Analysis of Bioactive Properties of Fish Protein Hydrolysates from *Scomber japonicus* Fin Wastes

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Abstract
Scomber japonicus is a salience fish species used in canned fish processing. Its wastes are discarded, causing economic losses and environmental pollution. Study aims on producing bioactive Fish Protein Hydrolysates (FPH) utilizing fish wastes as a remedy. First, Scomber japonicus fin waste was collected and blended. Aqueous extracts of fish protein were produced with 04 different ratios as sample: distilled water, 1:1, 1:2, 1:3, 1:4. Best extraction was selected using 10% Sodium Dodecyle Sulphate Poly Acrylamide Gel Electrophoresis. Extracts were hydrolyzed using Papain, Pepsin, Trypsin and Protease enzymes (1:100) at 37ºC under their optimum pH conditions for 0, 3, 6, 9, 12 and 24 hours followed by heat inactivation at 100ºC for 15 minutes. Hydrolysates were lyophilized and analyzed for antioxidant activities by Thiobarbituric acid reactive substances (TBARS) assay and diphenyl picrylhydrazyl (DPPH) scavenging assay, metal chelation activity by Fe (II) chelating activity and antibacterial activities by agar well diffusion method. No significant difference was observed among 04 ratios in yield ($p>0.05$). 1:1 ratio was selected for hydrolysis experiments. None of the FPHs showed antioxidant properties with TBARS assay ($p<0.05$). Radical scavenging activity demonstrated a significant difference among treatments ($p<0.05$). Fe (II) chelating activity revealed Fe releasing instead of chelation (1.84, 13.99, 16.48, 1.84 %,). Antibacterial activities against E.coli and Salmonella spp. were highly positive in all hydrolysates showing best activity against both strains by Trypsin hydrolysate. This concludes the FPHs of water extracted Scomber japonicus fin wastes contain strong antibacterial activity, weak antioxidant activity and iron releasing properties.

Keywords: *antibacteri, antioxidant, fish protein hydrolysates, metal chelating,*

1. Introduction

Pacific chub mackerel (Scomber *japonicas*), is a coastal pelagic growing to a maximum folk length of 50 cm and widely spread in Atlantic, Pacific, and Indian Oceans, where warm tropical water is abided (FAO, 2010 - 2017). They contain high amount of proteins including collagen (Nagai and Suzuki, 2000), and functional lipids (Phleger and Wambeke, 1994). Chub mackerelsare mainly processed by roasting, salting and canning in processing industry (Cho *et al*., 2014).

Offal from fish canning contains high quality fish proteins in different quantities. They can be utilized effectively to produce Fish protein concentrates (FPC) with many amino acids and bioactive peptides, biodiesel, glycerol, *omega*-3 fatty acids, fish meal, fish silage, lactic acid, ethanol and methanol (Ramakrishnan *et al*., 2013). Moreover, these types of wastes are generated in large quantities every year and mostly dumped in to the environment (Sheriff *et al*., 2014), causing environmental pollution. Fish protein hydrolysates (FPH) can be produced by enzymatic hydrolysis of fish offal. There is a belief that FPH can be used as a potential source of bioactive peptides in nutraceutical and pharmaceutical domains (Cudennec *et al*., 2008). They show strong anti-oxidative and ACE-inhibitory activities (Samaranayaka *et al*., 2010) preventing and treating agent of NSAID-induced and other gastrointestinal injurious conditions (Marchbank *et al*., 2008), cardio protective nutrient (Wergedahl *et al*., 2004) and as a digestibility of crude proteins increasing agent in animal feeds (Hevroy *et al*., 2005).

These types of FPH have already been prepared using sevaral types of fish wastes including skin, head, muscles, viscera, liver, frames, bones, roe and eggs (Chalamaiah *et al*., 2012). Fish fin wastes has been identified as a potential source for production of FPH (Benhabiles *et al*., 2012). Fin waste of *Scomber japonicus* can be analyzed for their bioactive properties such as antioxidant properties, antimicrobial properties, metal chelation properties, alone with the future goals of using those findings in medication and functional foods as a better way of facing emerging and existing health hazards (eg: cancers, diabetes) within human population. Therefore the objective of this research is to analyze the functional properties of Fish protein Hydrolysates produced from *Scomber japonicus* fin waste.

