Research Article

Optimization of Conditions for Extraction of Crude Protein from *Hilsa kelee* and Determination of Functional Properties of Crude Extract

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Abstract

Karattaya (Hilsa kelee) is a marine pelagic species, mainly found in Indo-Pacific to the coast of East Africa. It is a seasonal fish species with a high catch during the season. But the demand is low due to its bony structure thus it is mainly used to produce fish meal. The objective of this study was to develop a simple and nontoxic method for extracting crude protein from H. kelee and determine the functional properties of the crude extract. Collected fish samples was blend and treat with 04 different ratios of sample: distilled water as 1:1, 1:2, 1:3 and 1:4. Salt extraction of samples were obtained by mixing the precipitates of water extraction with 5%, 10%, 15% and 20% (w/v) concentrated NaCl solutions at 1:10 ratio. Crude extraction was lyophilized and selected the best ratio and concentrate by using the yield and SDS-PAGE analysis. Antimicrobial, antioxidant and metal chelating properties of selected crude extract samples were determined. In yield analysis of water extraction, 1:4 ratio showed a higher yield in compared to others ($p<0.05$). In salt extraction, there was no any significant difference between yields ($p>0.05$), therefore 5% (w/v) ratio was selected as the best. Salt extracted Fish Protein Concentrates (FPC) had higher antioxidant activities ($p<0.05$). FPC had negative response for the Fe^{2+} chelating activity and antimicrobial activity against E. coli and Total Plate Count (TPC). It concludes that extracted FPC contains strong antioxidant properties while they do not show Fe^{2+} chelating and antimicrobial properties.

Keywords: *Hilsa kelee, Fish Protein Concentrate, Protein extraction, Antioxidant property*

1. Introduction

At present, the demand for fish and fish products have increased. Out of 171 million tons of global fish harvest, roughly 88% or more than 151 million tons were used for direct human consumption in 2016 (FAO, 2018). In commercial fisheries, between 17.9 to 39.5 million tons (averaging 27 million tons) of fish were discarded annually

due to low market value (Davies *et al*., 2009). Fish products are an important source of animal protein for the population and they contribute to about 2% of GDP in Sri Lanka. About 67,300 tons of fish products (mostly dried and canned), worth US$ 59.4 million were exported from Sri Lanka to other countries (FAO, 2019).

Water, protein and lipids are the major components of fish muscle. Fish muscles contain about 78-80% of water, 18-22% of protein and 0.2-23.75% lipid (Venugopal and Shahidi, 1996). Proteins are classified into three categories based on skeletal muscle position and composition, which include sarcoplasmic, stroma, and myofibrillar proteins (Malva *et al*., 2018). Sarcoplasmic protein consists of 20-25% of the total fish protein and contains low molecular weight compounds such as albumin, myoglobin, hemoglobin and enzymes (Venugopal and Shahidi, 1996). Stroma protein consists of about 1.3% of the total protein and contains collagen and elastin (Belitz *et al*., 2009). Myofibrillar proteins are the main component of the skeletal muscle, which accounts about 50% of the total proteins, and are primarily made up of myosin and actin, which are involved in muscle contraction (Malva *et al*., 2018).

Fish and seafood products have high amount of valuable proteins (Sargent, 1997). In addition, these proteins are rich in several peptides such as carnosine (ß-alanylhistidine) and anserine (ß-alanyl-1-methylhistidine) (Wu *et al*., 2003) and also essential amino acids such as methionine and lysine which are easily digestible (Sarvenaz and Sabine, 2017; Tacon and Metian, 2013). Several studies (Dyerberg, 1985; Calder, 2004; Rudkowska *et al*., 2010; Lund, 2013) have shown strong link between fish and seafood consumption and positive health effects in particular with reduced risk of coronary heart disease and cardiovascular disease, reduced inflammatory diseases, arthritis, and cancer prevention (Sarvenaz and Sabine, 2017).

Hilsa kelee, is a marine, pelagic species. But when entering in to the estuarine it gains ability to tolerate quite low salinities (Eter, 1983). They are clupeid which occurs mainly in tropical coastal water and is locally called as “karattaya” (Rathnasuriya, *et al*., 2018). It is a seasonal fish species and has a high catch during April to September and low catch during October to December. Geographical distribution of Hilsa kelee ranges from Indo-Pacific to the coasts of East Africa (Panhwar *et al*., 2012). Estimated global catch of Hilsa kelee in 2006 was about 200 tons. Its standard length is 24.4 cm but it usually grows up to 15 cm to 18 cm (FAO, 2019). Demand for Hilsa kelee is low due to its bony structure and sharp tough bones which make it hard to eat (Revolvy, 2019). So, it has very low market price. Due to its low market demand, wastage is high during the season which also cause for the environmental pollution. Because of these reasons *Hilsa kelee* is used to produce fish meal. Further, there are only few researches carried out on this fish species. This fish species can be effectively use to produce fish protein concentrate (FPC) and generates high profit for the industry. Furthermore, by making fish protein concentrate, it can reduce the

environmental pollution which happens due to the wastage generate by excessive amount of fishes catch during the season.

