Research Article

Extraction of Crude Collagen from Yellowfin Tuna (*Thunnus albacares*) Skin and Determination of the Functional Properties of Its Hydrolysates

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Abstract

Collagen is a dominant protein in connective tissues and is valuable in food industry. Objective of this study was to develop a simple non-toxic method to extract collagen from Yellowfin tuna skin and to check functional properties of its hydrolysates. Extraction procedure was conducted using acetic, citric acid with 0.5 M concentrations. Based on 8% SDS-PAGE gel, type I collagen were identified. Enzymatic hydrolysis was done with protease, trypsin and pepsin with 0, 3, 6, 9, 12 and 24 hours times at 37°C after adjusting its optimum pH level. Best hydrolysate was selected for antioxidant activity with DPPH radical scavenging activity and TBARS assay. Iron chelating activity was evaluated using ferrozine indicator method and Antimicrobial activity was done using agar well diffusion method. Proximate analysis of raw skin was done for determine moisture, ash, protein, fat content and 59.44±0.013%, 1.91±0.37%, 28.55±1.19%, 6.83±0.30% values were obtained respectively. Hydrolysates produced after incubating for 0 hours at 37°C followed by heat inactivation was selected for further analysis. Hydrolyzed produced by collagen using citric acid showed lower scavenging activity compared to acetic acid ($p<0.05$). In TBARS assay citric acid showed high antioxidant activity than acetic acid ($p<0.05$). Both acetic acid and citric acid extractions did not show significant difference among the treatments in Fe^{2+} chelating activity ($p>0.05$). Good antimicrobial activity was obtained with acetic acid than citric acid ($p<0.05$). Accordingly, the hydrolysates incubated at 0 hours at 37°C showed good antioxidant activity with acetic acid extraction. This concludes that collagen hydrolysates produced using acetic acid showed good antioxidant activity.

Keywords: *Fish collagen, Yellowfin tuna, antioxidant activity, hydrolysates, non toxic*

1. Introduction

The Sri Lankan fisheries sector plays a vital role in provision of direct and indirect employment opportunities (Huss, 1994) and it has been considered as one of the major potential sectors for increasing the economy (Jayaweera *et al.,* 1988). The fish and fisheries products have export quality improved by the globalization process (Hemanta & Amarasinghe, 1983). To be a market leader in seafood exports in the South Asian region Sri Lanka follows strict quality assurance procedure for the European union (Hewapathirana & Bandulage, 2009). In the year 2017 total fish catch was 389,500 metric tons and out of that total marine production was 333,750 metric tons. Salmon, tuna, cod, skipjack tuna, marlin and shark are some marine fish that rapidly catches in Sri Lanka (MFARD, 2017). Tuna is a commercially important fish species (Sulthanbawa & Aksnes, 2006) widely distributed throughout tropical and temperate waters (Guerard *et al.,* 2002). The total catch of tuna and tuna like species was about 40,000 metric tons per year (Global valuation of tuna, 2017).

Tuna and tuna like species are considered as major commercial important species (FAO, 2013). Due to increment of the demand, global tuna market reached a value of US$ 11.38 billion in 2017 (Cision, 2018). There are large number of fish processing companies in the country (Helgi, 2017). Fish processing generates significant amount of waste (Jaiswal *et al.,* 2014) due to value addition and preservation, by portioning and removal of head, skeleton, skin, scale, gut and blood (Yogesh *et al.,* 2014). From whole fish two third of waste may be generated in canning process (Tuti *et al.,* 2012) and 65% of solid waste generated (Srinivasa *et al.,* 2008) which includes head, bone, viscera, gills, dark muscle and skin (FAO, 2012). Global waste generation was calculated as 130 million tons per year. Tuna processing waste is also a major component of coastal waste (Helgi, 2017). Tuna processing industry produces 30-35% product, 20-35% solid waste, 20-35% of liquid waste (Wondsakl *et al.,* 2006). Normally from one unit discarded 450,000 tons annually (Sulthanbawa & Asknes, 2006). Large amount of waste may generate from fish processing industry (Yogesh *et al.,* 2014) and mainly it contains the good quality proteins. Through that it causes the environmental pollution, because of the odor and high moisture content (Guerouali *et al.,* 1995).