2. Methodology

2.1 Production of Fish Protein Concentrates (FPC)

Fish fin wastes of *Scomber japonicas* canned processing were obtained from a commercial fish canning company, Mundalama, Sri Lanka and stored at -20°C. FPC was produced by two extraction solutions; water and phosphate buffer (Ramakrishnan *et al.*, 2013: as the standard protocol). Water extraction was done for four different ratios (fish fin waste: distilled water); 1:1, 1:2, 1:3 and 1:4 (n=3) and incubated overnight at 4ºC in refrigerator. Incubated water extractions were centrifuged (model: sorvall ST 40R, Thermofisher Scientific, Germany) separately at 3000 rpm, 4ºC for 15 minutes and the supernatants were lyophilized for 48 hours using freeze drier (model: 05512, iLShinBioBase Co.Ltd., Korea). The best ratio for water extraction was analyzed, using Sodium Dodecyl Sulphate-Poly

Acrylamide Gel Electrophoresis (SDS-PAGE) (QNX-700, C.B.S. Scientific) according to Sambrook and Russell (2006) and yield analysis. The yield of FPH was analyzed according to the following equation.

$$Yield\% = \frac{Final\ weight\ of\ lyophized\ sample}{Initial\ weight\ of\ FPC\ (or FPH)} \times 100 \quad (1)$$

2.2 Production of Fish Protein Hydrolysates

Fish Protein Hydrolysates (FPH) were produced by enzymatic hydrolysis of pre-prepared Fish Protein Concentrates (FPC). FPC (20 mg/mL solutions) were enzymatically hydrolyzed by four different enzymes; Pepsin (pH 2.5), Papain (pH 6.5), Trypsin (pH 7.8) and Alcalase (pH 6.5) using 1:100 (enzyme: sample) ratio for six different time lapse; 0 hour, 3 hours, 6 hours, 9 hours, 12 hours and 24 hours at 37ºC (n=3). They were then heat inactivated in a dry heat block at 100ºC for 15 minutes.

FPC taken from phosphate buffer extraction was hydrolyzed by Alcalase enzyme (pH 7.5) as described in Ramakrishnan *et al*., 2013. FPH were then lyophilized for 48 hours using freeze drier (model: 05512, iLShinBioBase Co.Ltd., Korea). Best hydrolysates were determined by SDS-PAGE (15 %) and analysis of physical appearance.

2.3Analysis of Functional Properties of the Hydrolysates

2.3.1Anti-Oxidative Properties

TBARS assay was conducted according to the method of (Abeyrathne *et al*., 2014) with some modifications. First, lyophilized Fish Protein Hydrolysate (FPH) was dissolved in distilled water (20 mg/ml). An oil-in-water emulsion was prepared by homogenizing 1 g of coconut oil and 100 µl of Tween-20 with 100 ml of distilled water for 2 minute in an ice bath. The emulsion was incubated at 37ºC. Then 8 ml of oil emulsion, 1 ml distilled water and 1 ml of FPH obtained from Scomber japonicus processing wastes was mixed and incubated at 37ºC for 16 hours.

After that 1ml of sample was transferred to a 50 ml Falcon tube, 2 ml of TBA/Trichloroacetic acid solution and 50 µL of 10% Butylated Hydroxyanisole in 90% Ethanol were added and vortex mixed. Next the prepared mixture was incubated at 90ºC in a water bath for 15 minutes for the purpose of color development. Then it was cooled for 10 minutes by using an ice bath and was centrifuged at 3000 x g for 15 minutes (temperature 50ºC). A blank was prepared by mixing 1 mL of distilled water, 2 mL of TBA/TCA solution and 50 µL of 10% Butylated Hydroxyanisole in 90% Ethanol. The absorbance of the solution was measured at 532 nm against the blank. Values of TBAR were expressed as milligrams of

malondialdehyde per liter of emulsion. DPPH scavenging assay was conducted according to the method of (Jiang *et al*., 2013) with some modifications. In here, 0.5 mL of FPH was added to 0.5 mL of DPPH (1,1-diphenyl-2picrylhydrazyl) solution which was prepared by dissolving 0.1 mM in methanol. The mixture was shaken thoroughly and incubated at 300ºC for 30 min in darkness. Absorbance was determined at 515 nm using UV-Spectrophotometer.

The scavenging effect was expressed as,

$$Scavenging\ activity\ of\ DPPH\% = [1 - (As - A1)A0 \times 100 \quad (2)$$

Where, As - the absorbance of the sample, $A0$- the absorbance of the control of the DPPH-methanol solution, $A1$- the absorbance of the sample added to methanol. Free radical-scavenging activity was quantified by a regression analysis of scavenging activity (%) versus peptide concentration and defined as an EC50 value.