FPC already extracted using several solvent extraction methods by various fish species (Akhade *et al*., 2016). Whole fish of fish waste is usually used to produce FPC (Ramakrishnan *et al*., 2013). FPC extraction processes are used to turn underutilized fish into more acceptable and valuable forms. FPC can be used as protein supplement to increase nutritive value of food (Cordova-Murueta *et al*., 2007). Research has shown that adding FPC to human diets has positive effect for kids and pregnant woman (FAO, 2016). Therefore, the objective of this research is to develop a simple nontoxic method for extract crude protein from H. kelee fish and to determine the functional properties of the crude extract.

2. Materials and Methods

2.1 Sample Collection and Sample Preparation

The experimental animal of the study was *Hilsa kelee* adults with average body length ranging from 15 cm to 18 cm. Fish were purchased from the coastal of manner, Sri Lanka. Whole fish were transported to Uva Wellassa University General Laboratory under freezing condition at -4℃, in a Styrofoam box with ice. Samples were packed to minimize the physical damages while transporting. All fish samples were washed properly and stored at -20℃ in a refrigerator until it used for the experiment. Proximate analysis of the fish was carried out according to the AOAC (2016) manual focusing on the Crude Protein, Crude fat and Ash content.

2.2 Extraction of Water-Soluble Protein from *Hilsa kelee* Fish

The collected fish were cut into small pieces and blended by using a blender (Model: HL1643/04, India). Then blended samples were taken and mixed with water in four different ratios (1:1, 1:2, 1:3 and 1:4) in triplicates. Then, samples were stored overnight at 4℃ in refrigerator and centrifuged at 3000 rpm at 4°C for 20 minutes by using a centrifuge (Model: ST 40R Thermo Fisher Scientific, Germany). Finally, supernatant was separated and precipitants were stored in refrigerator for further experiments. The supernatant was lyophilized by using a freeze dryer (Model: FD5512iLShinBioBase Co. Ltd., Korea) and measured the final weights of the samples. The best ratio of the extraction was determined by using yield analysis.

$$\text{Yield \%} = \frac{\text{Final weight of lyophilized sample}}{\text{Initial weight of fish sample}} \text{ X } 100 \qquad 1$$

2.3 Salt Soluble Protein extraction from *Hilsa kelee* Fish

Predetermined ratio of the water extraction was used to extract proteins. 40 g of the samples were used in three replicates. Each precipitate, which obtained from three replicates of the sample was divided in to four equal mass groups. Then weighed amounts of the precipitates were mixed in 5%, 10%, 15% and 20% (v/w) concentrated NaCl (Daejung chemicals and metals co. Ltd, Korea) solutions and incubated for overnight at 4°C in refrigerator. Then incubated salt extractions were centrifuged at 3000 rpm, 4°C for 20 minutes by using the centrifuge (Model: ST 40R Thermo Fisher Scientific, Germany). The supernatant was carefully separated from precipitate and supernatant was transferred to a dialysis tube and salt was removed using dialysis. After that samples were lyophilized by using a freeze dryer (Model: FD5512iLShinBioBase Co. Ltd., Korea) and final weights were taken. The best salt concentration of the extraction was determined by using yield analysis of samples.

$$\text{Yield } \% = \frac{\text{Final weight of lyophilized sample}}{\text{Initial weight of fish sample}} \text{ X } 100 \qquad 2$$

2.4 Analysis of Functional Properties of the Crude Extracts

2.4.1 Anti-Oxidative Properties

2.4.1.1 Thiobarbituric Acid Reactive Substance (TBARs) Assay

TBAR assay was conducted using method described by Abeyrathne et al. (2014) with some modifications. Oil in water emulsion was prepared by homogenizing 1 g of corn oil and 100 µl of Tween-20 (Research lab fine chem industries, India)) with 100 ml of distilled water for 2 minutes in an ice bath using a homogenizer at maximum power. 8 mlof oil emulsion, 1 ml of distilled water and 1 mlof crude protein sample was mixed to prepare the sample for lipid oxidation assay and it was incubated at 37°C for 16 hours in a water bath (Model: YCW-010E). After the incubation period, 1 ml of the sample was transferred to a 15 ml Falcon tube which contained 2 ml of TBA/Trichloroacetic acid (Sigma-Aldrich, USA) solution and 50 µl of 10% butylated hydroxy anisole (Sigma-Aldrich, USA) in 90% ethanol (Sigma-Aldrich, USA). Then mixture was mixed well using a vortex mixture (Model: ZX3, VELP Scientifics) and it was incubated at 90°C in a water bath (Model: YCW-010E) for 15 minutes to enhance the colour. Sample was cooled in an ice bath for 10 minutes. Then sample was centrifuged at 3000 x g for 15 minutes at 5°C by using centrifuge (Model: sorvall ST 40R, Thermofisher Scientific, Germany). After the centrifugation, absorbance of the supernatant was measured at 532 nm wave length against a blank using UV spectrophotometer (Model: Spectrophotometer UV-2005). Blank was prepared with 1 ml of distilled water and 2 ml of TBA/TCA solution. The amount of TBAR was expressed as milligrams of malondialdehyde per litter of the emulsion.