Collagen is a naturally occurring protein and major structural protein that is widely distributed in animal connective tissues (Ramshaw *et al.,* 1998). Their functions are different from those of globular proteins such as enzymes (Ehrlirh, 2007). Collagen has a high hydroxyprolin content and its amino acid composition is different from a protein (Tanaka *et al.,* 2018). Collagen is most abundant in invertebrates and vertebrates (Gallop, 1957). Collagen fibers exist in many places of the body and are a major component of the extracellular matrix that support most tissues and provide structure to the cells from the outside (Saito, 2001). Type I collagen are found in skin, liver and bones (Hema *et al,* 2013). Collagen are widely used in food, cosmetic,

biomedical and pharmaceutical industries. Preferential source of collagen are bovine skin and tendon as well as porcine skin (Wells, 1987). Porcine and Bovine originated collagen may be rejected because of infectious diseases (Senarathne *et al.,* 2006) like Bovine Spongiform Encephalopathy (BSE), Transmissible Spongiform Encephalopathy (TSE), and Foot and Mouth Diseases (FMD). Porcine originated collagen is also rejected due to religious reasons (Gomez, 2011). Therefore, the marine collagens are used as alternative sources (Leuenberger, 2015).

High availability of fish generates high amount of fish waste and it acts as a best alternative for collagen (Gordon & Hahn, 2010). Fish offal such as bones, skin, scales and fins can be serving as a collagen alternative source and there is no risk of disease transmission, no religious barriers and possibility of higher yielding collagen (Nagai, 2000). Generated waste from fish processing is just released to the rivers, canals or open environment. Those improper releasing of waste to environment cause a lot of environmental impacts. Efficient recovery and using by-products may lead to reduction of environmental pollution and it gives maximum economic benefits to waste (Kim & Mendis, 2006).

According to Hema *et al.,* (2013) acid soluble method and pepsin digestible method can be used for the extraction of collagen from the tuna skin, and isolated by salt precipitation. According to Paweena *et al.,* (2001) acid pepsin digestion method can be used and 0.067±0.06 g of collagen yield can be obtained from 1 g of tuna sample. According to Tanaka *et al.,* (2018) methanol extraction can be used for extraction and it mainly focused on pharmaceutical industry applications. Due to functional properties of hydrolyzed collagen it can be used in food processing as a binding agent with the functional properties. Therefore, the objective of this study was to extract collagen from yellow fin tuna skin with a low-cost method and identification of functional properties of its hydrolysates which are important in food and pharmaceutical industry.

2. Materials and Methods

2.1 Materials

Fresh samples of yellow fin tuna skin were provided by the Ceylon Fresh Seafood (Pvt) Ltd, Ja-Ela, Sri Lanka. Samples were transported to the university laboratory under the refrigerated conditions.

2.2 Sample Preparation

The skins were collected and scales, adhering tissues and residuals were removed manually by using a knife. After washing skins were cut into small pieces (2 cm × 2 cm) and stored at -20°C until used.

2.3 Proximate Analysis

The raw skin of yellow fin tuna was subjected to proximate analysis including moisture, Ash, Fat and protein contents according to AOAC standard methods (2016).

2.4 Pretreatment for the Skin

The skins were pretreated according to the Hema *et al.,* (2013) with modifications. The skins were weighed and treated with 0.4 M NaOH with sample to solution ratio of 1:10 and stirred for 24 hours to remove non collagenous materials from the skins. Then the treated mass was fully washed with chilled distilled water until neutral or faintly basic pH of wash water was obtained. Samples were subjected to pH measurement using pH meter (PL-700 PV) and residuals were collected for extraction procedure.

2.5 Collagen Extraction from Yellowfin Tuna Skin

The collagen was extracted by the method of Paweena *et al.,* (2001) with modifications. Two different acid combinations were used to select the best yielding extraction method (acetic acid pepsin method/citric acid pepsin method). Residuals were collected after the pretreatment and acetic acid/citric acid were added to solubilize non cross-linked collagen and break some of inter strand cross links. Then samples were blended and 0.9 M acetic acid/citric acid were added and shaken for 24 hours using an orbital shaker (OS 2000, JEIO TECH, Korea). Then viscous solutions were filtered and each extracted solution was centrifuged at 4000 rpm using a centrifuge machine (Thermo scientific, SORVALL ST 40R) and collected supernatants were subjected to pepsin digestion at 0.1% (w/w) for the collagen precipitation. Then extracted mass was salted out by adding NaCl to final concentration of 0.9 M in acetic acid/citric acid and stirred. Precipitate was collected using a centrifuge machine (Thermo scientific, SORVALL ST 40R) at 4000 rpm. Precipitate was dissolved in 0.5 M acetic acid/citric acid and stirred. Afterward resultant solution was dialyzed against the distilled water to remove minor fragments with non-helical form and lyophilized with freeze dryer (F05512, Korea).

