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Research Article

CHARACTERIZATION AND EVALUATION OF ANTIOXIDANT PROPERTIES OF SKIPJACK (*KATSUWONUS PELAMIS*) TUNA SKIN GELATIN HYDROLYSATE

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ABSTRACT

The present society is extremely conscious of the lifestyle-related ailments and the serious consequences that result from an unhealthy diet and lifestyle. A holistic approach to incorporating nutraceuticals into one's lifestyle has become the current trend. Protein hydrolysates with antioxidant potential have gained critical commercial importance. Protein hydrolysates are produced from food industrial remnants in order to reduce production costs and achieve sustainable resource management. The food industry generates a greater proportion of waste from fisheries, and with the consistent requisite for fish-based products, utilisation of remnants remains a critical concern. Fish hydrolysates have been discovered to have exceptional physical, chemical, and functional properties. It is noteworthy that fish hydrolysates have strong antioxidant properties against reactive oxygen species. In the current research, gelatin prepared from the processing waste of skipjack tuna (*Katsuwonus pelamis*) skin were hydrolysed by proteolytic enzyme papain. The obtained gelatin hydrolysate showed ~33% degree of hydrolysis (DH). The surface-active properties of Skipjack tuna skin gelatin hydrolysate (SSGH) revealed pronounced foaming and emulsion properties at low protein concentrations. SSGH demonstrated higher antioxidant efficacy by *in vitro* antioxidant assays. In conclusion, the resulting hydrolysates demonstrated remarkable antioxidant potential, as well as great development prospects for a variety of applications. Further studies with regard to isolation, purification and identification of and peptide sequences improve the quality of functional foods.

Keywords: Fish skin, Skipjack tuna, Gelatin, Papain, Gelatin protein hydrolysate, Antioxidant.

INTRODUCTION

Marine derived bio actives gain considerable importance due to the remarkable techno-functional and bioactive functionalities offered by them. Due to the diversity in the marine realm and the structural diversity of the compounds, bio actives derived from marine source remain unique in its functional attributes. Bioavailability, biodegradability and biocompatibility are the integral features concerned so far for the effective exploitation of marine derived bio-actives. These sorts of natural, environmental friendly bioactives have gained great developmental prospects for multitude of applications. Their technological and functional feature receives critical attention in the area of food, textiles, cosmetics, pharmaceutics and numerous others.

Mankind of today is critically conscious about the lifestyle associated ailments and the severe implications caused as a consequence of unhealthy diet and lifestyle. A holistic approach of involving nutraceutics in lifestyle has become the current trend today. Therefore, the market demand for nutraceutics has flourished since the past few decades. R and D in these lines are expanding exponentially world-wide. Bioactive peptides ascertaining antioxidant potential have gained critical significance on commercial aspects. Protein hydrolysates are known to contribute differential bio-functionalities (Peighambardoust *et al*., 2021). As an approach to minimise the production cost and to accomplish effective resource management, protein

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hydrolysates are generated from industrial remnants (Nazzaro *et al*., 2018).

In the food industry, larger fraction of waste generates from fisheries and as a result of the consistent increase requisite for fish-based products, exploitation of remnants remains as a critical concern (Ishak and Sarbon 2018). There exists no specific marginalization of fish by-products and are generally considered as remnants. The fish's skin remains a prominent, valuable and marketable collagen source exploited for employed to extract the gelatin. Gelatin is an integral, multifunctional ingredient used in the area of food. Gelatin derived from skin of fish is a potential repository of functional peptides with multitude of potential biological attributes achieved via enzymatic hydrolysis. Fish hydrolysates are found to possesses exemplary physical, chemical and functional attributes (Al-Nimry et *al*., 2021). Additionally, fish hydrolysates demonstrate pronounced antioxidant functionality against free radicals (Senevirathne and Kim, 2012). The enzymatically hydrolysed protein made from various fish protein sources, such as the round scad muscle (Jiang *et al*., 2014) Alaska pollack frame (Hou *et al*.,2011), yellowfin sole frame (Jun *et al*., 2004) and tuna cooking juice (Hung *et al*., 2014) have been shown to have antioxidant efficacy. In this regard, the current research approaches to prepare protein hydrolysates of antioxidant potential from fish skin processing wastes of skipjack tuna by enzymatic hydrolysis method. The resulting hydrolysate could be explored in the area of functional foods and pharmaceutics as a future line of research. The current approach is a sustainable approach against the waste management of fisheries resources of Cochin, Kerala, India.