2.4.1.2 DPPH Scavenging Assay

The DPPH scavenging activity of fish protein concentrate was conducted according to the method described in Jiang et al. (2014) with some modifications. Fish protein concentrate (0.5 ml) was added to 0.5 ml of DPPH solution which was prepared by dissolving 0.1 mM in methanol (Sigma-Aldrich, USA). Then mixture was shaken thoroughly and incubated at room temperature at 30°C for 30 minutes in dark environment. Absorbance was determined at 518 nm using UV-Spectrophotometer (Model: Spectrophotometer UV-2005). The scavenging effect was expressed as;

$$\text{Scavenging activity of DPPH \%} = 1 - \left[\frac{A_s - A_1}{A_0}\right] \text{ X } 100 \qquad 3$$

Where,

As - absorbance of the sample

A_0 - absorbance of the control of the DPPH-methanol solution

A_1 - absorbance of the sample added to methanol

2.4.2 Metal Chelation Properties

2.4.2.1 Fe (II) Chelating Activity

Fe^{2+} chelating activity was analyzed using the method described in Abeyrathne *et al*., (2014) with slight modifications. Fish protein concentrate (100 µl), 0.9 ml of distilled water and 1 ml of 10 mg/kg Fe^{2+} were mixed well by using vortex mixer (Model: ZX3, VELP Scientifica) in a 15 ml falcon tube and incubated for 5 minutes at room temperature. 11.3% (w/v) TCA was added to the mixture and after that centrifuged at 2500 x g for 10 minutes at 5°C by using centrifuge (Model: sorvall ST 40R, Thermofisher Scientific, Germany) to remove the precipitant. Then 1 ml of supernatant was transferred to a culture tube and 1 ml of distilled water, 800 µl of 10% ammonium acetate (HiMedia laboratories pvt. Ltd) and 200 µl of ferroin colour indicator (Daejung chemicals and metals co. Ltd, Korea) were added to the culture tube and mixed using the vortex mixer (Model: ZX3, VELP Scientifica). Again, it was incubated at room temperature for 5 minutes. The absorbance was measured at 562 nm. The Fe^{+2} chelating activity were expressed as;

$$Fe^{2+} \text{ chelating activity \%} = 1 - \left(\frac{\text{Sample absorbance}}{\text{Blank absorbance}}\right) \text{X } 100 \qquad 4$$

2.4.3 Antibacterial Property

Antibacterial properties were analyzed according to agar well diffusion method (Bendjeddou *et al*., 2016). First, Xylose lysine deoxycholate (XLD) and Eosin methylene blue (EMB) agar and plate count agar media were prepared and then autoclaved and allowed to solidified. After that lo isolated *Salmonella*, *Escherichia*

coli and plate count species were inoculated respectively on the media. Then plates were incubated at 37°C for 48 hours. After that Fish protein concentrate samples were prepared (20,000 ppm, 10,000 ppm, 5,000 ppm, 2,500 ppm, 1250 ppm, and 625 ppm). Finally, inhibition was measured using the cultured plates with different concentrations after 48 hours of incubation at 37°C. Augmentin® was used as the positive control and distilled water as negative control in the study.

2.5 Statistical analysis

Data were analyzed using Minitab 17 version. Functional properties of FPC were analyzed using one-way ANOVA in CRD (Completely Randomized Design) with three replicates for each sample. Mean comparison was calculated using Microsoft Excel 2013 version.

3.Results and Discussion

3.1 Proximate Analysis of *Hilsa Kelee* Fish

Table 1: Proximate composition of *Hilsa kelee* fish**.**

Constituent	Amount (%)
Crude protein	20.38 ± 2.38
Crude fat	10.18 ± 0.80
Ash	5.07. ± 0.92

Figure are mean values ± standard deviations

Fishes are major source of high-quality protein, fish occupies an importance place in human nutrition (Nargis, 2006). According to the previous studies fish protein composition was 18- 22% (Vanugopal and Shahidi, 1996) which varies depending on fish species. *Hilsa kelee* fish protein content (Table 1) is similar to the previous studies. Normally fish containing 0.20-23.75% of fat content (Vanugopal and Shahidi, 1996). Fish species of genus Clupeidae shows wide range of lipid content. According to the result, *Hilsa kelle* has 10.18± 0.80% fat content. Therefore *H. kelee* can be considered as a fatty fish. Ash is the measure of mineral content of any food including fish (Omotosho *et al*., 2011). The concentration of trace elements and minerals that contribute for the total ash contents are known to vary in fish depends on their environment, behavior of feeding, and ecosystem. In previous study genus Clupeidea, ash content varies from 2.1-4.9% (Vijayakumar *et al*., 2014). The ash content results of the present study of *H. kelee* were 5.07 ± 0.92%. *Hilsa kelee*'s bony structure may be reason for high value of ash content compared to the previous studies. Moisture content has reported earlier by Gopakumar, (1997).

3.2 Fish Protein Concentrate Extracted from *Hilsa kelee*

In present study, Yield of fish protein concentrate (FPC) extracted from Hilsa kelee by using two different extractions (water soluble extraction method and salt soluble extraction method) were compared.

