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Evaluation of the Physicochemical and Techno-functional Properties of Gelatin Extracted from Fish Processing Waste

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ABSTRACT

The current research was completed at the Indian Council of Agricultural Research-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India from August 2021 to August 2022 as a modified thermal extraction protocol for deriving gelatin from processing waste of skipjack tuna (*Katsuwonus pelamis*) skin. The study evaluated the effect of chemical composition and molecular weight contributing to the techno-functional attributes of the resulting gelatin. In the present study, we followed warm water-coupled NaCl pre-treatment to reduce the lipid content of the extracted gelatin. The extracted gelatin was characterized for Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The amount of gelatin extracted from the fish skin was 9.30±0.22%. The protein pattern of extracted gelatin on SDS-PAGE was identical to commercial grade gelatin, which implies the purity of extracted gelatin is comparable to that of commercial one. The physicochemical and functional properties were evaluated and pose remarkable techno-functional properties. Therefore, fish processing skin waste of skipjack tuna could be effectively exploited for the preparation of commercial gelatin. The extraction protocol remains environment friendly and this approach remains optimal for food technological applications. The effective exploration contributed to nutritional benefits, technological, functional, and biological functionalities for a large fraction of applications in the area of food technology, nutraceuticals, pharmaceutics, and numerous others as a future line of research.

KEYWORDS: Fish processing waste, physicochemical properties, pre-treatment, thermal extraction

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1. INTRODUCTION

Gelatin is a hydrophilic, thermo-reversible biopolymer derived by the hydrolytic degradation of collagen; a high molecular weight functional protein of the animal kingdom. For several years, the trade value of gelatin has attained a considerable increase due to its superior functional and technological properties offered by them. This functional biopolymer is having potential use in the area of food, pharmaceutical, cosmetic, and photographic applications (Al-Nimry et al., 2021). For food technological applications, it is widely used due to its remarkable properties including biodegradation, biocompatibility, non-toxicity, and non-immunogenicity (Dias et al., 2017, Said et al., 2021). The techno-functional attributes of gelatin are greatly affected by its source and type (Casanova et al., 2020). The mammalian source including the skin and bone from porcine and bovine are usually exploited for producing commercial gelatin (Usman et al., 2022) and they are generally ideal due to their viscosity, gel strength, and remarkable melting point (Yang et al., 2022). Presently, as a result of the Mad cow disease outbreak, certain sociocultural and some religious constraints, the mammalian source of gelatin production has received a negative impact (Ahmed et al., 2020). Therefore, gelatin derived from marine sources has received considerable demand as an alternative to mammalian counterpart.

It is reported that fish processing residues after processing remain at 36% of the total weight where a larger fraction contributes to bones and skins with a good number of proteins (Gómez-Guillen et al., 2002, Wang et al., 2019, Coppola et al., 2021). The principal benefit of exploiting fish processing waste for the extraction of gelatin relies on the bio-availability and utilization of processing waste (Mohanty et al., 2018, Huang et al., 2019, Masilan et al., 2021, Ikbal et al., 2022, Usman et al., 2022). The extraction protocol remains very significant in achieving the final quality of gelatin (Al-Hassan, 2020). Various methods are in existence for gelatin from different sources of raw material. The extraction methods are relayed on the removal of non-collagenous protein. This is achieved on differential treatment with alkali solution or neutral salt solution or enzymes (Kołodziejska et al., 2008, Shyni et al., 2014, Binsi et al., 2017, Kumar et al., 2017, Derkach et al., 2019, Moosavi-Nasab et al., 2020, Zhang et al., 2020, Talib et al., 2022, Boughriba et al., 2023, Derkach et al., 2022). For achieving less cross-linked collagens, acid treatment remains optimal whilst alkaline treatment is ideal for ascertaining more complex cross-linked collagens. These treatments are too laborious and are not environmentally friendly. Therefore, an alternative extraction method is required for the facile extraction of bioactives. Several studies have reported pre-treatments and enzyme-assisted extraction which is found to demonstrate superior yield and techno-functional attributes.

