady state in the last two hours of hydrolysis. The DH obtained after 4 hours of hydrolysis were 32.20%, 24.58% and 17.68% with pepsin, chymotrypsin and trypsin, respectively. Figure 1 showed the hydrolysis curves of *M. rume* muscle proteins by various proteolytic enzymes.

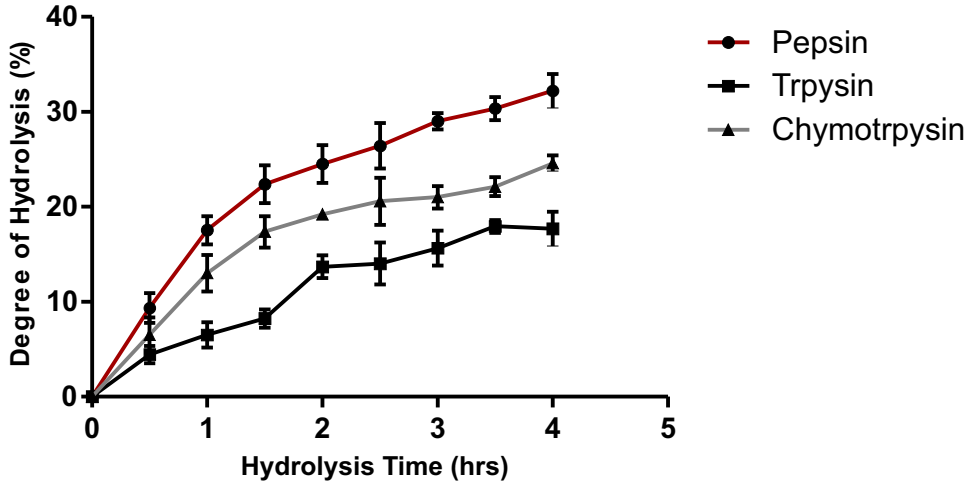

Figure 1: Degree of Hydrolysis Curves of *M. rume* Muscle Proteins

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

The DPPH radical scavenging activities of the protein hydrolysates obtained from muscle of *M. rume* are represented in figure 2. The scavenging abilities of all the hydrolysates were concentration- dependent. However, hydrolysates produced with pepsin displayed the highest DPPH radical scavenging activity with IC_{50} of 0.35 ± 0.003 mg/ml which is lower than the glutathione standard antioxidant used with IC_{50} of 0.03 ± 0.004 mg/ml. Scavenging ability of trypsin- and chymotrypsin-produced hydrolysates were found to be significantly different from that of glutathione while pepsin-produced hydrolysates were not significantly different at $P<0.05$.

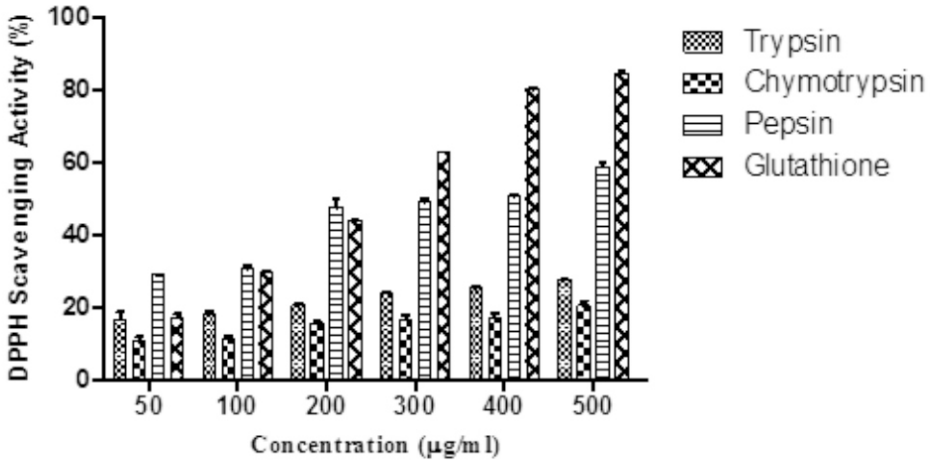

Figure 2: Scavenging Activity of Varying Concentrations of *Mormyrus rume* Muscle Protein Hydrolysates

Metal Chelating Activity

The iron (III) chelating activity of protein hydrolysates of the muscle of *M. rume* increased markedly with increasing concentration. The hydrolysates obtained with trypsin showed the strongest metal chelating ability with IC_{50} of 0.32 ± 0.01 mg/ml followed by chymotrypsin and pepsin's hydrolysates respectively. Metal chelating activity of protein hydrolysates of the muscle of *M. rume* is shown in figure 3. At $P<0.05$, the ability of trypsin-produced hydrolysates to chelate iron were found to be insignificantly different

from the standard chelating agent (EDTA) used while chymotrypsin- and pepsin-produced hydrolysates ability were significantly different.