3.2.1 Yield Analysis of Water-Soluble Protein Extraction

Water soluble protein extraction of the *Hilsa kelee* was conducted using four different dilution series with water as 1:1,1:2,1:3 and 1:4 ratio and to select the best and most cost-effective ratio for further experimentation. According to the result obtain from one-way ANOVA and tukey comparisons, it showed that there was a significant deference ($p<0.05$) between four extracted samples (Figure1). The yield increased with increasing sample: water ratio. The bonds between water molecule and water-soluble protein of fish sample increased, while increases the sample: water ratio (Ediriweera *et al*., 2017). Therefore, more proteins can extract with lower ratios. The highest water-soluble protein yield was recorded from 1: 4 (sample: water ratio) treated sample. Therefore, it was selected as suitable sample: water ratio for further experiment.

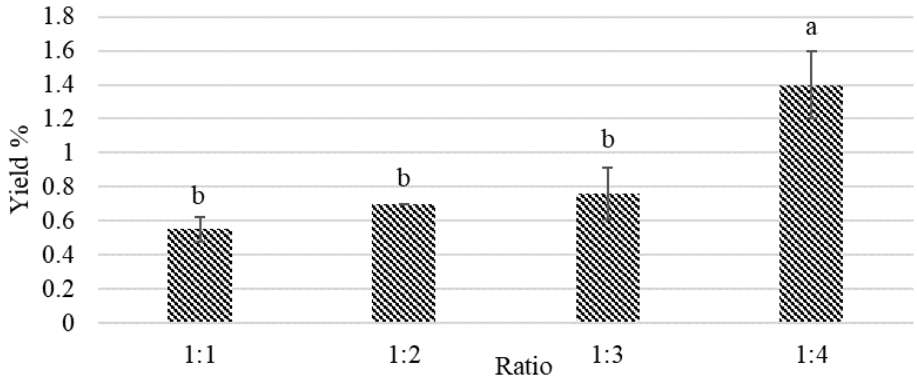
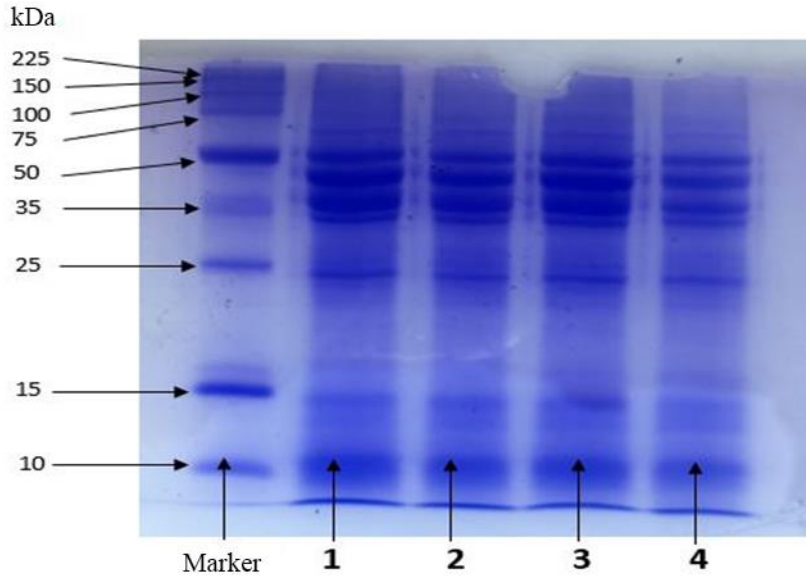


Figure 1: Yield analysis of water-soluble protein extracted from *Hilsa kelee*

3.2.2 SDS- PAGE Analysis of Water-Soluble Protein Extraction

SDS- PAGE gel patterns of water-soluble crude protein which were extracted from *Hilsa kelee* was analyzed by using 15% SDS-PAGE. According to SDS-PAGE analysis of water-soluble protein samples all four-sample had low molecular weight, and all of them have same band pattern. Several studies carried about SDS-PAGE analysis of more fish species however no any record data with *Hilsa kelee.* All band patterns were same because the same type of protein remaining in *Hilsa kelee* had been extracted from water in all four ratios (Plate 1). Therefore, select the suitable ratio (sample: water ration) of further experiment without considering

the SDS-PAGE analysis. Hence, considering yield analysis 1:4 ratio was selected for further analysis.



Lane 1- Marker; Lane 2- 1: 1; Lane 3- 1:2; Lane 4- 1:3; Lane 5- 1:4

Plate 1: 15% SDS PAGE profile of water-soluble protein extraction in different ratios of fish sample and water.

3.2.3 Yield Analysis of Salt Soluble Protein Extraction

Myofibrillar protein commonly called as salt soluble protein (Tahergorabi *et al*., 2011). Salt soluble protein extraction of samples were obtained by mixing the precipitates of water extraction with 5%, 10%, 15% and 20% (w/v) concentrated NaCl at 1:10 ratio. Crude extraction was lyophilized and selected the best salt concentrate for further experimentation. The yield decreased with increasing NaCl concentration and it showed that there was no any significant difference ($p>0.05$) among the four treatments (Figure 2). It demonstrated that yield does not change with increasing or decreasing of NaCl Concentration. Hence, the lowest concentration of NaCl helped to reduce the cost of extraction and it does not consume a lot of time. Therefore 5% (w/v) ratio was selected as the best sample for further experiment by yield analysis. Salt soluble protein extraction's yield depends on several factors such as homogenization time, salt concentration, species and muscle (Lan *et al*., 1993).

