Preparation and Characterization of Waste Fish Bone-Derived Gelatin/Chitosan Plastic Film Incorporated with Green Tea Extract

D. R. Barleany[1,*], D. K. Sari[1], R. S. D. Lestari[1], H. Alwan[1], M. Yulvianti[1], A. N. Afifah[1], N. Nisrina[1], and A. Gunawan[2]

[1] Department of Chemical Engineering, Faculty of Engineering, Universitas Sultan Ageng Tirtayasa,
[2] Department of Industrial Engineering, Faculty of Engineering, Universitas Sultan Ageng Tirtayasa

ABSTRACT: Fish-derived gelatin has recently been spotlighted as an alternative source following the negative issues of mammalian gelatin, but unfortunately it has poor mechanical properties that limit its application. Herein, an environmental-friendly film was prepared from milkfish (*Chanos chanos*) bone waste-derived gelatin and modified by adding chitosan, glycerol and sorbitol plasticizers as well as green tea extract to improve its mechanical properties. Gelatin was processed from milkfish bone waste using various concentrations of H_2SO_4 solution (2%, 3%, and 5%) (v/v) and soaking times of 18 h and 36 h for each of the solutions. The measurement test of pH, ash content, and moisture content were conducted on the gelatin product, and the characteristic functional groups were investigated using Fourier-Transform Infrared Spectroscopy (FTIR). The effect of green tea addition was also evaluated on the thickness, tensile strength, and elongation of the resulting films. The results showed that treatment using H_2SO_4 concentration of 3% with 36 hours of immersion time produced the best gelatin quality with a pH value of 5.9. Water content and ash content were 14.286% and 3.09%, respectively. The plastic film from this study has mechanical properties similar to polypropylene plastic so that it is expected to be a potential material for food packaging applications.

KEYWORDS: Film, waste fish bone, food packaging, gelatin, green tea.

[Received Sept. 25, 2023; Revised April 26, 2024; Accepted May 16, 2024] Print ISSN: 0189-9546 | Online ISSN: 2437-2110

I. INTRODUCTION

Since the 20th century, plastics and petroleum polymers have been widely used in the food industry as a packaging material due to their excellent properties such as flexibility, light weight and barrier. However, those polymers are mostly non-biodegradable and nonrenewable, thereby causing harm to humans and the environment (Mohamed *et al*., 2020). Previous report stated that during the COVID-19 crisis, pollution from plastic waste such as food packages increased the risk of infection and the possibility of global transmission (Oliveira *et al*., 2021). The worldwide lockdown enforced in response to the spread of COVID-19 at the beginning of 2020 prompted a notable increase in demand for packaged goods and take-home food, thereby significantly altering consumption trends (Hossain *et al*., 2024). This rise in demand has led to a corresponding uptick in the utilization of single-use plastics (Patrício Silva *et al*., 2021), typically derived from petroleum-based polymers (Oliveira *et al*., 2021). The challenge with the extensive utilization of these materials for one-time purposes lies in their non-biodegradable nature, which means they persist in the environment without breaking down. Given the escalating peril of plastic pollution, there is an urgent need to innovate new plastic formulations that prioritize renewability and biodegradability. In order to transition away from traditional plastics, food manufacturers are now prioritizing the widespread adoption of bio-alternative plastics, commonly known as bioplastics, as a key initiative (Ghasemlou *et al*., 2024). In recent years, biodegradable films used to package various foods to extend their shelf life have attracted more and more attention (Qazanfarzadeh & Kumaravel, 2023; Zehra *et al*., 2022). In addition to reducing the risk of environmental hazards, biological materials used for food packaging exhibit a great barrier against moisture and oxygen, thus increasing protection against microbiological spoilage (Etxabide *et al*., 2017).