2.3.2 Metal Chelation Property

Metal chelation properties were analyzed using Fe (II) chelating activity. It was determined using the ferrozine method according to Abeyrathne *et al*., (2014). 100 µL of the FPH, 0.9 mL of distilled water, and 1 mL of 10 ppm Fe^{2+} ($FeSO_4$) were mixed properly by using the vortex machine in a 15 mL Falcon tube. It was then incubated for 5 min at room temperature. After that 900 µL of 11.3% TCA was added to it and centrifuged at 2500 × g for 10 min to remove proteins and peptides present in the sample. Next 1 ml of the supernatant was transferred to a disposable culture tube. Then 1 mL of distilled water, 800 µL of 10% ammonium acetate (Fisher Scientific), and 200 µL of ferroin color indicator was added and mixed using the vortex machine. After that it was again incubated at room temperature for 5 minutes. Then absorbance was measured at 562 nm. The Fe^{2+} chelating activity was calculated using the following equation:

$$Fe\ chelating\ (\%) = [1-(Sample\ absorbance\ Blank\ absorbance)]\times 100 \quad (3)$$

2.3.3 Antibacterial Properties

Antibacterial properties were analyzed according to agar well diffusion method (Bendjeddou *et al*., 2016). Locally isolated food borne *Salmonella* and *Escherichia coli* bacteria cultures were used to analyze antimicrobial properties. First, locally isolated Salmonella and E.coli bacteria cultures were inoculated in XLD and EMB agar plates respectively and they were incubated at 37ºC for 48 hours. Meanwhile the FPH samples were prepared to analyze their antibacterial properties. A concentration series was prepared using each of the FPH as; 20,000 ppm, 10,000 ppm, 5,000 ppm,

2,500 ppm, 1,250 ppm and 625 ppm. Initially, 15-20 mL of agar was poured on petri plates and it was allowed to solidify. Then the bacterial strains were inoculated in on agar surfaces by streaking them on agar surface, using a sterile cotton bud. After that agar was punched with sterile cork bored of 4mm size followed by adding 100 µL of each sample with micropipette in to the bore.

Then the plates were kept for 30minutes and plates were incubated at 37ºC for 48 hours. Finally, the plates were observed for bacterial inhibition zones to analyze the antimicrobial activity. An antibiotic, named as Augmentin was used as the positive control and autoclaved distilled water was used as negative control for this test. The concentration of the positive control was equivalent to the smallest concentration of the series.

2.3.4 Statistical Analysis

Statistical analysis was conducted using Microsoft office excel and Minitab 17 statistical software packages.

3. Results and Discussion

3.1 Water Extraction

Although, there was no any significant difference among treatments, the yield percentages were increased with increasing sample: distilled water ratio (Figure 01). Therefore, 1:1 ratio was selected as suitable ratio for further experiments. According to the SDS-PAGE, all the four ratios of sample: water had demonstrated a poor number of protein bands and all the protein bands were same in pattern. Although the widths of protein bands have been slightly increased with the increasing ratio. Generally, all the fish processing factories use large amount of water for several purposes including cleaning, washing, draining. Specially, water used for washing and draining, highly contact with fish processing wastes for longer period of time. Therefore, most of the water-soluble fish proteins in fish processing wastes, are scrammed with water drainage. It is reasonable to elaborate the above result of poor number of protein bands were due to scramming of water-soluble proteins of fish processing wastes with draining water in the processing factory itself. All the band patterns were same because, water in all four different ratios had been extracted same types of protein remaining in the processing wastes. Since higher amount of H_2O molecules were conceded to be contacted with blended fish wastes samples in higher water amounts, with increasing sample: water ratio, widths of protein bands and smears have been increased slightly. Accordingly, it is fair to manifest that there is no any considerable importance of using higher sample: water ratio for protein extraction using Scomber japonicus canned fish processing wastes. Therefore, the decision taken in the yield analysis was confirmed and 1:1 (sample: water) was selected as the best ratio for protein extraction.