2.6 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis

SDS-PAGE was performed after the extraction of collagen according to Hema *et al.,* (2013) with modifications to identify best collagen hydrolysate and to confirm the extracted compound. 8% and 15% SDS-PAGE was used to evaluate extracted collagen and peptides derived from the hydrolysis. Resolving gel and stacking gel were prepared by using appropriate volume of solution with desired amount that are responsible for 8% and 15% gels.

2.7 Enzymatic Hydrolysis

Enzymatic hydrolysis was used to hydrolyze extracted collagen from Yellowfin tuna skin according to Abeyrathne *et al.,* (2014). Protease, trypsin and pepsin enzymes were used for hydrolysis. Lyophilized collagens were subjected to dissolve in 0.5 M acetic acid and pH were adjusted using pH meter (PL-700 PV) according to the optimal conditions of each enzyme (Protease 37°C, pH-6.5/ Trypsin 37°C, pH-7.8/ Pepsin 37°C, pH-2.5). Enzymes were added according to ratio of 1:100 (enzyme: substrate). Afterwards, samples were incubated in 37°C for 3, 6, 9, 12 and 24 hours. At the end of incubation samples were heated by using heat block in 100°C in 15 minutes to inactivate the added enzymes. Then 15% SDS-PAGE were performed to identify the best level of hydrolysis.

2.8 Measurement of Functional Properties

To measure the functional properties of collagen hydrolysates the best hydrolysate was selected based on the purity of the hydrolysate.

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity for hydrolyzed skin collagen may determine according to the method of Ace *et al.,* (2016) with modifications.750 µL of extracted hydrolyzed collagen sample was mixed with 300 µL of DPPH solution and kept for 30 minutes in dark environment at the room temperature for incubating. Ascorbic acid was used as positive control and the absorbance was measured through the 517 nm using UV spectrophotometer (4120020, J.P. SELECTA, Japan). The scavenging effect was calculated using the following formula,

$$\text{Scavenging activity of DPPH (\%)} = [1-(A_S-A_1)/A_0] \times 100 \quad (1)$$

where ,

A_S – Absorbance of the sample

A_0 – Absorbance of the control of the DPPH methanol solution

A_1 – Absorbance of the sample added to methanol

TBARS Assay

Hydrolysates antioxidant activity measured according to the method of Abeyrathne *et al.,* (2014) with modifications. Oil-in water emulsion were prepared with 1 g of corn oil/sunflower oil, 100 µL of tween-20 and homogenized for 2 minutes with 100 mL of distilled water by using a homogenizer (D-500). For lipid oxidation assay samples were prepared with 8 mL of oil emulsion, 1 mL of distilled water, 1 mL of hydrolyzed skin collagen and incubated at 37°C for 16 hours using a water bath (GEMMY CO-YCW-010E). Then incubated samples were cooled on ice bath for 10

minutes and centrifuged with centrifuge machine (Thermo scientific, SORVALL ST 40R) at 3000×g for 15 minutes at 5°C. Finally absorbance were measured by using UV spectrophotometer (4120020, J.P. SELECTA, Japan) for 532 nm. The blank was prepared by adding 2 mL of distilled water with 8 mL of oil emulsion and the procedure was followed as same as above.

Fe^{2+} Chelating Activity

Fe^{2+} Chelating activity of the collagen hydrolysates was measured according to Abeyrathne *et al.*, (2014). The 100 µL of the collagen hydrolysates, 0.9 mL of distilled water and 1 mL of 10 ppm $FeSO_4$ were vortex mixed in falcon tube and incubated for 5 minutes at the room temperature. For removal of protein and peptides in samples, 900 µL of 11.3% TCA (Trichloroacetic acid) was added and centrifuged at 2500 × g for 10 minutes using centrifuge machine (Thermo scientific, SORVALL ST 40R). Then 1 mL of supernatant were transferred to a disposable culture tube. Then 1 mL of distilled water, 800 µL of 10% Ammonium acetate and 200 µL of ferroin colour indicator (75 mg of ferrozin, 75 mg of neocupion and 1 drop of 6 N HCl in 25 mL of distilled water) were added and vortex mixed with vortex machine (Nemko, F202AO176). Then the samples were incubated for 5 minutes at the room temperature. Finally, the absorbance was measured at 532 nm using UV spectrophotometer. The Fe^{2+} Chelating activity was calculated using following formula.