Among biopolymers, gelatin, a chemically modified derivative of the animal protein collagen, is a very popular material for the preparation of biodegradable food packaging because of its excellent film formability, renewability, biodegradability, high transparency, biocompatibility, UV protection properties, and low cost (Bang *et al*., 2019). However, food packaging films made from gelatin have poor mechanical properties compared to petroleum-based plastic materials. Various attempts have been made to improve the mechanical, optical, physical and chemical properties of the film by combining gelatin with other biopolymers, such as chitosan (Duan *et al*., 2023; Fu *et al*., 2021; Rezaee *et al*., 2020; Tessaro *et al*., 2021). In addition, in order to provide more protection to food against oxidation and microbial spoilage, bioactive compounds can also be incorporated into the gelatin

*Corresponding author: ria.barleany@untirta.ac.id

doi: http://dx.doi.org/10.4314/njtd.v21i3.2063

film. The use of phenolic-rich plant extracts which act as antioxidant agents were carried out to enhance the functional properties of the films (Duan *et al*., 2023; Mortazavi Moghadam *et al*., 2023; Tessaro *et al*., 2021). Green tea, obtained from *Camellia sinensis L*. leaves, contains abundant polyphenol antioxidants, notably catechins (Martins *et al*., 2018). Green tea contains more catechins than black tea or oolong tea, which makes it possess stronger antioxidant properties compared to other types of tea (Vishnoi *et al*., 2018). Many studies have reported that the incorporation of green tea extract in packaging films enhances the antioxidant properties as well as the ability to inhibit microbial growth, improve mechanical, water-vapour barrier properties, and biodegradable rate (Jamróz *et al*., 2019; Martins *et al*., 2018; Sadeghi *et al*., 2022).

Gelatin consumed worldwide is mostly sourced from bones and skins of mammals (pigs and cows), and a small amount is obtained from fish-derived sources and poultry (Abedinia *et al*., 2017). However, there are religious and ethical concerns regarding the use of mammalian gelatin, as well as contamination of bovine gelatin by pathogens that cause mad cow disease, foot and mouth disease, and Bovine Spongiform Encephalopathy (BSE) (Mad-Ali *et al*., 2017). Gelatin extracted from fish processing waste including skin, bones, swim bladder and scale is a potential alternative to mammalian-based gelatins (Maihemuti *et al*., 2023; Nurilmala *et al*., 2021; Xia *et al*., 2022). They have been observed to have good film forming ability, resulting in transparent, nearly colorless films, and are highly scalable for environmentally friendly food packaging. Still, gelatin from fish has limited commercial application because of the lower gelling temperature and poorer gel properties than those derived from mammals (Nurilmala *et al*., 2022). The properties of fish gelatin are greatly determined by the species or tissue from which it is extracted, as well as by the conditions such as pH, temperature, and time during both pre-treatment and extraction process (Ahmad *et al*., 2017). Gelatin from warm water fish, which constitutes the majority of farmed fish species, was reported to show higher gel strength compared to gelatin from cold-water fish varieties (Nurilmala *et al*., 2021). In addition, a study showed that tropical fish gelatin had similar or even better gel strength, foaming properties, and emulsifying properties than mammalian gelatins (Peng *et al*., 2022).

This present study aims to extract gelatin from milkfish (*Chanos chanos*) bone waste, which is a type of warm water fish, for further processing into plastic film packaging with the addition of chitosan and green tea extract to improve its mechanical properties. Extraction of gelatin from fish with acid pretreatment can increase the yield up to several degrees compared to conventional methods, but on the other hand it reduces the gel properties. Acid pretreatments using sulfuric acid, citric acid, and acetic acid in gelatin extraction from various fish skin such as golden carp (*Probarbus Jullieni*) (Ali *et al*., 2018), pangasius (*Pangasianodon hypopthalmus*) ((Nurilmala *et al*., 2021), and codfish (*Gadus morhua*) (Alves *et al*., 2022) have previously been reported. Most of these studies have focused on the extraction of gelatin from fish skin. However, the extraction of gelatin from bone waste of milkfish (*Chanos chanos*) for packaging film application has not been carried out by any researcher. Hence, there is a need for studies on the use of sulfuric acid in the extraction of gelatin from bone of milkfish, considering the lack of information in this regard. In this work, the effect of sulfuric acid concentration on the yield and properties of the resulting gelatin was studied. The resulting gelatin was then applied to prepare plastic films at various green tea concentrations and the effect on the tensile strength and film elongation was examined.