The physicochemical properties of gelatin assure quality and marketable value. The proximal composition of gelatin chiefly relays on the efficiency of extraction (Uriarte-Montoya et al., 2011) and determines the edibility of the product (Anonymous, 2000). The gelatin color is a significant commercial parameter, which is influenced by factors like extraction conditions, fish type, and raw material (Boughriba et al., 2023). Additionally, molecular weight is an important characteristic that affects the attributes of extracted gelatin (Abedinia et al., 2020). The molecular weight chiefly depends on the extraction method followed (Eysturskarð et al., 2009). Therefore, the current study employs hot water-NaCl pre-treated thermal extraction as a modified method to derive gelatine from processing waste of skipjack tuna skin to evaluate the effect of chemical composition and molecular weight contributing to the techno-functional attributes of the resulting gelatin. This approach remains optimal for food technological applications as the extraction protocol remains environment-friendly.

2. MATERIALS AND METHODS

The current research work was carried out from August 2021 to August 2022 at the Indian Council of Agricultural Research-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India. The sample, Skipjack tuna (*Katsuwonus pelamis*) skin waste was obtained from NIFPHATT (National Institute of Fisheries Post Harvest Technology and Training) Kochi, Kerala, India, in a frozen state. The resulting samples were preserved at -80 °C till the extraction. The reagents employed in the entire approach remained exclusively of analytical grade (AR).

2.1. Gelatin extraction protocol

Tuna skin was cut into 1 cm^3 pieces and the gelatin was extracted as per the method of Kumar et al. (2017) with slight modification. The thawed skin was washed with warm water (40±2°C) for 10 m for removing superfluous material and reducing the fat content. Before the extraction step, the skin was soaked in 0.75M NaCl at room temperature with a 1:6 skin/solution (w/v) ratio for 10 m to remove the non-collagenous protein matter. After pre-treatment, the skin was thoroughly washed in water thrice. The washed skins were gently stirred with a 1:6 skin-to-water ratio (w/v) and kept in n water bath at 85°C for 60 m. After the heat treatment, skin residues were removed by filtering with Whatman No.1 filter paper. The freeze-dried filtrate was kept under desiccated condition until further analysis.

The gelatin yield was calculated as the ratio of gelatin

freeze-dried to wet skin weight of the sample employed for extraction (Equation 1).

Yield (%)=Freeze dried gelatin weight/Wet weight of skin employed for extraction×100 .....(1)

2.2. Chemical composition

The proximal composition of fresh fish skin (FS) and extracted fish gelatin (FG) were estimated (Anonymous, 2012). Calculation of crude protein was done using a nitrogen-protein conversion factor of 6.25.

2.3. pH and isoelectric point

The pH of fish skin gelatin (1%, w/v) was measured using a pH meter (EUTECH instruments, India). The pH of gelatin (2%, w/v) was adjusted to the consecutive sequence: 3, 5, 7, 9, and 11 using NaOH and HCl (1M). The zeta potential of filtered gelatin solution with different pH was analyzed in Zetasizer Nano ZS. The isoelectric point (pI) was determined from a pH rendering a zero potential at relative humidity (25±2°C).

2.4. Microstructure analysis

The surface morphology of the extracted gelatin was compared with commercial gelatin using SEM (Jeol, JSM 6390LA, Japan) with a voltage of 20KV.

2.5. Fourier transform infrared (FTIR) spectroscopy

The FT-IR spectra of fish skin gelatin were monitored using an FT-IR spectrometer (Nicolet iS50). The sample was analyzed for the existence of differential functional groups located specifically in the area of 4000–5000 cm^{-1} with 4 cm^{-1} resolution.