$$Fe^{2+} \text{ chelating activity } (\%) = [1 - (\text{sample absorbance/blank absorbance})] \times 100 \quad (2)$$

Antimicrobial Activity

Extracted collagen hydrolysates antibacterial activity was measured using the agar well diffusion method according to Patra *et al.*, (2009) with modifications. Agar was used to determine the anti-bacterial effects of collagen hydrolysates against common aerobic bacteria culture. The common aerobic bacteria culture was obtained from total plate count method. 20 mL of agar was poured on petri plate and allowed to solidify. Then surface was streaked using a cotton swab with the reference bacterial strains. Wells were prepared using cork borer. With the 5 mm diameter samples were poured in to wells and kept for complete diffusion. Then the agar plates were incubated at 37°C for 48 hours and measured the diameter of inhibition zone. Augmentin and distilled water were used as the positive and negative controls respectively.

2.9 Data Analysis

Each and every procedure were conducted separately for three replicates and the difference between mean values of three replicate groups were analyzed by one-way analysis of variance (ANOVA). Data was analyzed using Minitab 17 statistical software package.

3. Results and Discussion

3.1 Extraction of Collagen

Collagen extraction procedure were conducted according to the method of Paweena *et al.,* (2001). This new method was a simple nontoxic and cheap extraction method to extract crude collagen from Yellowfin tuna skin by products.

3.2 Yield Analysis for Collagen Extracted from Yellowfin Tuna Skin

According to the Figure 1 collagen extract with citric acid treatment (6.75±3.21 g) showed higher yield compared to the acetic acid treatment (6.63±2.53 g) and there is no significant difference between two treatments ($p<0.05$). A previous study also have manifested that collagen yield with citric acid treatment had higher yield than acetic acid treatment. But they have showed that the collagen yields of acid-enzyme extraction were very high and that values were about 51.4% (Japanese sea-bass), 49.8% (chub mackerel), and 50.1% (bullhead shark) respectively on the basis of lyophilized dry weight (Nagai *et al.,* 1999). The isolated Pacific Bluefin tuna skin collagen dry yield was 5.4% (Tanaka *et al.,* 2012). In comparison to the previous studies, present study shows lower yield, which could be due to the low purity of the extracts.

The main impurity here can be the presence of non-collagenous materials. The concentration of used acids and reduced solubility of collagen in the extraction solvent could be another factor. Further, the low yield of collagen content may be due to the denaturing of protein during the process and differences in environmental temperature. So, further study is needed to evaluate type of impurities present in the sample and reasons for lower yield.

As shown in Figure 1, among the two extractions citric acid extraction may be the appropriate, rapid and cheap method for extraction of collagens from Yellowfin tuna skin.

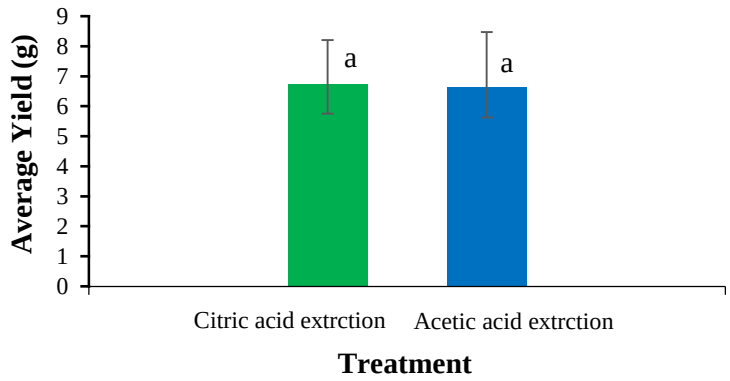

Figure 1: Average collagen yields from acetic acid and citric acid extractions

3.3 Determination of Collagen from the Extraction (SDS-Polyacrylamide Gel Electrophoresis)

Figures 2 and 3 shows that the molecular weight pattern of Yellowfin tuna skin collagen extracted with acetic acid and citric acid against the standard collagen. The subunit composition of the collagen was recognized by SDS-PAGE. Previous studies reported that most of fish species skin and bone collagen contain the type I collagen (Muyonga *et al.*, 2004; Yata *et al.*, 2001). It may comprise with two identical chains named as α1 and α2 (Duan *et al.*, 2004; Salarzadeh *et al.*, 2012; Tan & Chang, 2018).