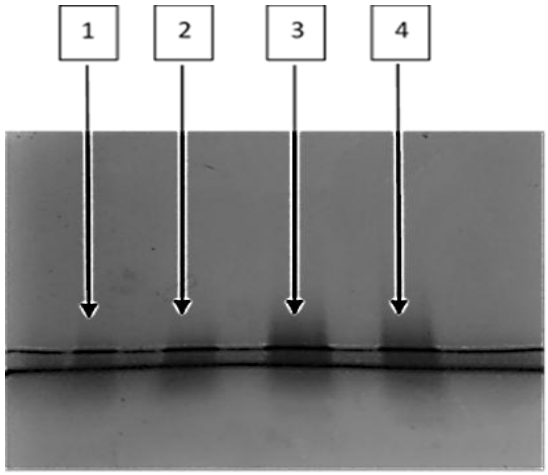

Figure 1: SDS-PAGE profile of water extraction in different ratios of fish fin waste and distilled water.
Note: Fish fin waste: distilled water as in ratio of, Lane1- 1:1, Lane 2- 1:2, Lane 3- 1:3 and Lane 4- 1:4 (n=3) incubated for overnight at 4°C in refrigerator

3.2 Production of Fish Protein Hydrolysates (FPH)

FPH were produced using enzymatic hydrolysis as previously described and analyzed by physical appearance and 15% SDS PAGE. All the FPC samples were opaque, prior to hydrolysis. After hydrolysis all most all the FPHs produced, in each enzyme, for each time lag showed at least a slight coagulation after heat denaturing process, while most has converted to clear and gold-like color solution. There was no much difference between samples hydrolyzed for 6 different time lags in all four different enzymes. Coagulation of heat denatured proteins occurred due to thermal denaturation of proteins (Boye *et al*., 1997). Since coagulation occurred in almost all the FPH samples, it did not play a critical role when the best time lag for hydrolysis was decided.

The shred which is compatible for FPC, has demonstrated a protein band in all four SDS-PAGE images. Because it was not subjected to any hydrolysis process and due to that it remains as the protein itself. In these SDS-PAGE images, some shreds compatible with different time lags of FPH also contain protein bands; Papain up to 12hours, Pepsin 0 hours, Trypsin 0 hours. Absence of protein bands and presence of smears, revealed that all the proteins have been hydrolyzed properly and they all became FPH. The best hydrolyzing time lags were determined as; Papain 24 hours, Pepsin 3 hours, Trypsin 3 hours and Protease 0 hours. The reason for these time differences to complete hydrolysis of extracted proteins is, the difference of used enzymes, revealing enzymes are substrate specific (Hu *et al*., 2013).

3.3 Analysis of Functional Properties

3.3.1 Antioxidant properties

3.3.1.1 TBARS assay

TBARS assay is widely applied in scientific researches to quantify the level of oxidation by means of measuring malonaldehyde (MDA), which is formed as the end product of lipid peroxidation caused by free radical induced reactions (Oakes and Van, 2003). As shown in Fig. 2, there was a significant difference ($P<0.05$) among treatments in TBARS assay, where Papain and Pepsin treated hydrolysates behaved alike, while all the other four different treatments were behaved heterogeneously. With this result, it was manifested that the produced FPHs contain oxidative properties, instead of antioxidant properties antagonistic to the expectation. The highest oxidative ability was recorded from the FPH treated by pepsin enzyme.

Since these FPHs accelerate the malonaldehyde formation these FPHs should not be incorporated in to heavy lipid containing foods. Because it induce the lipid oxidation, causing rancid aroma due to formation of secondary products including malonaldehyde, aldehydes and ketones.