2.6. Protein patterns

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) is used to determine the pattern of protein samples according to Laemmli (1970). The samples were prepared by adding 1% gelatin and 5% SDS (1:9 v/v) and heated in a boiling water bath for 3m. The resultant mixture and Laemmli sample buffer (Bio-Rad Lab, Inc., USA) were mixed (1:1, v/v) and heated for 3 min in a boiling water bath. 15 µL of resultant sample solution were injected with Invitrogen pre-casted Tris-Glycine gel (Thermo Fisher Scientific, Inc., USA). Novex Tris-glycine SDS running buffer (Thermo Fisher Scientific, Inc., USA) was used as the buffer solution. GelCodeBlue safe protein stain (Thermo Fisher Scientific, Inc., USA) was used to stain the loaded gel. Protein bands were identified using PageRuler™ Plus Prestained Protein Ladder (Thermo Fisher Scientific, Inc., USA).

2.7. Colour determination

The colour of the extracted fish gelatin was determined by employing Hunter lab Miniscan XE plus colorimeter. The measurement scale used to measure the color is as follows: Hunter L^* (lightness), a^* (redness), b^* (yellowness). For reference, a standard white plate was used with L^* (lightness) 93.99, a^* (redness) -0.82, and b^* (yellowness) 2.22 colour scale.

2.8. Turbidity and viscosity

A 6.67% gelatin (w/v) was used to determine turbidity and viscosity. Turbidity was estimated by adopting the method of Fernández-Díaz et al. (2001). Viscosity was measured by employing a Rheometer (Brookfield INC, Middleboro, USA) and the viscosity is expressed as m.Pas.

2.9. Foaming properties

The foaming capacity and foam stability were estimated as per the method of Sathe and Salunkhe (1981). The foaming capacity was calculated according to Equation 2:

Foaming capacity=(volume after whipping-volume before whipping)/volume before whipping×100

The whipped sample was exposed at 20 °C for 30 min undisturbed and volume was measured. Foam stability was measured according to Equation 3:

Foaming stability=(volume after standing-volume before whipping)/volume before whipping×100

2.10. Emulsion properties

The emulsion activity index (EAI) and emulsion stability index (ESI) were recorded in the study. The emulsion properties were analyzed as per equations 4, 5, and 6 (Pearce and Kinsella, 1978). A double-beam spectrophotometer was employed to record the absorbance of the solution at 500 nm.

$EAI=2T\,\Phi C$ .....(4)

Where, T - turbidity ($T=2.303A_{500}l^{-1}$; A_{500} is the absorbance at 500 nm; l is path length); Φ - oil volume fraction (0.25); C - protein concentration.

EAI (m^2/g)=2×2.303×A500/0.25×gelatin weight (g) ….(5)

ESI (min)=Ao×Δt/ΔA ….(6)

Where, ΔA=Absorbance at 0 m (A_0)-Absorbance after 10 m (A_{10}) and Δt=10 m

2.11. Fat binding capacity (FBC)

Fat binding capacity was carried out by the method of Cho et al. (2004). FBC was expressed as the percentage weight of dried gelatin and determined by Equation 7

FBC (%)=Weight of gelatin after draining oil/weight of dried gelatin×100

2.12. Water holding capacity (WHC)

Water Holding Capacity was determined according to Diniz and Martin (1997). WHC was expressed as the quantity of water (in ml) absorbed for 1 g gelatin and it

was estimated by the equation:

WHC (mg ml^{-1})=(Volume of water added intially-Volume of supernatant)/Weight of gelatin (g) ...(8)

2.13. Statistical analysis

The analysis was carried out in triplicates and the results were expressed as mean±standard deviation.

3. RESULTS AND DISCUSSION

3.1. Extraction yield

The percentage of extractable gelatin from the fish skin was found to be 9.30±0.22%. The extraction yield of gelatin from skins of Bigeye tuna (*Thunnus obesus*) and Croaker fish (*Johnius* sps.) were 8.42±0.32 and 7.15±0.19%, respectively (Dara et al., 2020, Kumar et al., 2017). According to Jamilah and Harvinder (2002) factors like characteristics of skin and collagen molecules, presence of varying soluble components in raw material, and reduction of extract content by leaching or partial hydrolysis are the factors that contribute to the variation in extraction yield of gelatin from different species. Extraction parameters like temperature, time, pH, pre-treatment process, and the storage condition of raw material influenced the hydrolysis of collagen to form gelatin (Karim and Bhat, 2009). The presence of non-collagenous protein in the skin would also influence the gelatin yield (Tumerkan et al., 2019). Kumar et al. (2017) stated that the temperature, ratio of extraction media to the skin, and duration of treatment influence the yield of extraction. It was found that 60 m heat treatment at 85°C with a 1:6 ratio of skin: water was ideal for maximum yield of gelatin.