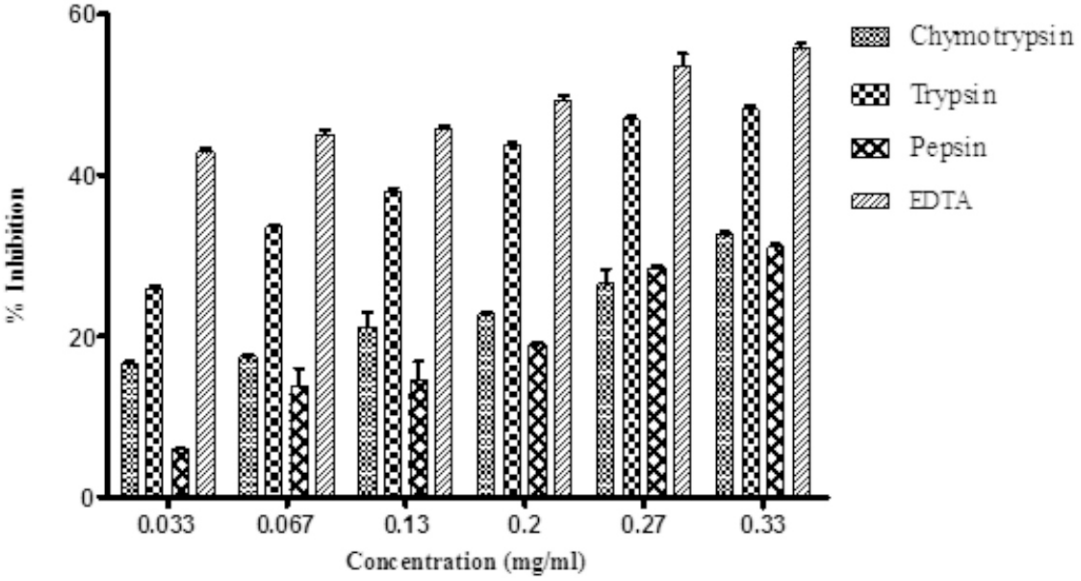

Figure 3: Metal Chelating Activities of Protein Hydrolysates of *Mormyrus rume* Muscle

Hydrogen Peroxide (H_2O_2) Scavenging Activity

The hydrogen peroxide scavenging assay of protein hydrolysates of the muscle of *M. rume* showed a concentration-dependent activity (Figure 4). Hydrolysates obtained using trypsin, displayed the highest hydrogen peroxide scavenging activity, IC_{50} of 0.18 ± 0.01 mg/ml. The abilities of all the hydrolysates to scavenge peroxide were significantly different, from that of the standard used (glutathione).

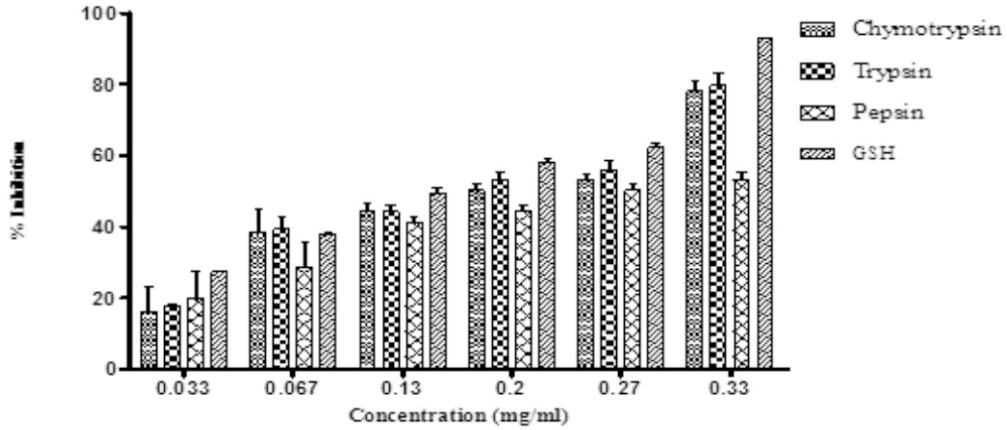

Figure 4: Hydrogen Peroxide Scavenging Activities of *Mormyrus rume* Muscle Protein Hydrolysates.