II. MATERIALS AND METHODS

A. Materials

Milkfish bone waste as the main raw material for the gelatin extraction was collected from one of the milkfish satay production houses in Serang, Indonesia, while food-grade chitosan with deacetylation degree (DD) of 87.2 % for film preparation was produced by PT. Biotech Surindo, Cirebon, Indonesia and green tea extract was from CV Lansida Group, Yogyakarta, Indonesia. Sulfuric acid (H_2SO_4) (Merck), sorbitol, and glycerol were purchased from Alfa Kimia, Cilegon, Indonesia.

B. Methods

1) Gelatin Preparation

Gelatin was prepared following the procedure outlined by Winarti *et al*. (2021) with some alterations. Gelatin preparation was done through degreasing, demineralization, and extraction stages. The degreasing stage was carried out to clean the bones from the remnants of meat and adhering fat. The milkfish bones were cleaned with running water until there were no remaining meat and fat attached, then soaked in boiling water (100 ºC) for 30 min with a 1:15 (w/w) of bone to water ratio. The fish bones were later ground to a size of 3-5 cm, and subsequently dried in the sun. The demineralization stage was carried out to remove calcium and other salts contained in the bones. Dried milkfish bones were soaked in sulfuric acid (H_2SO_4) solution with concentrations of 2%, 3% and 5% for 18 h and 36 h soaking time until ossein (soft bones) was formed. The utilization of H_2SO_4 during the demineralization stage in this study was grounded in the research of Prasetya *et al*. (2022). Ossein was then washed using distilled water to a pH of around 5-7. In the extraction stage, ossein was put into a beaker glass and extracted using distilled water in a ratio of 1:3 (w/w) in an overhead stirrer with a stirring speed of 516 rpm at a temperature of 70 °C within 2.5 h. The ratio of ossein to distilled water utilized was derived from the investigation conducted by Winarti *et al*. (2021), while the extraction temperature was determined according to the findings reported by Ismail *et al*. (2019). The filtrate formed from the extraction process was filtered, after which the liquid was taken and placed in a beaker glass, then concentrated using a rotary vacuum evaporator. The filtrate was put into the oven at a temperature of 80 °C for ± 6 h to obtain dry gelatin. Dry gelatin was mashed to become gelatin powder and then analyzed for yield, ash content, water content, pH, and characterized by using FTIR. The symbols and descriptions for the variables of gelatin preparation used in this work are shown in Table 1.

Table 1. Symbols of gelatin preparation.

No.	Symbol	Definition
1	Gelatin I	Gelatin treated with 2% H_2SO_4 and 18 h immersion time
2	Gelatin II	Gelatin treated with 3% H_2SO_4 and 18 h immersion time
3	Gelatin III	Gelatin treated with 5% H_2SO_4 and 18 h immersion time
4	Gelatin IV	Gelatin treated with 2% H_2SO_4 and 36 h immersion time
5	Gelatin V	Gelatin treated with 3% H_2SO_4 and 36 h immersion time
6	Gelatin VI	Gelatin treated with 5% H_2SO_4 and 36 h immersion time

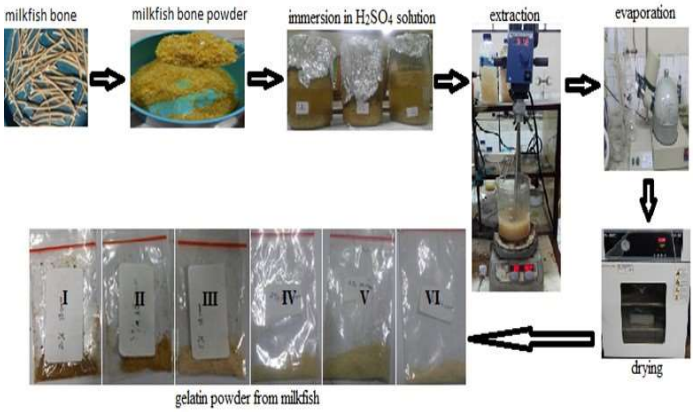


Figure 1. Preparation procedures of milkfish bone gelatin.

