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Chemical Properties of Fish Gelatin from Skin and Bone of Yellowfin Tuna (*Thunnus Albacares*)

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Abstract. This study aims to compare the chemical properties of extracted gelatin from the skin and bone of yellowfin tuna (*Thunnus albacares*) by using the acid method. The proximate and FTIR analyses of extracted gelatin (TSG and TBG) were conducted to determine their chemical properties. The acid condition successfully produced gelatin with a yield percentage of 3.24% and 2.61%, respectively. The proximate properties revealed that both extracted gelatin TSG and TBG contain a high total protein level (85.10% and 82.85%, respectively) and low lipid content (both 0.02%). The FTIR analyses showed that Amide A, I, II, and III appeared at 3452.58, 1637.56, 1517.98, and 1240.23 cm-1 in TSG FTIR spectra, while in TBG spectrum, those bands appeared at 3493.09, 1660.71, 1533.41, and 1238.30 cm-1. Both skin and bone of yellowfin tuna can potentially be used to produce gelatin for industrial and pharmaceutical purposes with further optimation.

Introduction

Gelatin is a polypeptide bond resulting from partial hydrolysis of collagen derived from bone, connective tissue and animal skin. Gelatin and collagen have the same physical and chemical properties, because gelatin is the product of collagen denaturation (Mariod & Adam, 2013).

Gelatin is used in various fields, such as food, pharmaceutical, cosmetic and photographic applications (Jamili et al., 2019). Generally, the gelatin used in various medicinal and food industries comes from bovine or porcine skin and bone. These materials have several drawbacks because there are still concerns about their healthiness and halalness (Elyasi et al., 2020; Shah & Yusof, 2014). Around 41% of gelatin produced in the world comes from pork skins, 28.5% comes from bovine skins, and 29.5% comes from bovine bones, while gelatin from fish accounts for only 1% of the total gelatin produced worldwide (Milovanovic & Hayes, 2018).

Yellowfin tuna waste is one of the by-products of fishery industry that have not been fully utilized. It can be used as gelatin because it contains large amounts of collagen, which can be seen from the elasticity of the skin structure (Jamili et al., 2019). Researches on the extraction and characterization of gelatin from the skin or bone of *Thunnus albacares* have been widely reported (Montero & Acosta, 2020). However, none study has compared the properties of *T. albacares* gelatin from both skin and bone parts.

Gelatin properties are related to its source. Muyonga et al. revealed that gelatin from the skin and bone of *Lates nicolitus* differed in their proximate composition due to their molecular weight (Muyonga et al., 2004a). These chemical characteristics will affect the functional properties of gelatin, such as gel strength, viscosity, melting point, and setting behaviour. In addition, the chemical properties of gelatin can also be studied from their Fourier Transform Infrared (FTIR) spectrum, which would reveal its molecular structure (Jridi et al., 2014).

This study aims to compare the proximate composition of extracted gelatin from the skin and bone of yellowfin tuna (*Thunnus albacares*) obtained from fish processing waste. Their FTIR spectra were also compared to commercial bovine gelatin to elucidate their structural

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properties.

Experimental

Sample Extraction

Sample Preparation. Samples in the form of skin and bone of tuna (Thunnus albacares) were cleaned of the remains of meat and fat that were still attached (degreasing) by soaking in boiling water for 30 minutes for fishbone and 1-2 minutes for fish skin while stirring. Then the samples were dried in an oven at 50 °C for 45 minutes and cut into small pieces (1.5 - 2 cm).

Hydrolysis. Both clean samples of skin and bone were then weighed 200 grams. The sample was immersed with 1000 mL of 6% HCl solution in a beaker for 48 hours until a soft sample (ossein) was formed. The soaking solution was changed every 24 hours. Then the ossein was washed using distilled water until the pH was neutral (pH 6-7).

Extraction. Each sample was dissolved in a ratio of 1:3 (w/w). The mixture was stirred at 80°C for 5 hours. The residue was filtered out, and the filtrate was then concentrated.

Drying. The gelatin solution was dried in an oven at 50 °C for 24 hours. After drying, it was ground until a fine powder resulted, TSG and TBG.

Proximate Analysis

Proximate analysis of extracted gelatin TSG and TBG were carried out based on the AOAC methods (AOAC Methods, 1990). Moisture content was measured by the oven method, while the total protein content of samples was evaluated by Kjeldahl method (conversion factor of 5.4). Total lipid content was determined by using soxhlet extraction, while ash content was measured by burning the dried sample in the furnace at 600 °C until white ash was formed.