MATERIALS AND METHODS

Sample collection

The sample, Skipjack tuna (*Katsuwonus pelamis*) skin waste was collected in frozen from NIFPHATT, Cochin. The resulting samples were preserved at −80 °C till the extraction achieves completion. The study employed analytical grade (AR) chemicals.

Production gelatin hydrolysate (SSGH)

Tuna skin was cut into $1cm^3$ pieces and the gelatin extraction was conducted based on the procedure of (Kumar *et al*., 2017) Slight modification were made to minimize the fat content. The thawed skin was washed with warm water (40 ± 2 ^{O}C) for 10 min for removing superfluous material and reducing the fat content. Proceeding to the extraction step, the fish skin was soaked in 0.75M NaCl for 10 min with the proportion of 1:6 skin (w)/solution (v) to eliminate unwanted proteinaceous constituents. The fish skin was subjected to thorough tape water washing thrice, after pre-treatment. The resulting skins sample were subjected to gentle stirring with skin to water ratio (weight/volume), 1:6 and exposing to 85°C for 60 min. The resulting skin sample after the heat treatment was subjected to filtration on employing Whatman No.1 filter paper. The gelatin solution's protein content was elucidated (AOAC, 1990). After adjusting the pH of gelatin solution to 6.5 employing 1M NaOH and 1M HCl the resulting solution was subjected to incubation at 60 ºC in water bath. Further, papain was supplemented to the resulting solution of gelatin at concentration of 1% of total protein. To terminate the hydrolysis, after 90 min, temperature elevated to 95ºC and maintained for duration of 10 min. The hydrolysate was subjected to filtration and the resulting solution was freeze-dried the samples were preserved at -20 ºC

Evaluation of the degree of hydrolysis (DH)

The measurement ofDH was carried out following the protocol of (Spellman *et al*., 2003). Hydrolysate (10 µl) was mixed with OPA reagent (3.4 ml), and the solution was permitted to rest at 25^{o} C for duration of 2 min. The DH was determined by measuring the optical density of the hydrolysate at 340 nm as follows:

$$DH\ (\%) = \frac{A \times 1934 \times d}{c}$$

Where, A-optical density of SSGH, d - dilution factor and c -protein content of SSGH (gL^{-1}).

Ultraviolet–visible (UV-Vis) spectroscopy

The UV-VIS absorption of tuna skin gelatin hydrolysis was monitored from 240 - 650 nm with the aid of a spectrophotometer (Shimadzu, UV-1800, Japan). UV-Vis absorption spectra were obtained using a graph of absorbance versus wavelength.

FTIR

Polyelectrolyte interaction and the chemical structure of the tuna skin gelatin hydrolysate were characterized with the aid of FT-IR Nicolet iS50 spectrometer in the 4000-500 cm^{-1} frequency range at four resolutions. The sample analysis was employed with this instrument to elucidate various functional groups (variable factors).

Foaming properties

The surface-active attribute involving foaming properties of SSGH at protein differential concentration (0.1, 0.5 and 1%) were evaluated on adopting the protocol of (Sathe and Salunkhe, 1981). The empirical formulae depicted herein are used to calculate foaming properties involving foaming capacity (FC) and stability (FS) respectively:

$$FC = \frac{\text{Whipped volume} - \text{Initial volume}}{\text{Initial volume}} \times 100$$

The samples subjected for whipping permitted to rest at 20 ˚C for duration of 30 min duration and the whipped sample volume was documented. The calculation of foam stability was done following the eq:

$$FS = \frac{\text{volume after standing} - \text{Initial volume}}{\text{Initial volume}} \text{x } 100$$

Emulsion properties

The determination of the emulsion propertiesof SSGH at protein different concentration (0.1, 0.5 and 1%) were conducted according to (Pearce and Kinsella, 1978). A double beam spectrophotometer was employed to determine the optical density at 500 nm (A500) of hydrolysate. The absorbance was recorded at 0^{th} min (A_0) and 10^{th} min (A_{10}) of emulsion formation. The emulsion activity index (EAI) and emulsion stability index (ESI) were calculated on the basis of the acquired reading

$$EAI\ (m2/g) = \frac{2 \text{ x } 2.303 \text{ x } A500}{0.25 \text{ x sample weight } (g)}$$

$$ESI\ (min) = \frac{Ao \text{ x } \Delta t}{\Delta A}$$

Where, $\Delta A = A_0 - A_{10}$ and $\Delta t = 10$ min

Determination of Anti-oxidant activity

DPPH scavenging activity

Protocol explained by Yen and Wu, 1999 were adopted to elucidate the DPPH functionality of SSGH. A double beam spectrophotometer was employed to measure the absorption at 517nm. Lower the absorbance kinetics of chemical mixture indicates DPPH scavenging activity at higher magnitude. The activity was measured by as follows:

$$\%\ \text{scavenging activity} = 1 - \frac{\text{Sample absorbance}}{\text{Control absorbance}} \text{ x } 100$$