3.2. Chemical composition

Chemical compositions of the fish skin (FS) and gelatin (FG) in percentage are summarised in Table 1A. The composition of freeze-dried gelatin is expressed as a dry weight basis. Fish skin contained moisture as the major component (62.05±1.68%) whereas fish gelatin had protein as the foremost element (91.75±4.04%). The crude fat content of fish skin is 6.60±0.20%. However, fish gelatin had 0.30±0.02% crude fat. Alkali/warm water pre-treated gelatin extraction from skipjack tuna skin showed a 95.52% and 98.15% reduction in fat content in the studies of Shyni et al. (2014) and Tumerkan et al. (2019), respectively. According to Montero and Acosta (2020) acid/warm water treated method of gelatin extraction from *Thunnus albacares* showed a 98.34% reduction in lipid content. Whereas NaCl without warm water pre-treatment gelatin extraction from *T. albacares* demonstrated a 39% reduction in fat content (Dara et al., 2020). Thus NaCl/warm water treatment employed in the current study reduced the lipid content by about 95.46%. The ash content of fish skin is 0.34±0.02% which was reduced to 0.07±0.01% in fish gelatin. The diminished ash content in FG indicates the effectual elimination of fish skin minerals and the quality of extracted gelatin (Uriarte-Montoya et al., 2011). A reduced amount of moisture (<6%) makes fish gelatin as per the recommended limit for food-grade gelatin (Anonymous, 2000).

Table 1: Physico-chemical properties of Skipjack tuna skin gelatin. (FS: Fish skin, FG: Fish gelatin)

A: Proximate composition (%)				
	Crude protein	Moisture	Fat	Ash
FS	26.26±3.19	62.05±1.68	6.6±0.20	3.34±0.02
FG	91.75±4.04	4.38±0.19	0.3±0.02	0.07±0.01
B: Physiochemical properties				
	pH	Turbidity (m.Pas)	Viscosity	
FG	6.63±0.02	1.08±0.01	11.08±0.21	
C: Colour analysis				
	L^*	a^*	b^*	
FG	78.64±0.11	0.85±0.02	11.42±0.17	
D: Interfacial properties (ml g^{-1})				
	Water holding	Fat binding		
FG	0.67±1.15	148.29±3.77		

3.3. pH and isoelectric point

The tuna skin gelatin (1%) has a pH of 6.63±0.03 (Table 1B). The pH was found to be influenced by the pre-treatment employed in the extraction process. Usually, gelatin extraction pre-treated with acid results in Type A gelatin, and alkali pre-treated extraction results in Type B gelatin. Baziwane and He (2003) reported pH values in the range of 3.8–5.5 and 5–7.5 for Type A and Type B gelatin respectively. Therefore, the gelatin derived from the skin of Skipjack tuna is Type B since the pH is 6.63.

The extracted gelatin is type B. The iso-electric point of fish skin gelatin is estimated as 5.75 (Figure 1). At acidic pH, gelatin shows a positive charge and at alkaline pH, gelatin shows a negative charge. The treatment condition adopted is an important aspect about the isoelectric point. For acid-processed gelatin, the isoelectric point remains in the pH range of 6.0–9.5, for alkali-processed ones, the isoelectric point is between 4.8 and 5.2 (Eastoe and Leach, 1977).

3.4. Microstructure analysis

The micro-texture appears to be a mesh of irregular fibrils with heterogeneous voids (Figure 2). The differential gelation characteristics could be a reason to ascertain the dense strands which may be formed as a result of protein orientation and aggregation (Jongjareonrak et al., 2006).