Reducing Power Activity

Based on the ability to reduce ferric cyanide to ferrous cyanide, the reducing power of protein hydrolysates of the muscle of *M. rume* was assessed and found to have a concentration-dependent activity as shown in figure 5. At the highest concentration of 1.0 mg/ml, hydrolysates obtained using trypsin had the highest reductive potential of 0.41 ± 0.002 units, but lower than ascorbic acid standard with a reductive potential of 0.460 ± 0.007 units at the same concentration.

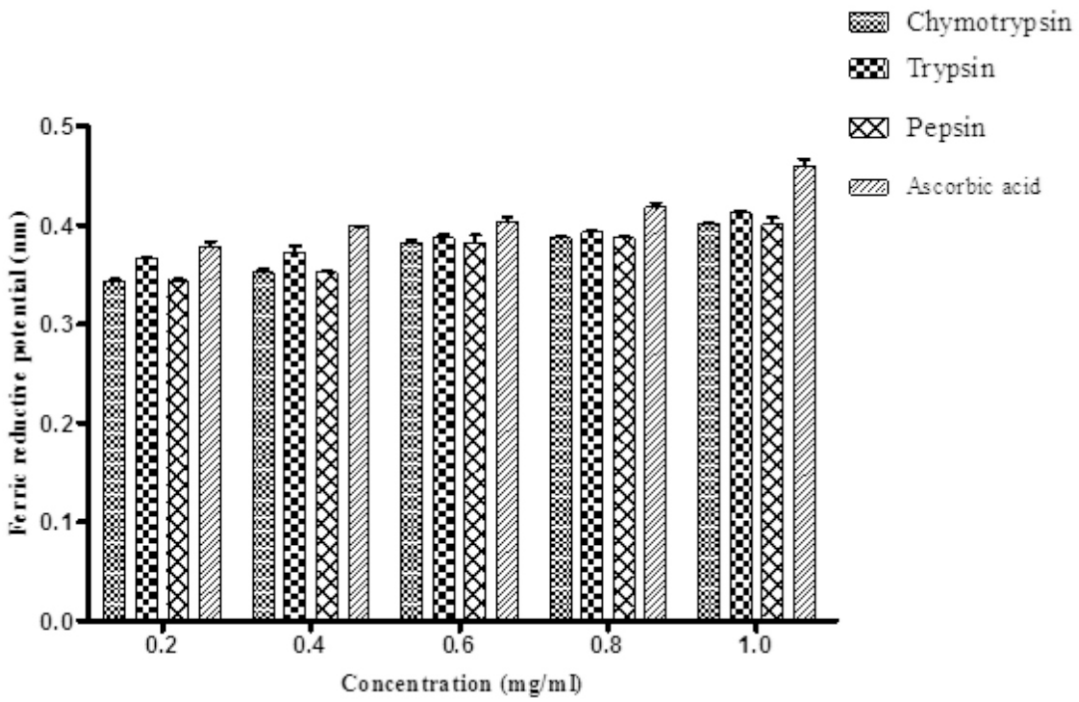

Figure 5: Reductive Potential of *Mormyrus rume* Muscle Protein Hydrolysates

DISCUSSION

A lot of fish species have been identified with potential medicinal values as it offers scope for use as functional food and as source of nutraceuticals. Fish serves as a good source of protein, rich in all the essential amino acids (particularly methionine and lysine) unlike plant proteins (Samanta, 2013). Bioactive activities especially antioxidant activities can be increased through hydrolysis with certain enzymes to yield functional hydrolysates (Lassoued *et al*., 2015). The type of enzyme used and its specificity is very important, as the antioxidant activity of the protein hydrolysates is dependent on it, since enzymes have specific cleavage positions on polypeptide chains, also, conditions of hydrolysis and protein substrates (fish species) are put into consideration (Bougatef *et al*., 2010). The overall antioxidant action of these hydrolysates is most likely attributed to the cooperative effects of several mechanisms including metal ion chelating, free radical scavenging, inhibition of lipid peroxidation, singlet electron transferring (Chen *et al*., 1998) which is mainly due to the diversity of antioxidant action of the bioactive peptides.