FRAP

Determination of the ferric reducing power was carried out on following the protocol of (Oyaizu, 1986). The optical density of the resultant sample was monitored at 700 nm employing spectrophotometer. Higher reducing power was ascertained for the reaction mixture on the basis of higher absorbance evaluated.

Statistical analysis

The experimental results shown are the averages of triplicates (n = 3), and they were each executed separately. The data were represented as mean ± standard deviation.

RESULTS AND DISCUSSION

DH is a quantitative assay that indicates the extent to which the polypeptide chain has been hydrolysed by breaking the peptide bonds. Proteolytic cleave of native polypeptide chain of skipjack tuna skin gelatin *via* papain resulted in 32.51 ± 1.28 % of hydrolysis. The DH affects the hydrolysates' technological, functional, and biological characteristics (Islam *et al*., 2022). The absorption spectra of the resulting sample employing UV spectrophotometer were recorded in the range of wavelength 200 – 700 nm (Figure 1). The distinctive peptide bond fragments of the gelatin backbone were detected in the absorption profile of chromophore groups at 210–240 nm (Hermanto and Fatimah, 2013).

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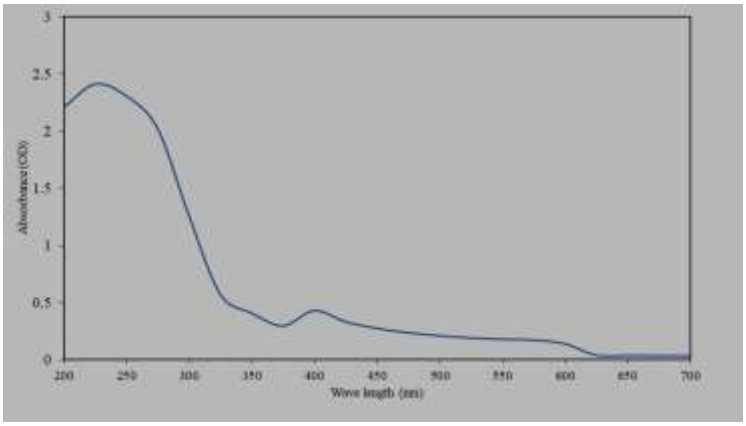

Figure 1. UV-Vis spectra of Skipjack tuna skin gelatin hydrolysate (SSGH).

For the FT-IR analysis, Peaks at different amide regions (4000 to 500 cm^{-1}) were demonstrated for skipjack tuna skin gelatin hydrolysate and prominent amide depicted as A, B, I, II and III bands were seen (Figure 2). The associated reason and wavenumber of these bands were tabulated in Table 1.

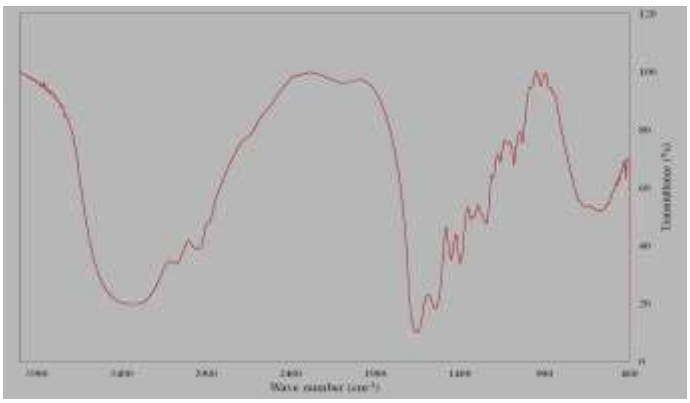

Figure 2. FTIR spectra of Skipjack tuna skin gelatin hydrolysate (SSGH).

Table 1. Inferences of FTIR bands in Skipjack tuna skin gelatin hydrolysate (SSGH) and associated reasons.