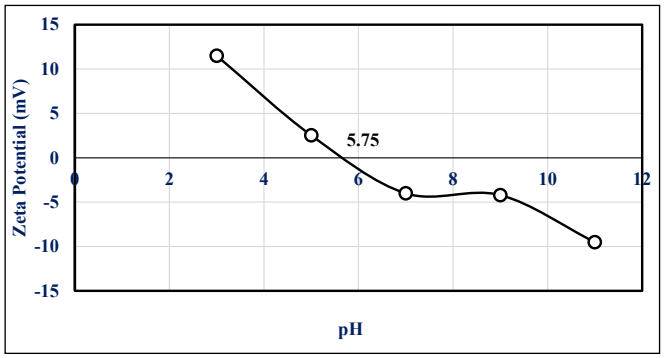

Figure 1: Zeta potential of Skipjack tuna skin gelatin

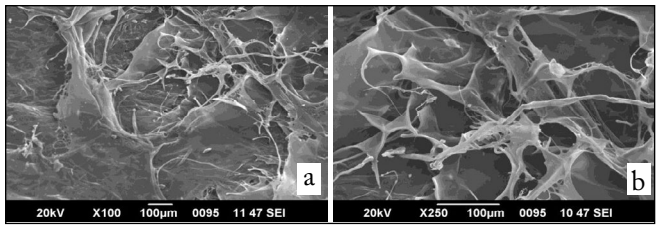

Figure 2: Microstructure of Skipjack tuna skin gelatin at (a) ×100 magnification (b) ×250 magnification

The physical characteristic such as gel strength values is influenced by the assembling of the denser strand in the protein microtextured matrix. The existence of larger voids in the matrix results as a consequence of the higher temperature-mediated extraction protocol. This observation is a clear sign of the existence of water in the observed matrix in a steady form. The irregular fibrillar structural orientation of the matrix is observed as a consequence of the existence of hydrogen, hydrophobic and ionic interactions among themselves (Nomura et al., 2000).

3.5. Fourier transform infrared (FTIR) spectroscopy

FTIR pattern of tuna skin gelatin (Figure 3) reveals the characterization of variable factors and gelatin secondary structure. The higher absorption in the peptide region implies the proteinaceous nature of extracted fish skin gelatin. The peptide I band was recorded at wavenumbers 1630.62 cm^{-1}. From the FTIR spectrum, it is observed that peptide I bands are formed in association with carbon and oxygen (C=O) stretching vibration of the carboxylic moiety coupled bending vibrations of nitrogen and hydrogen (N-H) of the NH moiety of the gelatin polypeptide backbone and C-N (single bond) stretching of the covalent bond were recorded in particular ascertained due to stearic non hindrance (Payne and Veis, 1988). The peptide I band indicates the higher interaction of C=O (area of ester linkage) with the adjacent polypeptide chains. This was attributed to the expulsion of telopeptide from the polypeptide backbone. The coil structure of gelatin is characterized about the peak at the peptide 1 region of the spectrum (Nagarajan et al., 2012). Determining the secondary protein structure for infrared