In the present study, protein hydrolysates were prepared from *M. rume* muscle by treatment with different proteolytic enzymes which include pepsin, trypsin and chymotrypsin to obtain peptides with different amino acid sequences and composition, since Vignesh *et al*. (2011) reported that antioxidant activities of protein hydrolysates depends on levels and composition of both free amino acids and peptides. Also, Lassoued *et al*. (2015) mentioned that the antioxidant activity is related to the amino acid constituents, sequence and hydrophobicity. Ren *et al*. (2008) mentioned that bioactive peptides, which are inactive within the sequence of parent protein, can be released by enzymatic hydrolysis.

Degree of hydrolysis (DH) shows the level of peptide bond hydrolysis and it depends on the nature of the protein, especially the amino acid composition and sequence. Duration of hydrolysis, optimum pH and temperature of the enzyme as well as the enzyme specificity are also crucial in obtaining good hydrolysis. Degree of hydrolysis of *M. rume* protein was closely related to the DH obtained for smoothhound (*Mustelus mustelus*) muscle protein which ranges between 9.2% and 20.3% using various enzymes (Bougatef *et al*., 2009). Though, the DH reported for goby

muscle protein using crude enzymes from different sources (Nasri *et al*., 2014) was lower compared to the present result, the shape of hydrolysis curves is similar. The degree of hydrolysis reported in the present work compared well with the DH published by Li *et al.* (2017) for scalloped hammerhead (*Sphyrna lewini*) cartilage which ranges from 18.33% to 23.72%. In contrast to the above reports, higher DH (76.29% and 63.49%) was published for red tilapia (*Oreochromis niloticus*) byproduct protein using thermolysin and alcalase for hydrolysis, respectively (Roslan *et al*., 2014).

DPPH free radical is widely used to assess the antioxidant activity of natural compounds. Peptides responsible for the scavenging activity act as electron donors and convert free radicals to more stable products and terminate radical chain reaction (Li *et al*., 2013). In DPPH assay, proton-donating substrate such as antioxidants scavenges the radicals upon encounter and reduces the absorbance as the colour is changed from deep violet colour to faint purple or pale yellow colour. The degree of discolouration indicates the radical scavenging potential of the antioxidant. In the present study, hydrolysates obtained using pepsin showed the strongest DPPH scavenging ability with an IC_{50} of 0.35 ± 0.003 mg/ml but GSH, which was used as a positive control, had a better DPPH scavenging ability than all the fish protein hydrolysates, with a lower IC_{50} value of 0.03 ± 0.004 mg/ml. This result is in contrast with the results of Cao *et al*. (2013) who reported that DPPH scavenging activity of hydrolysates of the three protein fractions from *Paphia undulate* were lower than in the current report. The IC_{50} value of 4.82, 9.19, and 9.31 mg/ml, were reported for the proteins respectively. Jia *et al*. (2010) reported that DPPH scavenging activity of the hydrolysates from Alaska Pollack skin (IC_{50} = 2.5 mg/ml) was lower than that of glutathione (IC_{50} = 0.03 mg/ml) and also pepsin-, trypsin- and chymotrypsin- derived hydrolysates in this study. Peptides, with moderate contents of hydrophobic amino acids such as leucine, alanine, tryptophan, valine and cysteine may have contributed to the DPPH scavenging activity of the fish protein hydrolysates (Cao *et al*., 2013).

Metal chelating activity of peptides are being tested to evaluate their antioxidant potential, it measures how effectively the peptide can compete with ferrozine to form complexes with iron (II), thereby disrupting the formation of ferrozine-Fe^{2+} complex. Colour reduction shows the metal ion chelating activity and as the purple colour decreases, the chelating activity increases. Ferrous ions can catalyse the Haber-Weiss process and induce superoxide anion to form more hazardous hydroxyl radicals; hence the scavenging of hydroxyl radicals by antioxidant is effective mainly via the chelating of ions (Norshazila *et al*., 2010). In the present study, lower IC_{50} of EDTA (0.202 ± 0.01 mg/ml), used as a positive control, showed it is more effective in scavenging hydrogen peroxide radicals than all the hydrolysates. Of all the fish protein hydrolysates, hydrolysates obtained with trypsin had the lowest IC_{50} of 0.32 ± 0.01 mg/ml. Similarly, *Sardinella longiceps* showed metal chelating activity (Jeeviata *et al*., 2014).