Bands	Wavenumber (cm^{-1})	Reason
Amide I	1656.09	C = O stretching vibration of the carboxylic moiety coupled bending vibrations of N-H of the NH moiety of the gelatin polypeptide backbone Nagarajan *et al*.,2012
Amide II	1245.38	Stretching vibrations of C-N moieties in primary amides Guillén *et al*.,2010
Amide III	566.76	Out-of-plane CO bending (Kong and Yu, 2007)
Amide A	3338.62	Stretching vibrations of the N-H group Guillén *et al*.,2010
Amide B	3075.81	Asymmetric stretch vibration of =C-H and $-NH^{3+}$ Nagarajan *et al*.,2012

The EAI and ESI of SSGH measured with respect to protein content is shown in figure 3. At a protein concentration of 0.1%, higher EAI was recorded and at 1 % protein content higher ESI was recorded. There remains substantial change in EAI values for different protein concentration of FGP samples. This specifies that FGP at 1 % had higher EAI than other concentrations. EAI measures how well a protein contributes to the production and stabilisation of a newly created emulsion by apportioning units of stabilised interface area per unit protein weight (Giménez *et al*., 2009). Similar pattern has been shown for round scad protein hydrolysates (Thiansilakul *et al*., 2007), and it might be caused by increased concentrations of protein-protein interactions, which over time lead to a decline in protein concentration at the oil-water interface. The prominent factors that influence the emulsion potential comprises of solubility, amino acid sequence and molecular size whilst, other factors are degree of hydrolysis (DH), enzyme type employed, the extraction solvent and environmental pH (Halim *et al*., 2016). The FGP has a positive correlation with regard to surface activity and concentration of protein.

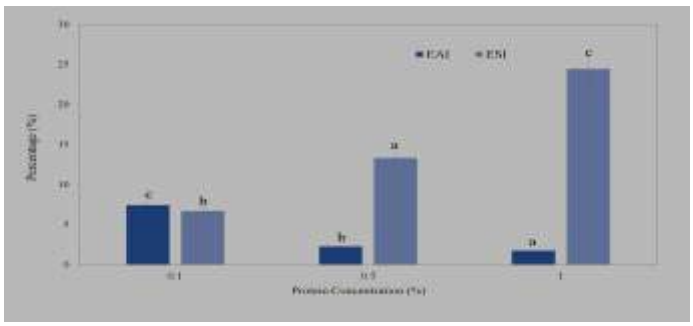

Figure 3. Emulsifying properties of Skipjack tuna skin gelatin hydrolysate (EAI-Emulsion activity index, ESI-Emulsion stability index). The bars with different letters are significantly different from each other at $P \leq 0.05$ according to Duncan's Multiple Range test.

The FC and FS at different protein concentrations of skipjack tuna skin gelatin protein hydrolysate are depicted in Figure 4. The higher foaming capacity was observed in 1 % concentration of FGP. The optimum protein concentration to reach higher stability of hydrolysates was 1% when compared to 0.5 and 0.1%. The findings showed that the concentration of hydrolysates influences both foaming capability and foaming stability. For the formation of foam, the capacity of protein to absorb at the air-water interface at rapid phase by reducing the interfacial tension is critical. Food foams constitute dispersion of air droplets enveloped by surfactants thereby lowering the interfacial tension (Kinsella and Melachouris, 1976). This effect is ascertained by protein or protein hydrolysates as a result of their amphiphilic characteristic. The FS relays on the type of proteins or peptides restricting the collapse of liquid and air. In the study of (Zhu and Damodaran, 1994), increased foaming capacity and reduced foaming stability in whey proteins are being reported. This may be due to the incorporation of air into the solution of smaller peptides, on acquiring weaker strength to form foams. There exists a positive correlation between the DH and foaming properties. It is demonstrated that peptides possessing low molecular weight were incapable of maintaining a well-ordered molecular interface orientation that results to weak foaming properties (Kristinsson and Rasco, 2000). Other factors like penetration, transportation and molecular reconfiguration at the air-water interface regulates the (Halim *et al*., 2016).

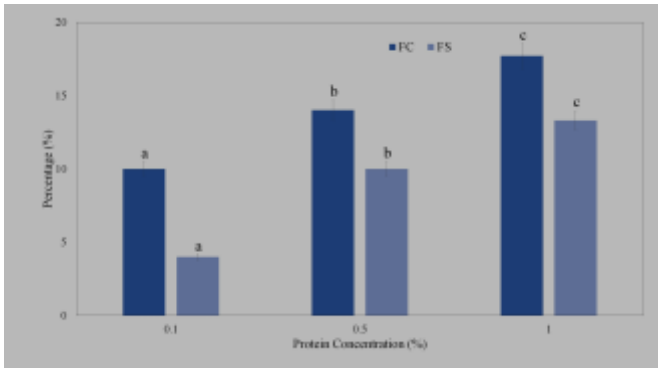

Figure 4. Foaming properties of Skipjack tuna skin gelatin hydrolysate (FC-Foaming capacity, FS-Foaming stability). The bars with different letters are significantly different from each other at P ≤ 0.05 according to Duncan's Multiple Range test.