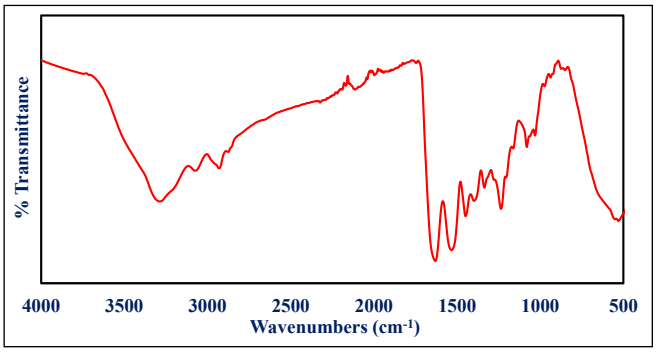

Figure 3: FTIR spectra of skipjack tuna skin gelatin

spectroscopic analysis of absorption at the peptide I region is relevant (Kittiphattanabawon et al., 2016). Peptide II region was recorded at wavenumber 1533.62 cm^{-1}. The peptide II band generates as a result of the bending vibration of N-H moieties and stretching vibrations of C-N moieties (Nagarajan et al., 2012). The detection of peptide III bands was recorded near to the wavenumber of 1235.33 cm^{-1} for the fish skin gelatin. The combination peaks formed between C-N stretching vibrations and N-H deformation of the polypeptide moieties are represented by the peptide III bands (Jackson and Mantsch, 1995). During the denaturation of collagen to gelatin molecular disorganization exist as a result of α-helix to a random coil structure. This indicated the greater change of molecular structure due to the transformation of the helix to random coil transition to align the secondary structure (loss of triple helix state). This transformation occurred during extraction employed at higher temperatures (Muyonga et al., 2004). Amide A band is generated as a consequence of the stretching vibrations of the N-H group, identified at 3287.32 cm^{-1}. The hydrogen bonds are formed as a result of free N-H stretching vibrations (3400–3440 cm^{-1}) of the amide A band (Sinthusamran et al., 2014). The N-H group of the peptide involved in a hydrogen bond has been observed to shift the position to a lower frequency. During gelatin extraction, hydrogen bond stabilizing collagen structures are found to be disintegrated (Kittiphattanabawon et al., 2016). The amide B band for the sample corresponding to wavenumbers is depicted as 3074.72 cm^{-1}, by asymmetric stretch vibration of=C-H and $-NH^{3+}$ (Nagarajan et al., 2012).

3.6. Protein patterns

The pattern of proteins and molecular weight of extracted Skipjack tuna fish skin gelatin (FG) and commercial porcine gelatin (PG) were determined by SDS-PAGE and portrayed in Figure 4. The protein pattern of PG and FG are identical with β, α_1, and α_2 bands, which reflects the purity of extracted gelatin comparable to that of commercial one. The α_1 and α_2 chains correspond to the prominent band depicted in the molecular weight region of 130 and 120

kDa. The deprivation of indigenous collagen through the gelatin extraction results in α and β chain components in the protein pattern. The β component of collagen is represented by a faint band that corresponds to a molecular weight range of 200 kDa, more or less similar to the report of Gomez-Guillen et al. (2002). Slow-slung bands are deprived in PG and FG protein patterns, specifying the effective purging of non-collagenous peptides during gelatin extraction. The comparative contents of band components, high-weight protein masses, and lower-mass peptide fragments reflect the physical properties of gelatin.

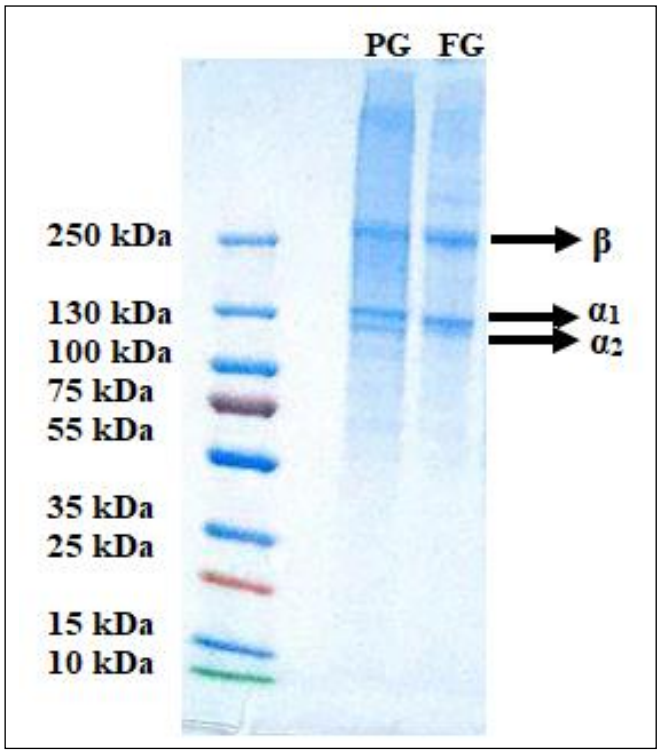

Figure 4: SDS-PAGE Skipjack tuna fish gelatin (FG) and porcine gelatin (PG)