Table 1. Properties of TSG and TBG

Properties	TSG	TBG	Standard (SNI 06-3735-1995)
Yield	3,24%	2,61%	-
Ash	1,70%	2,65%	< 3.25%
Moisture	10.62%	10.95%	< 16%
Protein	85.10%	82.85%	-
Lipid	0.02%	0.02%	-
Colour	yellowish	brown	No colour
Odour	normal	normal	normal

FTIR Analysis

Shimadzu IRPrestige 21 FTIR spectrometer was used to record FTIR spectra between wavenumbers of 500 and 4000 cm-1. The sample was prepared by obtaining the KBr pellet from the mixture of gelatin and potassium bromide (1:10).

Result and Discussion

Proximate Properties

The data on the characteristics of gelatin from yellowfin tuna (*Thunnus albacares*) bone (TBG) and skin (TSG) are presented in Table 1.

The conversion of collagen into gelatin is affected by temperature, heating time and pH (Montero & Acosta, 2020). It was found that gelatin yield from the skin part was higher than from the bone, which is in accordance with the previous report (Tinrat & Sila-Asna, 2017), but still lower than the yield of other fish sources (Rawdkuen et al., 2013). It may be due to the difference in pretreatment conditions in gelatin extraction. The type and concentration of acid or alkali used in the pretreatment process can affect the yield of extracted gelatin (Zhou & Regenstein, 2005), because some collagenous protein could also be destroyed during sample preparation (Hao et al., 2009).

Gelatin colour TSG and TBG indicate that the colour/whiteness of the extracted gelatin is different from the SNI standard for gelatin (1995). The obtained colour gelatin is thought to come from the colour of the dried fish skin and bone samples after drying, which was yellowish and brown, respectively. Skin and bone colour after pretreatment affected the colour of gelatin, in which gelatin from fishbone has a higher turbidity value than from fish skin (Muyonga et al., 2004a).

The resulting gelatin's moisture content showed that they meet the quality standard range of gelatin that is the maximum of 16%. The moisture content can affect not only the texture of gelatin products but also affect the shelf life of the products (Haryati et al., 2019).

The ash content of food indicates the number of minerals contained (Weng et al., 2014). The ash content of both TSG

and TBG were suitable to the SNI standard of gelatin, which is not more than 3,25%.

Both TSG and TBG contained lipid in very low concentrations. This result is less than the lipid percentage of other fish gelatin from previous reports (Muyonga et al., 2004a; Sahoo et al., 2015; See et al., 2010).

The protein content is one of the parameters that determine gelatin quality. Based on the results of protein analysis, the protein content of TSG was higher than TBG. However, both protein contents were still high compared with the protein level of gelatin from other sources such as Nile perch bones (78.40%) (Muyonga et al., 2004a), alaska pollock skins (83.90%) (Zhou & Regenstein, 2005), pangasius catfish skins (81.61%), and snakehead skins (75.63%) (See et al., 2010), but still lower than gelatin from surimi waste (88.40%) (Norziah et al., 2014), rabbitfish skin (94.72%) (Haryati et al., 2019), and catfish skin (97.71%) (Nasution et al., 2018). The high levels of the protein will increase gelatin ability to form a gel and will increase the viscosity (Montero & Acosta, 2020).