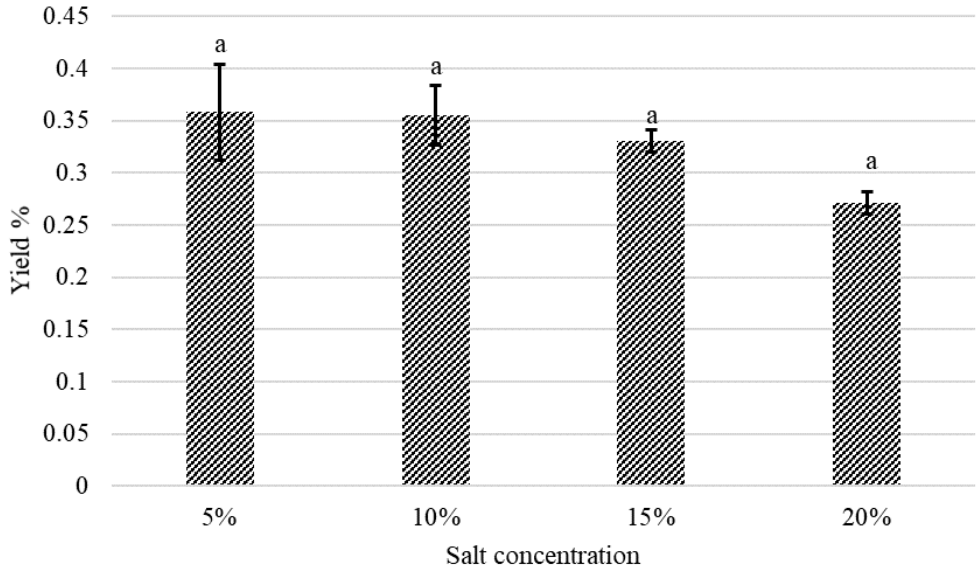


Figure 2: Yield analysis of Salt soluble protein extracted from *Hilsa kelee*

3.3 Analysis of Functional Properties

3.3.1 Anti-Oxidative Properties

3.3.1.1 TBARs Assay

The TBARs assay is well-recognized, established method for lipid peroxide calculation (Hyun *et al*., 2014). Also, TBARs assay is widely applied in scientific researches to quantify the level of oxidation by mean of measuring malondialdehyde (Oakes and Van Der Kraak, 2003). In TBARs assay malondialdehyde is a secondary end product of lipid peroxidation and react with thiobarbituric acid (TBA) and form a pink pigment (Bulut *et al*., 2007). All the samples were bright pink colour, and pure corn oil was with light pink colour at the end of the chemical reactions of TBARs assay. According to the result obtained from one-way ANOVA and Tukey Pairwise comparisons, it showed that there was a significant deference ($p<0.05$) between treatments (Figure 3). However, salt soluble protein had low malondialdehyde concentration compared to the water extraction.

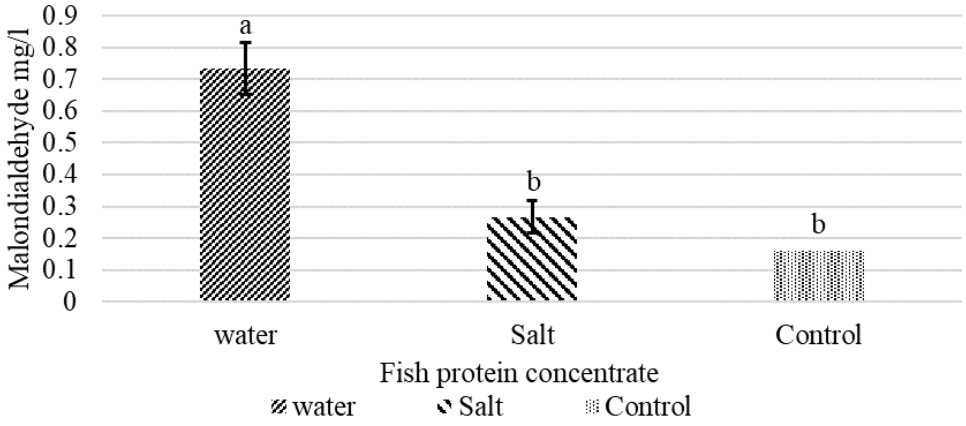


Figure 3: Graphical representation of results obtained for TBARS assay

3.3.1.2 DPPH Radical Scavenging Activity Assay

Free radical-scavenging is a primary mechanism by which antioxidants inhibit oxidative processes. The DPPH radical scavenging assay is a widely used method for evaluating the ability of hydrolysates to scavenge free radicals generated from DPPH reagent. The DPPH is a stable free radical which contains a free electron in one of the atoms in its nitrogen bridge (Kedare *et al*., 2011). When DPPH radical encounters a proton-donating substrate such as an antioxidant, the radical is scavenged and the absorbance is reduced (Rajapakse *et al*., 2005). Due to this reaction a colour change is occurred and this colour change is measured by spectrophotometer. Fish protein concentrate produced according to protocol and Ascorbic acid exhibited positive response for DPPH scavenging assay. According to the result obtain from one-way ANOVA and Tukey pairwise comparisons, it showed that there was a significant deference ($p<0.05$) between treatments (Figure 4). In present study DPPH radical scavenging activity was measured for the crude proteins which were extracted from water and salt extraction methods.