3.7. Colour determination

The colour values of fish skin gelatin as depicted in Table 1C. The method of extraction and resource type influences the gelatin colour (Ockerman and Hansen, 1999). Even though the colour is not a functional character; it has importance in the food industry. The negative a* values of gelatin samples indicate a greenish tint and positive b* values indicate the yellowness of gelatin samples. The L* values shown by fish gelatin were considerably lower for reported gelatin derived from the black tilapia skins, sin croaker, red tilapia, and shortfin scad, whose L* values from ranged 89.3 to 93.3 (Cheow et al., 2007, Jamilah and Harvinder, 2002). The inherent skin pigments may be co-extracted with gelatin and eventually affect the colour of gelatin (Nagarajan et al., 2012).

3.8. Turbidity and viscosity

Turbidity has substantial importance when gelatin is used as a thickening agent. Turbidity is influenced by the effectiveness of filtration and pH during gelatin extraction (Muyong et al., 2004). Table 1B depicts the turbidity of fish gelatin (6.67%, w/v) obtained as 1.08±0.01. The temperature of gelatin extraction is positively correlated to protein clumping, it also reflects turbidity (Kittiphattanabawon et al., 2016). The retention or contamination of inorganic, proteinaceous, many substances, and unfiltered particulate matter during gelatin extraction may also influence the turbidity of the product (Eastoe and Leach, 1977).

Viscosity is the measure of resistance to flow. Table 1B shows the viscosity of fish gelatin (6.67%, w/v), and the value is 11.08±0.21. The viscosity of the testified gelatin where in the range of 20–70 Cp (Johnston-Banks, 1990). The parameters like the molecular weight of gelatin peptides, concentration and polydispersity of gelatin, temperature, and pH of extraction, and aging of solution will potentially alter the viscosity of gelatin.

3.9. Foaming properties

Foam expansion (FE) and foam stability (FS) of extracted fish skin gelatin at different protein concentrations (0.1, 0.5, and 1%) are shown in Figure 5. FE and FS of both gelatin samples depicted a positive correlation to the concentration of gelatin. The foaming capacity of fish skin gelatin at 1% concentration was higher than 0.1 and 0.5 %. At the newly-created air-liquid interface as a result of bubbling, proteins undertake rapid absorption, and those ascertaining structural re-organization, demonstrate remarkable foaming potential in comparison to proteins possessing slow absorption potential and acquire resistance to unfolding at the interface (Damodaran, 2017). The foaming potential of a protein solution generally possesses a positive correlation to the molecular weight of peptides (Jellouili et al., 2011).

Foam stability was demonstrated considerably lower in 0.1% gelatin in comparison to 0.5 and 1% gelatin. Foams

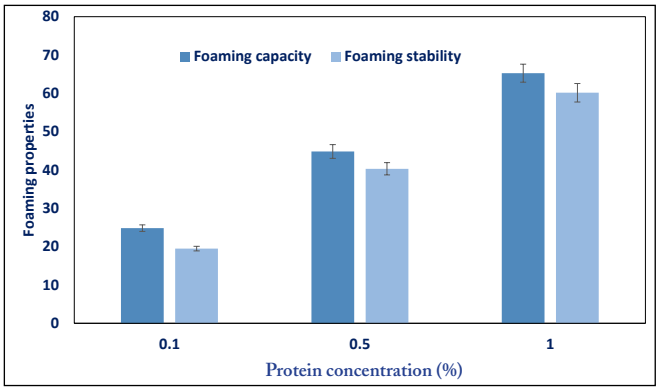

Figure 5: Foaming properties: Foaming capacity and foaming stability of Skipjack tuna skin

with higher concentrations of proteins appeared denser and more stable as a result of interfacial film thickening (Zayas, 1997). Additionally, the foaming stability relay on the orientation of the film and represent the extent of protein-protein interaction within the matrix (Koli et al., 2012). The foam stability relies on different factors like bulk and surface viscosities, electrical repulsion between the layers of the foam, the rate of attaining equilibrium surface tension, steric stabilization, etc (Liu et al., 2003).