The DPPH radical scavenging potential of SSGH as a factor of concentration has been evaluated. The free radical scavenging potential of bioactive is well known with regard to reactive oxygen species scavenging potential Harnedy and FitzGerald (2012). The IC50 value of FGP is recorded as 9.8302 mg/mL as depicted in Figure. 5. Previous studies reported IC50 values of 13.66 mg/ml in skin gelatin protein hydrolysates of tilapia (Zhang *et al*., 2012) and 11.98 mg/ml in thornback ray (Lassoued *et al*., 2015) which considerably higher when compared to present study results. This indicated the remarkable scavenging potential of free radicles by the FGP. The current study shows that FGP has the ability to scavenge DPPH radicals at higher concentrations.

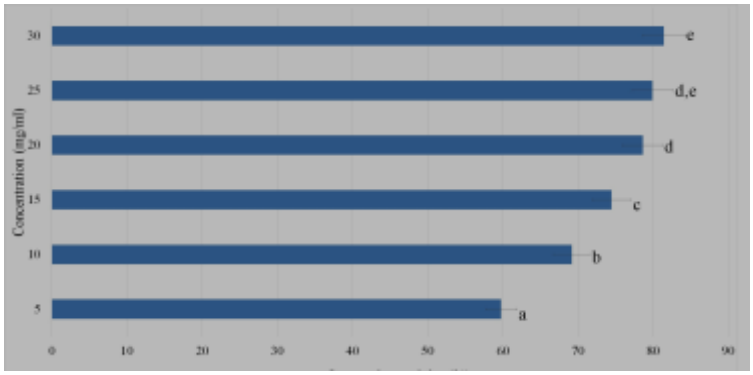

Figure 5. DPPH Scavenging activity of Skipjack tuna skin gelatin hydrolysate. The bars with different letters are significantly different from each other at P ≤ 0.05 according to Duncan's Multiple Range test.

The Figure 6 depicts the FRAP of different concentrations of skipjack tuna skin gelatin protein hydrolysate. The results indicated that FGP possessed increased reducing potential in a concentration dependant manner. It is demonstrated that samples possessing higher reducing power is observed to have better potential to donate electrons. Works have already demonstrated the correlation on antioxidant property and reducing power. FRAP is typically used to quantify a molecule that reduces Fe3+complex to Fe2+complex (Binsan *et al*., 2008).

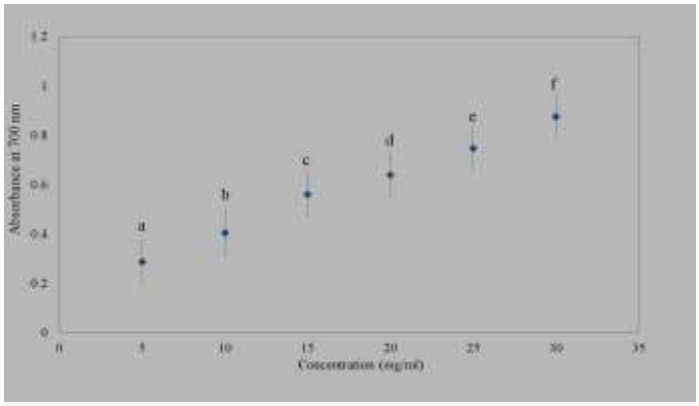

Figure 6. Ferric reducing activity of Skipjack tuna skin gelatin hydrolysate. The dots with different letters are significantly different from each other at $P \leq 0.05$ according to Duncan's Multiple Range test.

CONCLUSION

The current study approached to derive potential bioactive peptides from the skipjack tuna skin gelatin. The resulting peptide demonstrated remarkable surface-active properties. At low concentrations, the hydrolysates demonstrated better foaming and emulsion properties. The stability of the fish gelatin hydrolysate emulsions and foams was also remarkable. The resulting hydrolysates demonstrated to pose remarkable antioxidant potential, pose great developmental prospects for varied applications. Further studies are required on regard to the purification and characterization.

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