The reducing power assay is used to evaluate the ability of an antioxidant to donate electron, it measures the ability of antioxidant to reduce iron (III) to iron (II), which can be detected by the formation of a Perl's Prussian blue. The stronger the absorbance, the better the reducing power and reducing power of peptides is associated with the specific amino acids and peptide sequences (Cao *et al*., 2013). Trypsin-produced hydrolysates exhibited the highest reductive potential with IC_{50} of 0.41 ± 0.002 units at 1 mg/ml among all the *M. rume* muscle protein hydrolysates. This is similar to the reductive potential of ascorbic acid (standard), which had a reductive potential of 0.46 ± 0.007 units but still higher than the reductive potential reported by Cao *et al*. (2013) for three types of proteins at 2 mg/ml (0.25 ± 0.01, 0.27 ± 0.04 and 0.14 ± 0.01 units). The reducing power increased with increasing concentration of the hydrolysates in this study. This is in accordance with Kumar *et al*. (2012) who reported same for horse mackerel skin and croaker skin hydrolysates and also for the three types of proteins mentioned by Cao *et al*. (2013).

Hydrogen peroxide can cross the cell membrane rapidly and it reacts with iron (II) or copper (II) ions to form hydroxyl radicals, which may be the

origin of many toxic effects. It is very important for cells to control the amount of hydrogen peroxide that is allowed to accumulate (Babu *et al*., 2013). Hydrolysates obtained using trypsin, showed a higher IC_{50} of 0.18 ± 0.01 mg/ml than the glutathione standard (IC_{50} of 0.15 ± 0.01 mg/ml). This makes glutathione, a more potent antioxidant than the protein hydrolysates.

CONCLUSION

In the present study, protein hydrolysates of the muscles of *M. rume* have been found to possess potent antioxidant activities at varying concentrations. These antioxidant hydrolysates can serve as ingredients in preparation of health-care products and prevent oxidation reactions in food processing. Further research needs to be carried out to isolate, purify and characterize the peptides responsible for the diverse functional properties of the hydrolysates.

REFERENCES

Adedeji, F. A., Ibrahim, Z. B., Abdulsalami, S. A. and Akinkunmi, A. A. (2014). Comparison of microbiological and proximate analysis of *Synodontis nigrita*, *Chrysichthys nigrodigitatus* and *Mormyrus rume* in Olomore market, Abeokuta, Ogun State, Nigeria. *Journal of Fisheries and Aquatic Science*, 9: 496-500.

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.

Bougatef, A., Hajji, M., Balti, R., Lassoued, I., Triki-Eliouz, Y. and Nasri, M. (2009). Antioxidant and free radical-scavenging activities of smooth hound (*Mustelus mustelus*) muscle protein hydrolysates obtained by gastrointestinal proteases. *Food Chemistry*, 114: 1198-1205.

Bougatef, A., Manni, L., Barkia, A., Nasri, M., Nedjar-Arroume, N., Ravallec, R. and Guillochon, D. (2010). Purificatiion and identification of novel antioxidant peptides from enzymatic hydrolysates of sardinelle (*Sardinelle aurita*) by-products proteins. *Food Chemistry*, 118: 559-565.

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

Chen, H. M., Muramoto, K., Yamauchi, F., Fujimoto, K., and Nokihara, K. (1998). Antioxidative properties of histidine-containing peptides designed from peptides fragmentsfound in the digests of a soybean protein. *Journal of Agricultural and Food Chemistry,* 46: 49–53.

Cristina T.F., Manuel A. and Vioque J. (2012). Iron-chelating activity of chickpea protein hydrolysate peptides. *Food Chemistry*, 134**:**1585-1588.

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.

Daud, N. A., Babji, A. S. and Yusop, S. M. (2013). Antioxidant activities of red tilapia (*Oreochromis niloticus*) protein hydrolysates as influenced by thermolysin and alcalase. *AIP Conference Proceedings* 1571: 687-691.

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.

Fawole O.O. (2002). Morphometry and diet of *Mormyrus rume* in the Lekki Lagoon, Nigeria. *Reviews in Tropical Biology*, 50 (2): 689-694.

Hoyle, N. T. and Merritt, J. H. (1994). Quality of fish protein hydrolysates from herring (*Clupea harengas*). *Journal of Food Science*, 59: 76-79.

Idowu, T. A., Umar A. B., Jidauna S. B., and Sogbesan O. A. (2015). Nutritive values of *Mormyrus rume* and *Protopterus annectens* in Lake Gerio, Yola. *BAOJ Food Sciences and Technology,* 1(1): 004-009.

Jia, J., Zhou, Y., Lu, J., Chen, A., Li, Y. and Zheng, G. (2010). Enzymatic hydrolysis of Allaska Pollack (*Theragrachal cogramma*) skin and antioxidant activity of the resulting hydrolysate. *Journal of the Science of Food and Agriculture,* 90(4) : 635-640.