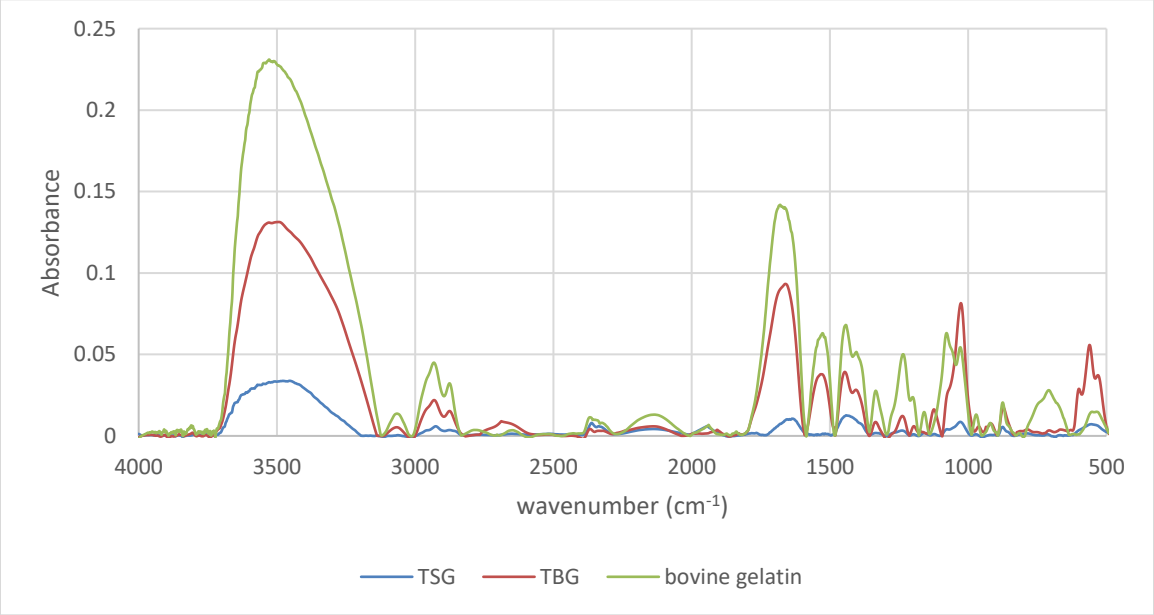

Figure 1. FTIR spectra of extracted gelatin samples and bovine gelatin

Table 2. Amide bands of gelatin samples from FTIR spectra

Samples	Amide A	Amide I	Amide II	Amide III
TSG	3452.58	1637.56	1517.98	1240.23
TBG	3493.09	1660.71	1533.41	1238.30
Bovine gelatin	3527.80	1680.00	1525.69	1234.44

FTIR Spectra

In order to understand the molecular difference of extracted gelatin (TSG and TBG), an FTIR spectroscopy study was applied in this study. Moreover, both spectra were also compared to observed spectra from bovine gelatin, which are shown in Figure 1. The FTIR spectra of protein compounds can be identified from the presence of nine bands, known as amide A, B, and I-VII. Among them, amide A, amide I, II, and III bands are the most prominent and important bands in the characterization of collagen and gelatin protein structures (Cebi et al., 2016). Hameed et al. (2018) state that Amide A and Amide I bands in FTIR spectra have been used to identification of gelatin structure. Thus, important amide bands of both spectra are shown in Table 2.

Overall, the TBG spectrum is quite similar to that observed in bovine gelatin. It may be due to the same part of the gelatin source, in which both TBG and bovine gelatin were made from bone parts. However, the spectrum of bovine gelatin showed higher intensity in almost all bands than TBG and TSG.

Amide A bands which appeared between 3600-3200 cm^{-1}, indicates N-H stretching vibration. It was shown as broadband in all spectra. However, in the TSG spectrum, this band was appeared as the broadest band and at the lowest wavenumber, which suggested that TSG has more

N-H groups of shorter peptide fragments (Salem et al., 2020).

C=O stretching vibration of peptide linkage, which is known as amide I band, appeared in the range of 1600-1700 cm^{-1}. It is also coupled to C-N stretching and N-H bending vibration (Salem et al., 2020). Thus, this band has a high correlation with the secondary structure of a protein. TBG spectrum showed a higher wavenumber of amide I band than TSG. It may be due to the different types of crosslinks in both types of tissue (Muyonga et al., 2004b).

The spectra band in the range of 1565-1520 cm^{-1} is known as the amide II band, which is associated with N-H bending couple with C-N stretching vibration (Ali et al., 2018). The amide II band in the TSG spectrum appeared at a lower wavenumber than TBG, indicating that TSG has more hydrogen bonding between N-H bonds with adjacent α-chains.

Amide III band (1301-1229 cm^{-1}) is also associated with C-N stretching and N-H bending vibrations (Kong & Yu, 2007). Muyonga et al. (2004b) report that the band around 1240 cm^{-1} is related to the triple helical structure of the sample. The intensity of this band in the TBG spectrum was higher than the similar band in the TSG spectrum, which corresponds to the more intermolecular interaction in its triple helical structure than TSG.

In addition, the region of 1100-1000 cm^{-1} has been used to discriminate the fish and mammalian gelatins (Cebi et al., 2016), as well as the skin and bone of fish gelatins (Muyonga et al., 2004b). This region is attributed to C-O vibration due to carbohydrate moieties. The different pattern of the band in this region between tuna and bovine gelatin spectra was significant. Moreover, the TBG spectrum showed higher intensity of the band, which is associated with more pentosidine crosslinks than in TSG.

Conclusion

Fish-based gelatin was successfully extracted from skin and bone of yellowfin tuna waste which skin gelatin has a higher yield (3.24%) than bone gelatin (2.61%). Ash and moisture content of extracted gelatin were following the standard values. Both gelatin samples have a high level of total protein and very low lipid content. Based on the FTIR spectra, bone gelatin has more similarity with bovine gelatin in which indicate that bone gelatin has a similar chemical structure to bovine gelatin. Further studies are needed to optimize the extraction yield and properties.

Conflict of Interest

The authors declare that there is no conflict of interest.

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