2) Gelatin Powder Yield

The gelatin powder yield (GPY) was determined and calculated with the formula shown in Eqn. (1):

$$\text{GPY}(\%) = \frac{weight\ of\ sample\ after\ drying}{weigh\ \ of\ raw\ material}\ x\ 100 \qquad (1)$$

3) Active Film Synthesis

The process of making active films from gelatin and chitosan in this study followed the procedures of Chen et al. (2021), with modifications made to the addition of green tea extract and the concentration of the ingredients used. Gelatin solution was prepared by mixing 3 g of gelatin with 50 mL of distilled water in a 100 mL beaker, then stirring and heating at 45 ºC for 2 h. 3 g of chitosan and 0.5 mL of acetic acid were added to a 100 mL beaker and then stirred for 2 h to form a chitosan solution. Gelatin solution and chitosan solution were mixed and then added with 12 mL of glycerol, 5 mL of sorbitol, and pure green tea extract with various concentrations of 1; 1.5; and 2.5 % (v/v) then stirred with an overhead stirrer for 1 h. The film solution formed was then put into a 250 mL beaker glass, poured into a 15 x 15 cm plastic mold, after which it was dried at room temperature for 48 h.

III. RESULTS AND DISCUSSION

A. Characteristics of Gelatin from Milkfish

Figure 1 shows the process of milkfish bone gelatin production using sulfuric acid pretreatment and the application for active food packaging film. The color of commercial gelatin usually ranges from pale yellow to dark yellow which is influenced by raw materials and extraction conditions (Alfaro *et al*., 2015). All gelatin products extracted from milkfish bones from this study provided color characteristics that matched those of commercial gelatin.

B. Gelatin powder yield from milkfish bone

The yield of gelatin powder indicates the amount of dehydrated gelatin obtained from raw materials or the relative ratio of dehydrated gelatin (Kim *et al*., 2020). The yield of gelatin from different raw material sources can be affected by several factors including temperature, pH, and extraction time (Ahmed *et al*., 2020). Gelatin powder yield from milkfish bone in this study ranged from 0.3 to 1.1 % (Figure 2). The yields obtained from this study are in line with another study, where Ismail *et al*. (2019) extracted gelatin from milkfish bones using acetic acid and sodium hydroxide and obtained yields around 0.2 to 4.6 %, depending on the type and concentration of acid used, while milkfish bone gelatin using acetic acid and citric acid objected yields ranged between 2.48 to 10.48 %, and showed an interaction between the acid type and acid concentrations in the analysis of variance (ANOVA) results (Winarti *et al*., 2021). The low yield of fish gelatin in the present study may be due to incomplete hydrolysis of collagen or the loss of collagen during the washing process (Alfaro *et al*., 2015).

In general, there is an increase in the yield of milkfish bone gelatin in acid treatment with increasing concentrations, as previously reported by some researchers Ismail *et al*., 2019; Rafael *et al*., 2021. The reason for this is that a high concentration acid can disassemble the triple-helix structure into single chains, thereby enhancing the solubility of gelatin in water during the extraction process Winarti *et al*., 2021; Zuraida & Pamungkas, 2020. However, treatment with sulfuric acid of the present study showed that the gelatin yield increased at a concentration of 3% but decreased at a concentration of 5%. Similar results were also obtained when using acetic acid treatment for milkfish bone extraction (Winarti *et al*., 2021). Furthermore, it has been explained that the greater the concentration of acid, the more it can enlarge and open up the collagen structure. This increased opening rate results in a higher yield of extracted collagen. The quantity of acid plays a crucial role in dissolving calcium salts and consequently augmenting both the collagen content in ossein and the production of gelatin during extraction. This process involves the hydrolysis of the triple helical chain by H+ ions,

converting it into a single water-soluble chain. Excess acid concentration leads to additional hydrolysis, resulting in the degradation of some gelatin and subsequently reducing its quantity. Comparable results were reported, where higher gelatin yields were obtained when using an acid concentration of 0.15 M compared to 0.2 M, valid for both types of acid used (acetic acid and hydrochloric acid) Sántiz-Gómez *et al*., 2019. In addition, See *et al*. (2015) observed a comparable pattern of acid extraction, wherein weak organic acids such as acetic and citric extracted a greater amount of gelatin from African catfish (*Clarias gariepinus*) skin compared to a strong acid like sulfuric acid.