3.10. Emulsion properties

Emulsion property has an important role as they are integral, particularly in emulsified products, where gelatin is widely employed. In Figure 6, the emulsion activity index (EAI) and emulsion stability index (ESI) of fish skin gelatin at different protein concentrations (0.1, 0.5, and 1%) are depicted. Emulsifiers are potential surface-active constituents that adsorb to interfaces and enable the formulation of minute droplets by decreasing the interfacial tension upon homogenization (Walstra, 2003). The EAI was evaluated concerning the turbidity of the emulsion recorded at a wavelength of 500 nm, estimating the potential of the protein to assist the orientation and stabilization of the newly formed emulsion by permitting units of the area of the interface. The emulsifying capacity of gelatin was found to be inversely proportional to the concentration of gelatin. The EC at a high value estimated at a lower concentration may be ascertained due to a higher degree of polypeptide unfolding as a result of the shearing involved in the process of emulsification (Kinsella and Melachouris, 1976).

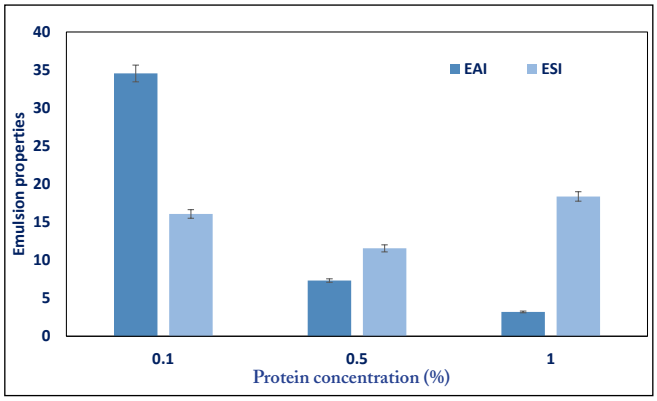

Figure 6: Emulsion properties: Emulsion activity index (EAI) and emulsion stability index (ESI) of Skipjack tuna skin gelatin

An increase in ESI of all gelatin samples was observed with an increase in concentration. At interfaces, higher concentrations of proteins enabled more adsorption (Yamauchi et al., 1980). At elevated concentrations, gelatin with superior hydrophilic characteristics might interact with each other, therefore there exists a minimal ratio of gelatin remaining to be localized at the oil-water interface.

3.11. Fat binding capacity and water holding capacity

Water-holding and fat-binding capacities are important functional attributes, which are related to texture as a result of interaction amongst water, oil, and other components (Cho et al., 2004). Table 1D shows the FBC and WHC of fish skin gelatin. The efficacy of fat binding remains superior in gelatin extracted from tuna skin. The higher efficacy is attributed to factors involving a degree of exposure to hydrophobic moieties and a higher ratio of tyrosine (Ninan et al., 2011). Noncovalent bond is involved in lipid-protein interaction and remains an external requisite for the FBC (Lawal, 2004).

The water-holding capacity is the measure of the ability of the protein to imbibe water and retain it against a gravitational force within the protein matrix, remaining as a critical part of proteins' functional attribute in the food system (Koli et al., 2012). Water-holding capacity is anticipated to be influenced by the amount of hydrophilic amino acids, higher hydroxyproline content, and the persistence of pores within the gelatin structure (Ninan et al., 2011, Kaewruanga et al., 2014).

4. CONCLUSION

There exists a considerable reduction in the lipid content of the extracted gelatin on employing the warm water coupled NaCl pre-treated method. This approach remained optimal for food technological applications and remain eco-friendly. The current method ascertained remarkable yield and techno-functional properties for extracted tuna skin gelatin.

5. ACKNOWLEDGEMENT

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