Jeeviata, K., Priya, M. K. and Khora, S. S. (2014) Antioxidant activity of fish protein hydrolysates from *Sadinella longiceps*. *International Journal of Drug Development and Research,* 4(6):137-145

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.

Kumar, N. S., Nazeer, R. A. and Jaiganesh, R. (2012) Purification and identification of antioxidant peptides from the skin protein hydrolysate of two marine fishes, horse mackerel (*Magalaspis cordyla*) and croaker *(Otoli thesruber). AminoAcids,* 42(5): 1641-1649.

Lassoued, I., Mora, L., Nasri, R., Jridi, M., Toldra, F. and Aristoy, M. (2015) Characterization and comparative assessment of antioxidant and ACE inhibitory activities of thornback ray gelatin hydrolysates. *Journal of Functional Food*s, 13: 225-238.

Li, X. R., Chi, C. F., Li, L. and Wang, B. (2017). Purification and identification of antioxidant peptides from protein Hydrolysate of Scalloped Hammerhead (*Sphyrna lewini*) cartilage. *Marine Drugs,* 15: 61-76.

Li, Z., Wang, B., Chi, C., Gong, Y., Luo, H. and Ding, G. (2013) Influence of average molecular weight on antioxidant and functional properties of cartilage collagen hydrolysates from *Sphyrna lewini, Dasyatis akjei and Raja porosa*. *Food Research International,* 51: 183-193.

Lowry, O. H., Rosebrough, N. J., Farr, A.L. and Randall, R. J. (1951). Protein measurement with the Follin phenol reagent. *Journal of Biological Chemistry* (USA), 193 : 265-275.

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.

Najafian, L. and Babji, A. S. (2012). A review of fish-derived antioxidant and antimicrobial peptides: Their production, assessment, and Applications. *Peptides*, 33: 178-185.

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.

Nasri, R., Ben Amor, I., Bougatef, A., Nedjar-Arroume, N., Dhulster, P., Gargouri, J., Chaabouni, M. K. and Nasri, M. (2012). Anticoagulant activities of goby muscle protein hydrolysates. *Food Chemistry*, 133: 835–841.

Nasri, R., Jridi, M., Lassoued, I., Jemil, I., Ben Slama-Ben Salem, R., Nasri, M., and Chaabouni, M. K. (2014). The influence of the extent of enzymatic hydrolysis on antioxidative properties and ACE-inhibitory activities of protein hydrolysates from goby (*Zosterisessor ophiocephalus*) muscle. *Applied Biochemistry and Biotechnology,* 173: 1121–1134.

Norshazila, S., Zahir, I., Suleiman, K., Aisyah, M. and Rahim, K. (2010). Antioxidant levels and activities of selected seeds of Malaysian tropical fruits. *Malaysian Journal of Nutrition,* 16(1):149-159

Onimisi, M. M. and Shittu, O. A. (2015). Some Aspects of the Biology of African Electric Fish *Mormyrus rume* Valenciennes, 1846 (Osteoglossiformes; Mormyridae) in Lower River Niger at Idah, Kogi State, Central Nigeria. *International Journal of Innovative Research and Development,* 4(8): 45 – 48.

Ren, J., Zhao, M., Shi, J., Wang, J., Jiang, Y., Cui, C. and Kukuda, Y. (2008) Optimization of antioxidant peptide production from grass carp sarcoplasmic protein using response surface methodology. *Food*

Science and Technology, 41: 1624-1632

Roslan, J., Yunos, K. F., Abdullah, N. and Kamal, S. M. M. (2014). Characterization of fish Protein Hydrolysate from Tilapia (*Oreochromis niloticus*) by-product. *Agriculture and Agricultural Science Procedia*, 2: 312-319.

Samanta S. K. (2013). Marine fish-derived bioactive peptides and proteins for human therapeutics – a review. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(3): 0975-1491.

Sarmadi, B. H. and Ismail, A. (2010). Antioxidative peptides from food proteins: A review. *Peptides*, 31: 1949-1956.

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.

Ugoala, C., Ndukwe, G. and Audu, T. (2009). Investigation of the constituent fatty acids of some Freshwater fishes common in Nigeria. *Brazilian Journal of Aquatic Science and Technology*, 13(1) : 65-70.

Vignesh, R., BadhulHaq, M. A., Devanathan, K. and Srinivasan, M. (2011). Pharmacological potential of fish extracts. *Archives of Applied Science Research*, 3(5): 52-58.