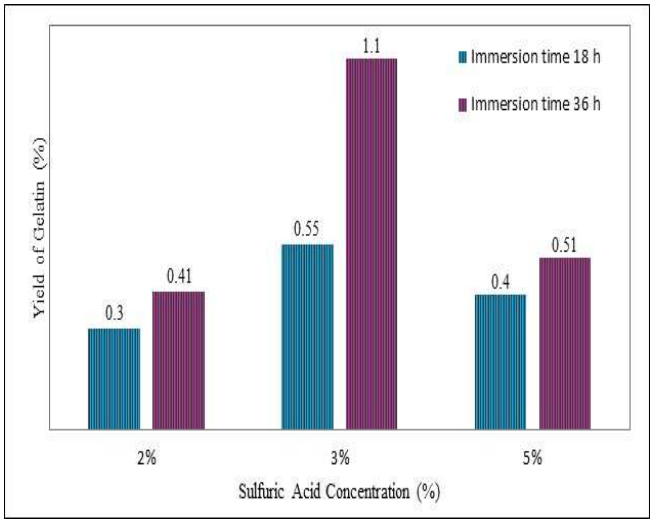


Figure 2. Yield of gelatin powder at various

The pretreatment time also affects the yield of gelatin. Hence, various pretreatment times (18 and 36 h) were studied. When using sulfuric acid in various concentrations (2, 3, and 5%), soaking time in the pretreatment stage showed a significant difference in the extraction results. Pretreatment using longer immersion time in sulfuric acid solution (36 h) gave greater yields compared to shorter immersion time (18 h). This increase in gelatin yield could result from organic acids solubilizing uncrosslinked collagens and disrupting inter-chain cross-links within the collagens, ultimately facilitating additional solubilization of collagen during the extraction process (Liu *et al*., 2015). Yessirita & Prima Putra (2023) also reported that gelatin yield increased continuously with increasing the soaking time from 6 to 24 h in 3 % of acetic acid. In another report, gelatin yield from shortfin scad (*Decapterus macrosoma*) heads increased from 1 to 8 h of soaking time in citric acid and declined at 12 h (Kuang & Mohtar, 2018).

C. FTIR Study

Carbonyl (C=O), amine (NH), and hydroxyl (OH) groups are common in the gelatin structure and give rise to five main peaks, which lie in the wavenumber region from 3440 to 3201 cm^{-1} (amide-A), from 3000 to 2923 cm^{-1} (amide-B), from 1698 to 1633 cm^{-1} (Amide-I), from 1543 to 1447 cm^{-1} (Amide-II) and the wavenumber region at 1365 to 1200 cm^{-1} (Amide-III) (Ashrafi *et al*., 2023).

Figure 3 shows the similarity between the functional groups that appear in milkfish bone extract gelatin and commercial gelatin. In commercial gelatin, the peak of Amide-A is found at a wavelength of 3406.20 cm^{-1}, while in milkfish bone gelatin at a wavelength of 3537.45 cm^{-1}. Amide-A corresponds to the vibration of the NH group coupled with hydrogen bonding. The amide-A peak of milkfish bone gelatin appeared at a higher wavelength compared with the commercial gelatin. This may due to the higher intermolecular interaction via hydrogen bonding between commercial gelatin molecules. The absorption peaks of Amide-B (illustrating CH and -$NH3^{+}$ stretching) for commercial gelatin and milkfish bone gelatin appear at wavelengths of 2927.56 and 2941.44 cm^{-1}, respectively. Amide-I of milkfish bone gelatin appears at 1631.78 cm^{-1} while for amide-II there are 2 peaks namely at 1546.91 and 1409.96 cm^{-1}. The absorption peak of amide-I also appears in commercial gelatin sample, which is at the wavelength of 1624.81 cm^{-1}. Amide-I represents the C=O stretching/hydrogen bonding coupled with COO^{-} and amide-II illustrates the NH bending coupled with CN stretching (Theerawitayaart *et al*., 2019). Because there are similarities in functional groups with commercial gelatin, it can be ascertained that the sample obtained from milkfish bone extraction in this study is gelatin.