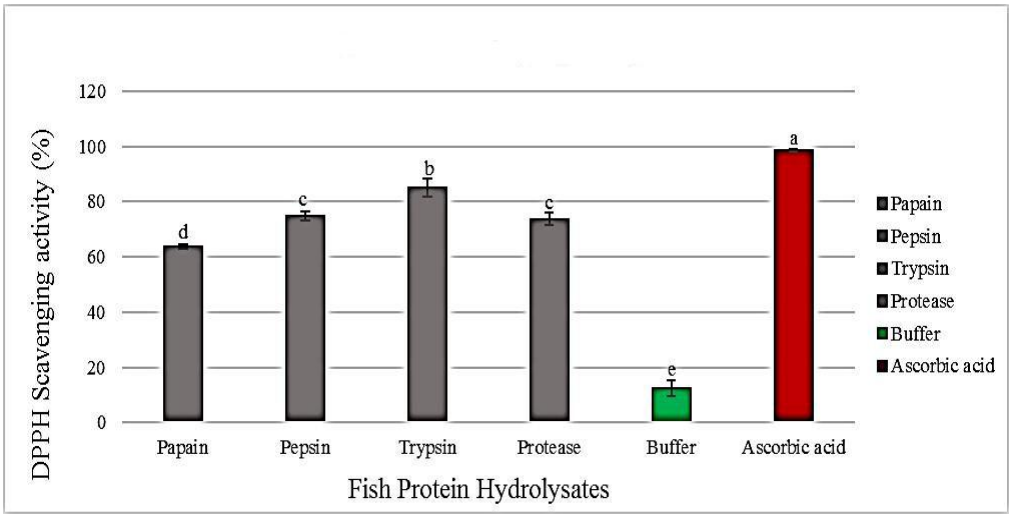

Figure 2: TBARS assay in enzymatically hydrolyzed FPC by four different enzymes; Pepsin (pH 2.5), Papain (pH 6.5), Trypsin (pH 7.8) and Alcalase (pH 6.5) using 1:100 (enzyme: sample) ratio for six different time lapse; 0 hours, 3 hours, 6 hours, 9 hours, 12 hours and 24 hours at 37ºC with control

3.3.1.1 DPPH scavenging assay

DPPH scavenging assay is a standard chemical assay that used for analysis of antioxidant activities of biological compounds. The 1, 1 - Diphenyl 2 - Picryl Hydrazyl (DPPH) is a stable free radical which contains a free electron in one of the atoms in its nitrogen bridge (Eklund *et al*., 2005). All the FPH treated by four different enzymes and FPH produced according to the standard protocol and Ascorbic acid (as the referenced antioxidant) exhibited high radical scavenging activities in DPPH scavenging assay and there was a significant difference between treatments ($p<0.05$). Further, Pepsin and Protease hydrolysates showed similar behavior, while all the other treatments behaved heterogeneously. However, none of the FPH were able to demonstrate a higher scavenging activity than Ascorbic acid. FPH obtained from phosphate buffer extraction, displayed a considerable level of scavenging activity against DPPH free radical (Fig 3).

DPPH assay results were antagonistic to the results of inequitable to decide that these FPHs contain an antioxidant activity or not. Hence, it is fair to manifest that, even though they bear a kind of antioxidant activity, it is not a strong activity. TBARS assay. This contrariness can be explicated by analyzing the principles of these two kinds of assays. Even though, these activity. TBARS assay. This contrariness can be explicated by analyzing the principles of these two kinds of assays. Even though, these FPH involved in accelerating the formation of malonaldehyde, they scavenge the DPPH free radical. It is fair to manifest that, even though they bear a kind of antioxidant activity, it is a weak antioxidant property. The values obtained for scavenging activity of FPHs produced by pepsin (74.7%) and papain (63.6%), using Scomber japonicus fins were considerably higher than that of FPHs produced by Pepsin (46%) and papain (36%), using backbones of *Rastrelliger kanagurta* (Indian mackerel) (Sheriff *et al*., 2014).

Besides, the results of DPPH scavenging assay in both studies confirm that the FPH hydrolyzed by Pepsin contains a higher antioxidant activity than FPH produced using papain. Hence, this conclusion is antagonistic to the conclusion obtained from TBARS assay. This contrariness can be explicated by analyzing the principles of these two kinds of assays. Even though, these FPHs involved in accelerating the formation of malonaldehyde, they scavenge the DPPH free radical. It is a possible phenomenon. However, it is inequitable to decide that these FPHs contain an antioxidant activity or not.

Hence, it is fair to manifest that, even though they bear a kind of antioxidant activity, it is not a strong activity. Though, many marine fish derived FPHs have demonstrated the

strong antioxidant properties. Examples include *Scomber austriasicus* and *Thunnus obesus* derived FPHs. Those two fish species belongs to the family Scombridae in which *Scomber japonicus* also exists. FPHs produced from *Scomber austriasicus* under hydrolysis by protease N enzyme (Wu *et al*., 2003) and FPHs produced from *Thunnus obesus* under alcalase, α-chymotrypsin, neutrase, papain, pepsin, and trypsin enzymes have disclosed strong antioxidant properties (Je *et al*., 2008). However the result obtained from this study is compatible with the findings of Lin *et al*., (2014), regarding the antioxidant properties of five of marine fishes, including Pacific mackerel, Spanish mackerel, hairtail, Japanese anchovy and horse mackerel. Above mentioned research study has revealed that all the FPHs produced using all five fish types demonstrated low antioxidant activities.