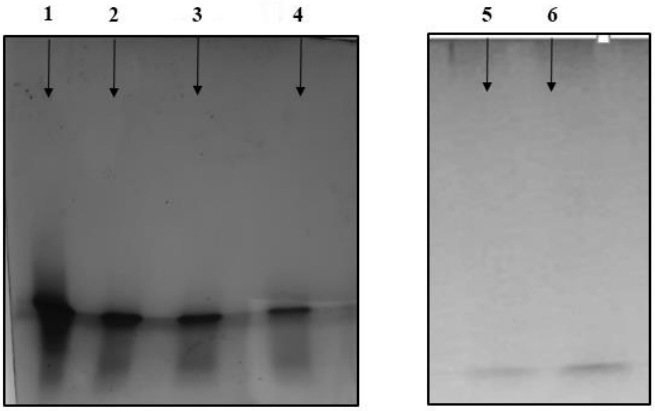

Figure 2: SDS-PAGE of Yellowfin tuna skin collagen extracted with acetic acid Lane 1 = Standard Collagen; Lane 2 = Acetic acid treated collagen extract (Replicate 1); Lane 3 = Acetic acid treated collagen extract (Replicate 2); Lane 4 = Acetic acid treated collagen extract (Replicate 3); Lane 5 = Acetic acid treated collagen extract (Replicate 4); Lane 6 = Acetic acid treated collagen extract (Replicate 5)

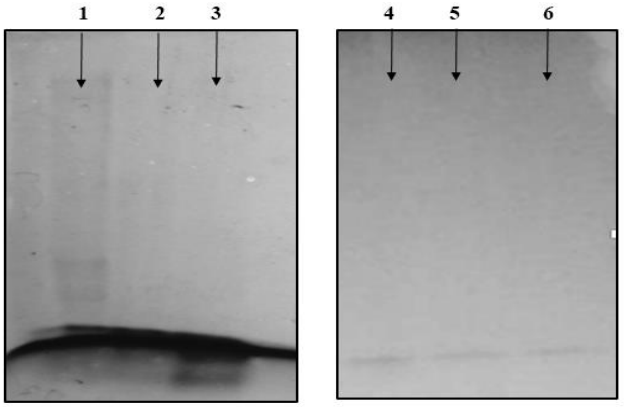

Figure 3: SDS-PAGE of yellowfin tuna skin collagen extracted with citric acid
Lane 1 = Standard Collagen; Lane 2 = Citric acid treated collagen extract (Replicate 1); Lane 3 = Citric acid treated collagen extract (Replicate 2); Lane 4 = Citric acid treated collagen extract (Replicate 3); Lane 5 = Citric acid treated collagen extract (Replicate 4); Lane 6 = Citric acid treated collagen extract (Replicate 5)

The skin collagen of Big eye snapper (Kittiphattanabawon *et al.*, 2005), Brown banded bamboo shark (Kittiphattanabawon *et al.*, 2010), Nile perch (Ogawa *et al.*, 2003), Walleye Pollock (Yan *et al.*, 2008) all consist of α1 and α2 chains. In present study according to the SDS-PAGE 10% gel images it shows that yellowfin tuna skin collagen also composed of two α chains similar to previous studies. On the other hand, these patterns were similar to the type I collagen from marine sources. All these observations conclude that the isolated collagen is a typical type I collagen.

3.4 Enzymatic Hydrolysis

Enzymatic hydrolysis of protein is a complex process due to the several peptide bonds and their specific accessibility to enzymatic reactions. The specificity of enzymes is not the only factor that affects the peptide structure of the final product. Environmental factors such as temperature and pH play an important role. Both factors, temperature and pH can gently affect the enzyme reaction kinetics and the effect of these factors is different for each enzyme. Normally there is an optimum combination of both pH and temperature, where an enzyme is the most active. Temperature and pH maximize deactivating of the enzymes by denaturing (Hordur *et al.*, 2000). In the previous study the extracted collagen was hydrolyzed using alcalase, α-chymotrypsin, neutrase, papain (Je *et al.*, 2007). In the present study the extracted Yellowfin tuna skin collagen was hydrolyzed using Pepsin, Trypsin and Protease enzymes -with different time combinations.