However, considering extracted samples none of them showed significantly high antioxidant properties compared with the ascorbic acid. But when consider the result of salt soluble extracted sample and water-soluble extracted sample, it shows that *Hilsa kelee* have good antioxidant property. The values obtained for DPPH scavenging activity of fish protein concentrates in water extract was 76% and in salt extract it was 86.63%. However, salt soluble protein showed highest DPPH radical scavenging activity compared to water soluble protein.

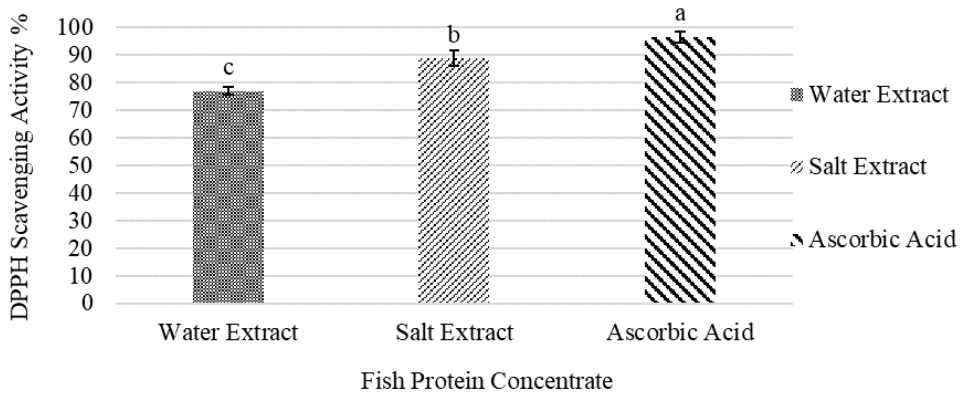


Figure 4: DPPH Radical scavenging activity of water soluble extracted and salt soluble extracted crude proteins compare with the ascorbic acid as the positive control

3.4 Metal Chelation Properties

3.4.1 Fe (II) Chelating Activity

Ferrous ion (Fe^{2+}) is the most powerful pro-oxidant among metal ions (Ktari *et al*., 2012). In a fenton reaction, this ion can interact with hydrogen peroxide to create the reactive oxygen species and the hydroxyl- free radical (OH), leading to the initiation and/or acceleration of lipid oxidation (Stohs and Bagchi, 1995). Metal chelating activity is used to determine the chelating ability of hydrolysates to chelate the metals such as iron, copper and cobalt. These transition metals, which are normally present in the food, increases the auto oxidation and breakdown hydro 46 peroxides. By donating an electron, transition metal reacts with peroxides to form alkoxyl radical (Gallegos-Tintoré *et al*., 2011). Therefore, the chelating properties included in the hydrolysates will retard the oxidative activities taken place mainly in food products. According to the results obtained from this study, both samples did not show any metal chelating property (data not shown). Instead of that both samples showed metal releasing properties. When considering the both samples there was a significant difference between two treatments ($p<0.05$). Salt extracted sample had a high metal releasing property than water extracted sample.

3.5 Antimicrobial Properties

3.5.1 Antimicrobial Activity against *E. coli* sp. and TPC Bacteria

Food spoilage is a major problem in food production. The effects of food spoilage are caused by microorganisms. Preservatives are used to prevent food spoilage. So, we can use dietary protein as preservatives because it helps to improve the consumer's health. Antimicrobial peptide plays key role in native immunity by interacting with bacteria and killing them (Zhang *et al*., 2008). Researches have

reported that almost all fish antimicrobial peptides have function against several gram (+) or gram (-) strains (Su, 2011). In this study antimicrobial analysis was conducted using the agar well diffusion method. Locally isolated E. coli and TPC bacteria were used to determine the antimicrobial property. After incubating for 48h under optimum conditions diameter of inhibition zones were measured. According to the results, both extracted fish protein samples did not show any antimicrobial property against the *E. coli* and TPC bacteria. Therefore, it is better to produce fish protein hydrolysate from the extracted fish protein concentrate to check the antimicrobial properties.

4. Conclusions

Best yield of water extraction was gained through 1:4 ratio and best yield of salt extraction was gained through addition of 5% (w/v) salt. Extracted crude fish protein concentrates (FPC) contains strong antioxidant properties however, demonstrated an Iron releasing activity. Also, FPC produced using *Hilsa kelee* did not showed any Antimicrobial Properties.

5. References

Abeyrathne, E.D.N.S., Lee, H.Y., Jo, C., Nam, K.C. and Ahn, D.U., (2014). Enzymatic hydrolysis of ovalbumin and the functional properties of the hydrolysates. *Poultry science*, 93(10), 2678-2686.