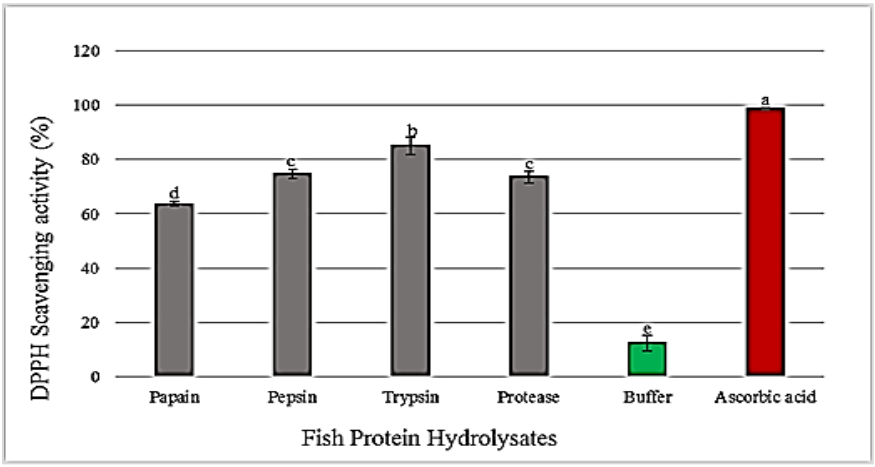

Figure 3: DPPH scavenging assay in enzymatically hydrolyzed FPC by four different enzymes; Pepsin (pH 2.5), Papain (pH 6.5), Trypsin (pH 7.8) and Alcalase (pH 6.5) using 1:100 (enzyme: sample) ratio for six different time lapse; 0 hours, 3 hours, 6 hours, 9 hours, 12 hours and 24 hours at 37ºC with controls.

3.3.2Metal chelation properties

3.3.2.1 Fe (II) chelating activity

All most all the FPH derived from water extracted FPC, had procured Fe releasing activity, over chelation as exhibited in figure 4. Besides, the FPH obtained from Phosphate Buffer Extraction demonstrated a slight Fe (II) chelating activity. Moreover, there was a grouped with Protease while FPH hydrolyzed significant difference ($p<0.05$) among treatments. FPH hydrolyzed by Papain Pepsin and Trypsin belongs to another group according to their Fe (II) releasing behavior. The highest Fe (II) releasing activity was recorded from FPH hydrolyzed by Trypsin.

Ferrozine can quantitatively form complexes with Fe^{2+} ion. In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease of colour formation. The results indicated that protein hydrolysates had a pronounced capacity for iron binding. Transition metals, such as Fe, Cu and Co, in foods affect both the speed of autoxidation and the direction of hydroperoxide breakdown to volatile compounds (Nawar, 1996). It has known that the chelation of transition metal ions by anti-oxidative peptides involve in retarding the oxidation reaction (Sherwin, 1990) and the size and sequence of amino acids in the resulting peptides most likely determine the antioxidant activity of protein hydrolysates (Chen *et al*., 1998). Proving the above mentioned statements, the results of TBARS assay and Fe (II) chelating activity are alike in the present study. Because, transitional metal ions, such as Fe (II) can catalyze the generation of reactive oxygen species which oxidize unsaturated lipids (Stohs and Bagghi, 2005).