3.5 Determination of Best Hydrolysates

Collagen that extracted from Yellowfin tuna skin from both acetic acid and citric acid extractions was hydrolyzed with Pepsin, Protease and Trypsin enzymes under 37°C for 0, 3, 6, 9, 12 and 24 hours and the best hydrolysate were selected. The purity of the hydrolysates produced from the Yellowfin tuna skin collagen was observed visually. Generally, in SDS-PAGE collagen may result two α chains. But in this case of hydrolysates, it did not show any band in gel image. Previous studies revealed that the peptides with molecular weight < 2kDa do not show bands in 15% SDS-PAGE (Abeyrathne *et al.*, 2014). So, it can be concluded that the protease, pepsin and trypsin enzymes can produce peptides with molecular weight <2kDa from collagen even with 0-hours incubation time.

According to the gel images it was confirmed that all proteins have hydrolyzed during the process. Because of that the all hydrolysates show no band patterns and turbidity. So, it can be concluded that the hydrolysates derived from protease, pepsin, trypsin enzymes with 0 hours incubation period was the best hydrolysate to determine the functional properties.

3.6 Functional Property Analysis of Collagen Hydrolysates

DPPH Radical Scavenging Activity

Figure 4 shows the results according to the DPPH radical scavenging activity for Yellowfin tuna skin collagen hydrolysates from acetic acid and citric acid extractions. Generally according to the obtained results all the treatments show more than 85% DPPH radical scavenging activity which showed a significant difference between acetic acid extraction hydrolysates and citric acid extraction hydrolysates ($p<0.05$). When compared with the control group of ascorbic acid there is no significant difference between control group and the acetic acid extract hydrolysates group ($p>0.05$). With consideration of mean values, the highest radical scavenging activity is shown by the acetic acid extract hydrolyzed with pepsin enzyme. So, results shown in Figure 4 concludes that the Yellowfin tuna skin collagen hydrolysates can be used as a good antioxidant compound and better substitute for ascorbic acid.

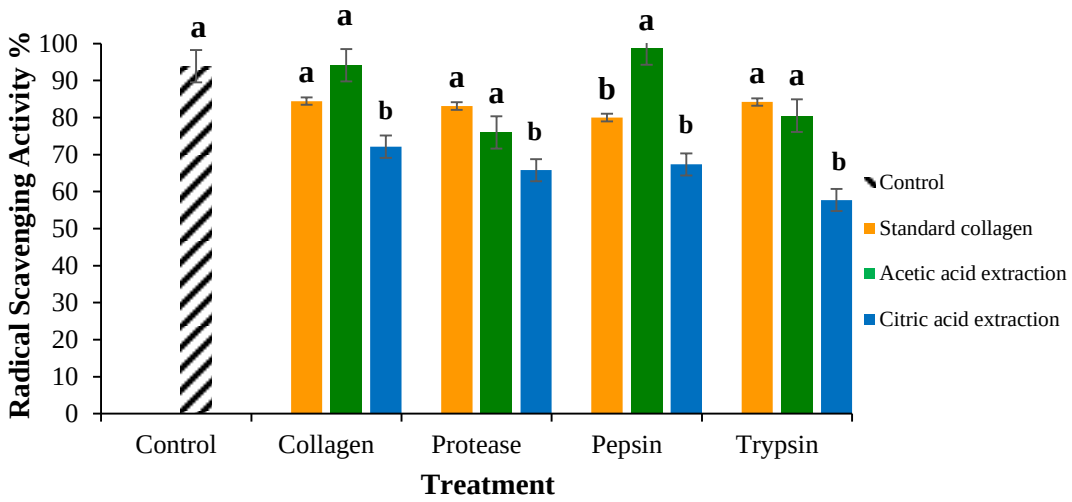

Figure 4: DPPH radical scavenging activity of Yellowfin tuna skin collagen hydrolysates, hydrolyzed with protease, pepsin and trypsin at 37°C for 0 hours.
a-b values are means with standard error. Values with different letters are significantly different

TBARS Assay

According to Figure 5, there was a significant difference between acetic acid extraction and citric acid extraction ($p<0.05$), but when compared with the control there was no significant difference between the control group and citric acid hydrolyzed collagen with pepsin enzyme. Low malonaldehyde concentration may responsible for the high antioxidant activity, and results shown in Figure 5 concludes that collagen hydrolysates are suitable alternative

for reduction of oxidative stress. According to past studies on Yellowfin tuna bone collagen, it shows that collagen hydrolysates with protease enzyme did not show the antioxidant activity against oxidation of lipids. However, in the present study with citric acid extraction it shows a good antioxidant activity against the lipids. It could be because of the difference of extraction steps, time durations taken for the steps and the characters of used acids.