Akhade, A.R, Koli, J.M., Sadawarte, R.K. and Akhade, R.R., (2016). Functional properties of fish protein concentrate extracted from ribbon fish, *Lepturacanthus savala* by different methods. *Processing and Post-Harvest Technology*, 7(2), 274-283.

Belitz, H.D., Grosch, W. and Schieberle, P., (2009). Food Chemistry, 4th Eds. Pp 625-626 Springer, Berlin Heidelberg.

Ben Khaled, H., Ktari, N., Ghorbel-Bellaaj, O., Jridi, M., Lassoued, I. and Nasri, M., (2011). Composition, functional properties and in *vitro* antioxidant activity of protein hydrolysates prepared from sardinelle (*Sardinella aurita*) muscle. *Food Science and Technology*, 51(4), 622-633.

Bendjeddou, A., Abbaz, T., Ayari, A., Benahmed, M., Gouasmia, A. and Villemin, D., (2016). Antibacterial activity and global reactivity descriptors of some newly synthesized unsymmetrical sulfamides. *Oriental Journal of Chemistry*, 32(2), 799-806.

Bulut, M., Selek, S., Gergerlioglu, H.S., Savas, H.A., Yilmaz, H.R., Yuce, M. and Ekici, G.J., (2007). Malondialdehyde levels in adult attention-deficit hyperactivity disorder. *Psychiatry Neurosci*, 32(6), 435-438.

Calder, P.C., (2004). N-3 fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clinical Science*, 107(1), 1-11.

Cordova-Murueta, J.H., Navarrete Del Toro, M. de los A. and Garcia Carreno, F.L., (2007). Concentrates of fish protein from by catch species produced by various drying processes. *Food Chemistry*, 100(2), 705-711.

Davies, R.W., Cripps, S.J., Nickson, A. and Porter, G., (2009). Defining and estimating global marine fisheries by catch. *Marine Policy*, 33(4), 661-672.

Dyerberg, J., (1985). Coronary health aspects of fish food lipids. *Voeding*, 46(12), 388-391.

Ediriweera, T. K., Aruppala, A. L. Y. H. and Abeyrathne, E. D. N. S., (2017). Analysis of bioactive properties of fish protein hydrolysates from Scomber japonicus Fin Wastes. *Technology and Value Addition Volume*, 1(1), 31-45.

Eter, J. G. J. S., (1983). Reproduction, age and growth of the *Kelee shad, Hilsa kelee* (Cuvier, 1829) (Pisces: Fam. Clupeidae) with information's on its fishery in Maputo Bay, Mozambique by Department of Fisheries Biology and Instituto De Desenvolvimento Pesqueiro Maputo.

FAO Fishery Country Profile - Sri Lanka. 2019. FAO Fishery Country Profile - Sri Lanka. [Online] Available at: http://www.fao.org/fi/oldsite/FCP/en/LKA/profile.htm. [Accessed 13 November 2019].

FAO, 2018. The State of World Fisheries and Aquaculture. Rome: Food and Agriculture Organization of the United Nations, FAO Fisheries and Aquaculture Department; p. 04.

FAO.org. 2019. FAO Fisheries & Aquaculture - Aquatic species. [Online] Available at: http://www.fao.org/fishery/species/2097/en [Accessed 14 Oct. 2019]

Gallegos-Tintoré, S., Torres-Fuentes, C., Martínez-Ayala, A. L., Solorza-Feria, J., Alaiz, M., Girón-Calle, J. and Vioque, J., (2011). Antioxidant and chelating activity of *Jatropha curcas* L. protein hydrolysates. *Science of Food and Agriculture*, 91(9), 1618–1624.

Gopakumar, K., (1997). Biochemical composition of Indian food fish. Central Institute of Fisheries Technology, Cochin

Hamann, D.D., (1994). Rheological studies of fish proteins, in; Food Hydrocollids. Eds. K. Nishinaki and E. Doi, Pp. 225, Plenum Press, NEW YORK, U.S.A.

Hyun, T.K., Kim, H.C. and Kim, J.S., (2014). Antioxidant and antidiabetic activity of *Thymus quinquecostatus* Celak. *Industrial crop and production*, 52, 611–616.

Jiang, H., Tong, T., Sun, J., Xu, Y., Zhao, Z. and Liao, D., (2014). Purification and characterization of antioxidative peptides from round scad (*Decapterus maruadsi*) muscle protein hydrolysate. *Food chemistry*, 154, 158-163.

Kedare, S. B. and Singh, R. P., (2011). Genesis and development of DPPH method of antioxidant assay. *Food Science and Technology*, 48(4), 412–422.

Ktari, N., Jridi, M., Bkhairia, I., Sayari, N., Ben Salah, R., and Nasri, M., (2012). Functionalities and antioxidant properties of protein hydrolysates from muscle of zebra blenny (*Salaria basilisca*) obtained with different crude protease extracts. *Food Research International*, 49(2), 747–756.

Lan, Y. H., Novakofski, J., Carr, T. R. and McKeith, F. K., (1993). Assay and storage conditions affect yield of salt soluble protein from muscle. *Food Science*, 58, 963-967.

Lund, E. K., (2013). Health benefits of seafood; is it just the fatty acids. *Food Chemistry*, 140, 413-420.