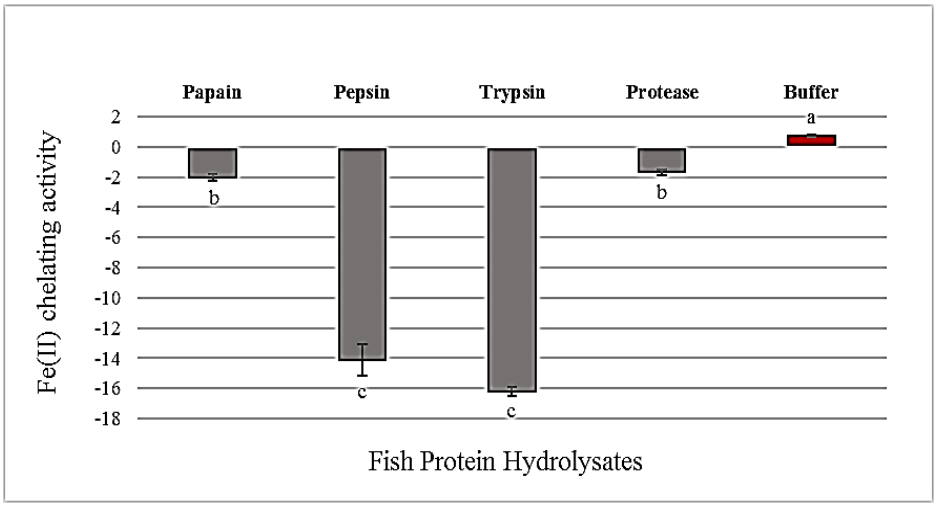

Figure 4: Fe (II) chelating activity assay in enzymatically hydrolyzed FPC by four different enzymes; Pepsin (pH 2.5), Papain (pH 6.5), Trypsin (pH 7.8) and Alcalase (pH 6.5) using 1:100 (enzyme: sample) ratio for six different time lapse; 0 hours, 3 hours, 6 hours, 9 hours, 12 hours and 24 hours at 37 ºC with controls.

3.3.3 Antibacterial properties

3.3.3.1 Antibacterial activity against *E.coli* spp.

According to the results obtained, it revealed that all the FPHs, except FPH derived from phosphate buffer extraction had exhibited inhibition zone seven at the smallest concentration used. There was a significant difference ($p<0.05$) between four treatments (enzymes) in

all the concentrations in the concentration series. According to Tukey pairwise comparison, it revealed that the Trypsin treated protein hydrolysate had been demonstrated best antibacterial activity in both highest and least concentrations. The highest inhibition was observed in the highest concentration of FPH produced using Trypsin enzyme (Fig. 5).

Antibacterial properties of peptides derived from FPHs can be identified due to their interaction with membranes of bacterial pathogens with the aid of cationic moietiesthat peptides contain (Bardan *et al*., 2004). Further, these results were compatible with the results of two previous studies which were conducted on antibacterial activity of FPHs produced using *Scomber scombrus* (Atlantic mackerel). Those studies revealed that the FPHs produced using commercial enzymes including protamex, neutrase, papain, and flavourzyme demonstrated antibacterial properties against *Listeria innocua* and *E.coli* (Ennas *et al*., 2015a; Ennas *et al*., 2015b). Moreover, the FPHs obtained from half-fin anchovies subsequently the hydrolysis by papain, pepsin, trypsin, alkaline protease, acidic protease, and flavoring protease, had demonstrated an antibacterial activity against *E.coli* (Song *et al*., 2012).

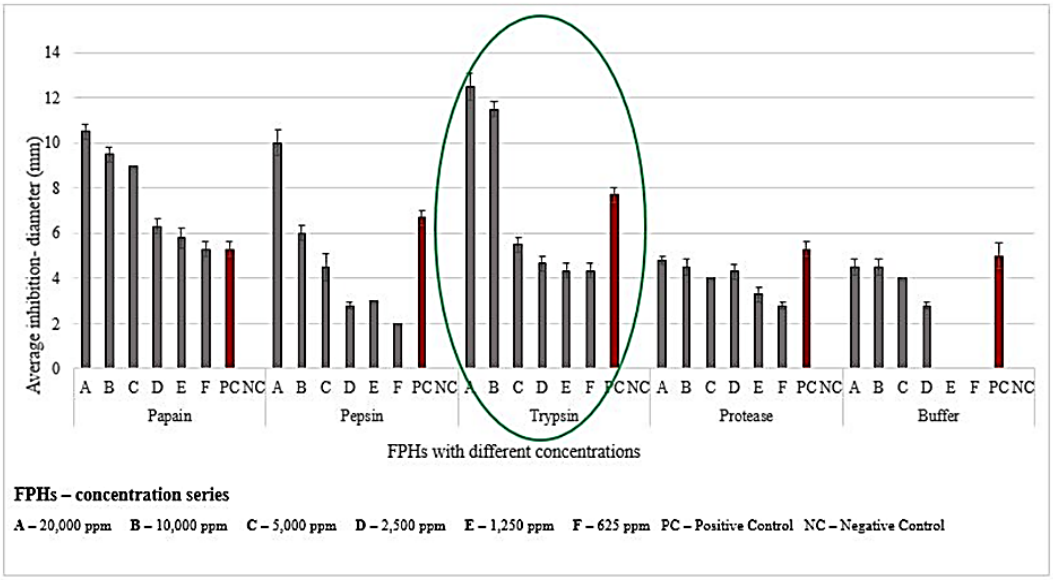

Figure 5:Antibacterial activity in enzymatically hydrolyzed FPC by four different enzymes; Pepsin (pH 2.5), Papain (pH 6.5), Trypsin (pH 7.8) and Alcalase (pH 6.5) using 1:100 (enzyme: sample) ratio for six different time lapse; 0 hours, 3 hours, 6 hours, 9 hours, 12 hours and 24 hours at 37ºC with controls against E.coli spp. for 24 hours.