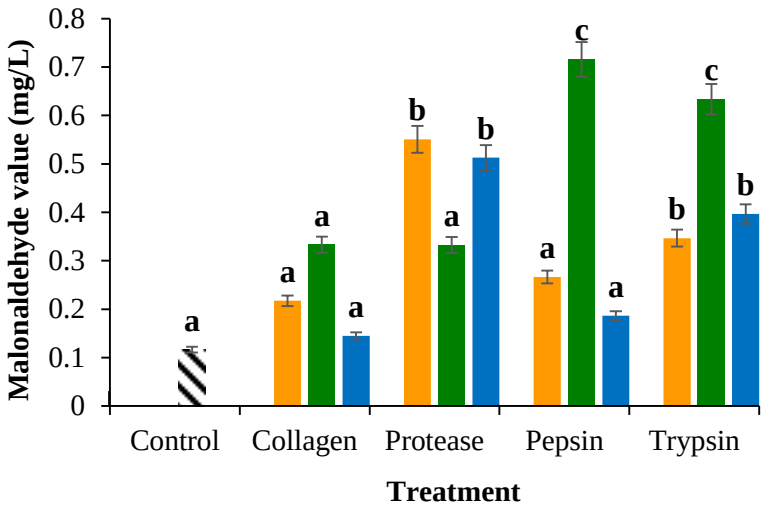

Figure 5: TBA reactive substance value of Yellowfin tuna skin collagen hydrolysates hydrolyzed with protease, trypsin and pepsin at 37°C for 0 hours.
a-c values are means with standard error. Values with different letters are significantly different

Fe^{2+} Chelating Activity

According to the Figure 6 there is no significant difference between acetic acid treatment and citric acid extraction treatment ($p>0.05$).When compared with the standard collagen also, there is no significant difference ($p>0.05$). Figure 6 shows that there are slightly high chelating agents present in the collagen hydrolysates produced with protease, trypsin and pepsin enzymes. When considering the mean values slightly high chelation activity was shown with acetic acid extract hydrolyzed with trypsin enzyme. Metal binding proteins are potent chelates of heavy metals. Some foods and proteins have been suggested to reduce absorption or reabsorption of toxic metals and to support natural detoxification pathway (Sears, 2013). Accordingly, our results conclude that collagen hydrolysates produced from the Yellowfin tuna skin can be used as a source to remove metals from human body and it could be highly beneficial for food and pharmaceutical industry.

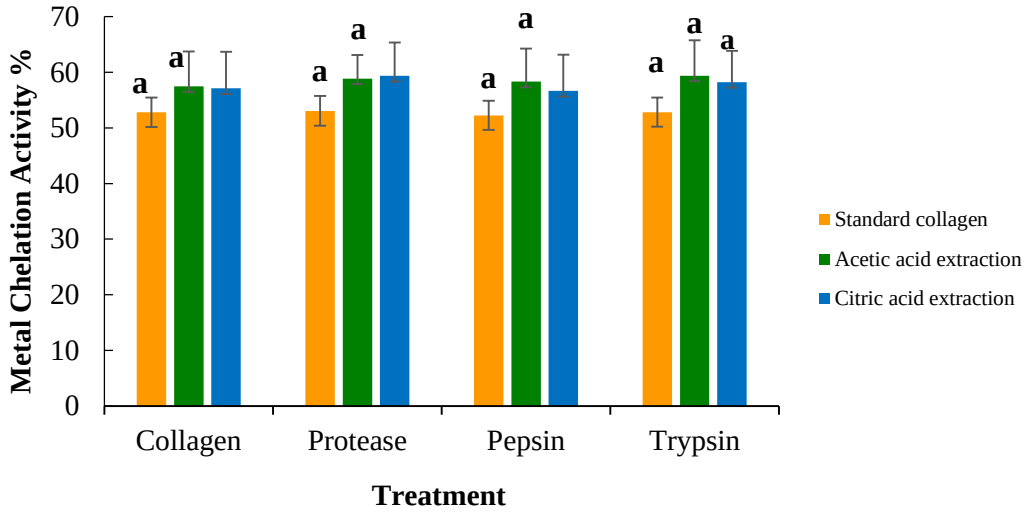

Figure 6: Fe^{2+} chelating activity of Yellowfin tuna skin collagen hydrolysates hydrolyzed with protease, pepsin and trypsin enzymes.
a values are means with standard error. Values with different letters are significantly different