Malva, A.D., Albenzio, M., Santillo, A., Russo, D., Figliola, L., Caroprese, M. and Marino, R., (2018). Methods for extraction of muscle proteins from meat and fish using denaturing and nondenaturing solutions. *Food Quality*, 847847, 1-9.

Nargis, A., (2006). Seasonal variation in the chemical composition of body flesh of koi fish *Anabas testudineus* (Bloch) (Anabantidae: Perciformes). *Scientific and Industrial Research*, 41(3), 219-226.

Oakes, K.D. and Van Der Kraak, G.J., 2003. Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. *Aquatic Toxicology*, 63(4), 447-463.

Official Methods of Analysis of AOAC International, 2016. George W., Latimer, Jr. 20th Edition.

Omotosho, O.E., Oboh, G. and Iweala, E.E.J., (2011). Comparative effects of local coagulants on the nutritive value, invitro multi enzyme protein digestibility and sensory properties of Wara cheese. *Dairy Science*, 6, 58-65.

Panhwar, S. K., Siddiqui, G. and Ayub, Z., (2012). Gonads annual development of *Kelee Shad*, *Hilsa Kelee* (Cuvier, 1829) from the coastal waters of Pakistan and its spawning ecology. *Marine Biology*, 38(1), 79–87.

Rajapakse, N., Jung, W.K., Mendis, E., Moon, S.H. and Kim, S.K., (2005). A novel anticoagulant purified from fish protein hydrolysate inhibits factor XIIa and platelet aggregation. *Life Sciences*, 76(22), 2607-2619.

Ramakrishnan, V.V., Ghaly, A.E., Brooks, M.S. and Budge, S.M., (2013). Extraction of proteins from mackerel fish processing waste using alcalase enzyme. *Bioprocessing Biotechniques*, 3, 2-9.

Rathnasuriya, M. I. G., Haputhantri, H. S. S. K., Balawardhana, D. G. T. C., Wimalasiri, H. B. U. G. M. and Weerasekera, S. J. W. W. M. M. P., (2018). 'Stock status and biological aspects of data poor *kelee shad*, *Hilsa kelee* (Cuvier, 1829) small scale purse-seine fishery off Mannar Peninsula, Sri Lanka', National Aquatic Resources Research and Development Agency (NARA). (2018):17.

Regenstein J. M. and Regenste1in C. E., (1992). Introduction to Fish Technology, Van Nostrand Reinhold, New York.

Revolvy, L., (2019). "*Ilish*" (Online). Available at: https://www.revolvy.com/page/Ilish?cr=1 [Accessed 24 Nov. 2019]

Rudkowska, I., Marcotte, B., Pilon, G., Lavigne, C., Marette, A. and Vohl, M. C., (2010). Fish nutrients decrease expression levels of tumor necrosis factor-alpha in cultured human macrophages. *Physiol Genomics*, 40, 189–194.

Sambrook, J. and Russell, D.W., (2006). Detection of protein-protein interactions using the GST fusion protein pulldown technique. Cold Spring Harbor Protocols, 2006(1):3757.

Sargent, J. R., (1997). Fish oils and human diet. *British Journal of Nutrition*, 78(1), S5-SI3.

Sarvenaz, K.T. and Sabine, S., (2017). Nutritional value of fish: Lipids, proteins, vitamins, and minerals. *Reviews in Fisheries Science & Aquaculture*, 26(2), 243-253.

Srikanya, A., Dhanapal, K., Sravani, K., Madhavi, K., Yeshdas, B. and Praveen, K.G., (2018). Antioxidant and antimicrobial activity of protein hydrolysate prepared from tilapia fish waste by enzymatic treatment. *Current Microbiology and Applied Sciences*, 7(10), 2891-2899.

Stohs, S. J. and Bagchi, D., (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biology & Medicine*, 18(2), 321–336.

Su, Y., (2011). Isolation and identification of pelteobagrin, a novel antimicrobial peptide from the skin mucus of yellow catfish *(Pelteobagrus fulvidraco*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 158(2), 149-154.

Tacon, A. G. J. and M. Metian., (2013). Fish matters: importance of aquatic foods in human nutrition and global food supply. *Reviews in Fisheries Science*, 21(1), 22–38.

Philips, G.O. and Williams, P.A., (2011). Hand book of food proteins, Pp 116-149. Woodhead, West verginia.

Venugopal, V. and Shahidi, F., (1996). Structure and composition of fish muscle. *Food Reviews International*, 12(2), 175-197.

Vijayakumar, N., Sakthivel, D. and Anandhan, V., (2014). Proximate composition of clupeidae and engraulidae inhabiting thengaithittu estuary puducherry- South East coast of India. *Science Invention Today*, 3(3), 298-309.

Wu, H.-C., Chen, H.-M. and Shiau, C.Y., (2003). Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). *Food Research International*, 36(9-10), 949–957.

Zhang, Y. X., Zou, A. H., Manchu, R. G., Zhou, Y. C., and Wang, S. F., (2008). Purification and antimicrobial activity of antimicrobial protein from brown-spotted grouper, Epinephelusfario. *Zoological Research*, 29(6), 627-632.