3.3.3.2 Antibacterial activity against *Salmonella* spp.

Food derived and locally isolated *Salmonella* spp. was used for the assay and the assay was conducted following same procedure practiced in *E.coli* inhibition assay. Though, the inhibition zones were measured at both 24 hours and 48 hours, due to complete inhibition of *Salmonella* in some FPHs at 48 hours (Eg: Trypsin, Protease), only the results obtained at 24 hours have been presented. According to the results acquired, it is manifested that there was a significant difference ($p<0.05$) in between four treatment for all the concentrations of the FPH concentration series. The best activity had been demonstrated by Trypsin treated hydrolysate in all the concentrations of the FPH concentration series. The highest inhibition had demonstrated by the highest concentration of FPH produced by Trypsin, which is compatible with the results of *E.coli* inhibition assay. Even the lowest concentrations of Papain and Trypsin had exhibited a higher inhibition than the positive control; Augmentin at 24 hours. Though, there was no any significant difference ($p>0.05$) in between four treatments for the Positive Control (PC), Augmentin. After considering results obtained from both assays, it can be concluded that the highest concentration of FPH produced by Trypsin contained best antibacterial properties against *Salmonella* spp. It can be concluded that all most all the FPHs had exhibited a considerably strong antibacterial properties against both *E.coli* and *Salmonella* spp.

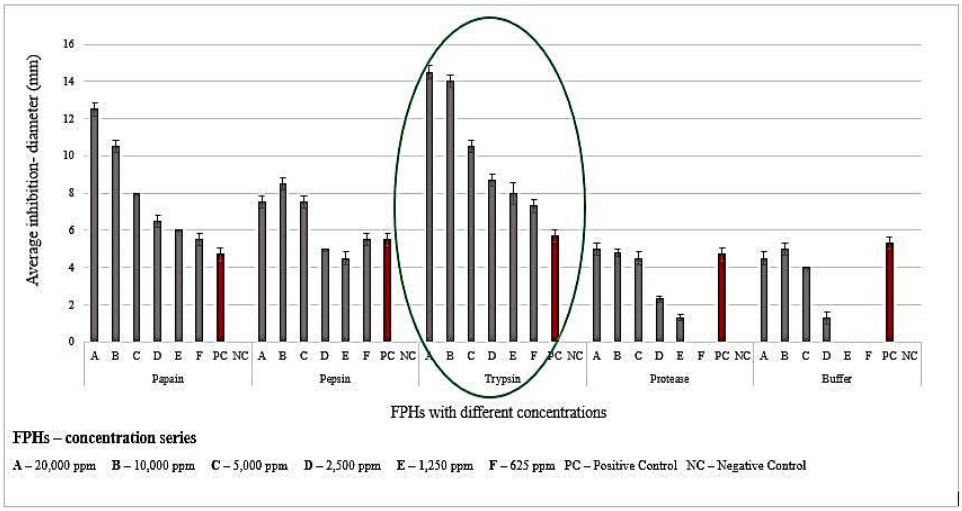

Figure 6: Antibacterial activity in enzymatically hydrolyzed FPC by four different enzymes; Pepsin (pH 2.5), Papain (pH 6.5), Trypsin (pH 7.8) and Alcalase (pH 6.5) using 1:100 (enzyme: sample) ratio for six different time lapse; 0 hours, 3 hours, 6 hours, 9 hours, 12 hours and 24 hours at 37ºC with controls against Salmonella spp. for 24 hours.

4. Conclusions

All the FPH produced by enzymatic hydrolysis (Papain, Pepsin, trypsin and protease) of *Scomber japonicus* fin waste, contained strong antibacterial properties and weak antioxidant properties as favorable bioactivities. All of the FPH produced demonstrated iorn releasing activity. FPH produced by enzymatic hydrolysis, using Trypsin enzyme, demonstrated best antibacterial and antioxidant activities among all four types of FPH.

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