Antimicrobial Activity

Hydrolyzed peptides from Yellowfin tuna skin collagen shows slight inhibition against aerobic common bacteria after 48 hours. According to the obtained results acetic acid extraction with trypsin enzyme shows high inhibition than rest. There was no significant difference between 10,000 ppm and 20,000 ppm. There was a significant difference between treatment and positive control at remaining concentrations (5000 ppm, 1250 ppm, 625 ppm) ($p>0.05$).

Antibacterial activity of hydrolysates from Yellowfin tuna skin collagen have shown gradual decrement with the decreasing of concentration of samples however, there was a significant difference between two treatments and in comparison, to the control (Augmentin®). When consider antibacterial activity, several factors need to be taken into account such as amino acid composition, sequence molecular weight and type of bacteria (Dibernardini *et al.*, 2001). In the case of this the future studies are needed to confirm antimicrobial activity of hydrolysates of Yellowfin tuna skin collagen against *E coli* and *Salmonella* (Figure 7).

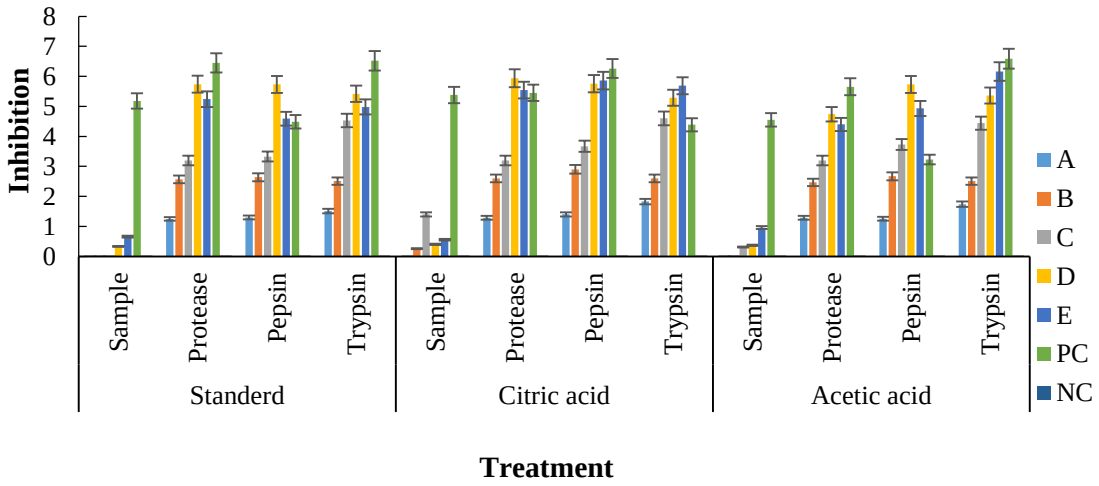

Figure 7: Antibacterial activity of Yellowfin tuna skin collagen hydrolysates hydrolysed with protease, trypsin, and pepsin enzymes at 37°C for 0 hours against common aerobic bacteria culture
A : 625 ppm, B : 1250 ppm, C : 5000 ppm, D : 10,000 ppm, E : 20,000 ppm, NC : Negative Control, PC : Positive Control

4. Conclusion

By using two different acid extractions, the Yellowfin tuna skin collagen was extracted successfully. It reveals that Yellowfin tuna by product waste could be utilized as better alternative for collagen extraction in industries. The hydrolysates produced from yellowfin tuna skin collagen with acetic acid extraction shows high radical scavenging activity than citric acid extraction. Citric acid extracted collagen hydrolysates shows high antioxidant activities against the lipid oxidation. Slightly high chelating activity were shows with both extractions. Both extracts with trypsin shows high antimicrobial activity and acetic acid extracted collagen hydrolysates showed more. So acetic acid extract collagen hydrolysate can be used as an effective radical scavenger, good metal chelater and can be consider as a good microbial inhibitor.

5. Acknowledgement

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