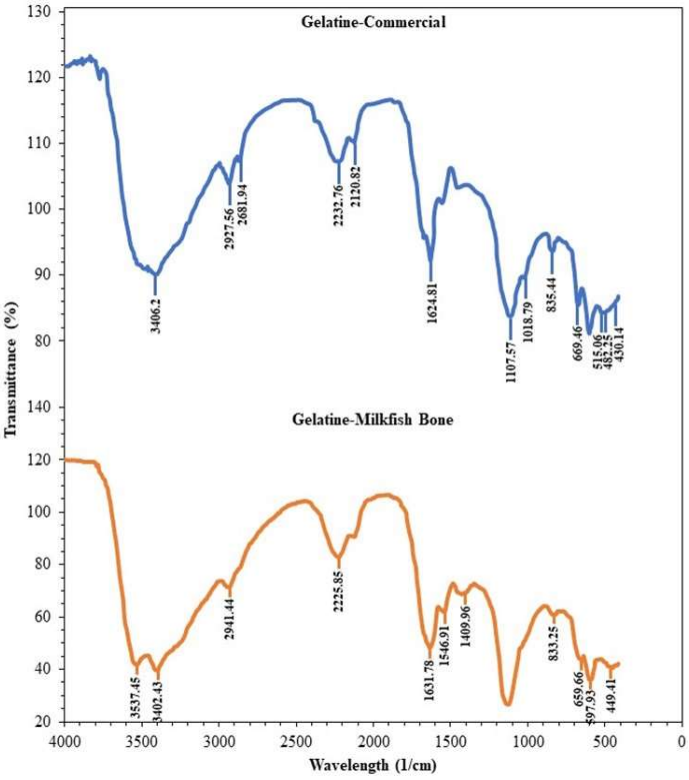


Figure 3. FTIR spectra of commercial gelatin and gelatin from milkfish

D. Degree of acidity (pH), water content, and ash content of gelatin

This study produced gelatin from milkfish bones in the form of dark yellow to yellowish-white powder, with the characteristics shown in Table 2.

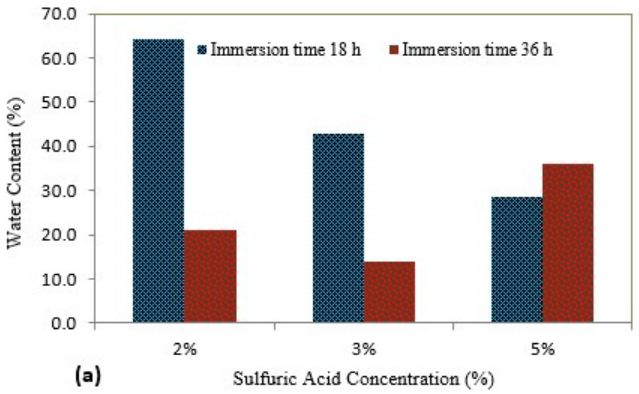
Table 2. Gelatin characteristics from the milkfish bones

No.	Product	pH	Water content (%)	Ash content (%)	Color
1	Gelatin I	5.74	64	6.4	dark yellow
2	Gelatin II	5.81	43	7.3	dark yellow
3	Gelatin III	5.27	29	9.1	pale yellow
4	Gelatin IV	5.06	21	4.6	pale yellow
5	Gelatin V	5.51	14	3.1	pale yellow
6	Gelatin VI	5.90	36	5.5	pale yellow

The pH value of gelatin is affected by the soaking time and washing process during bone preparation, before the extraction stage. Moreover, the pH of the gelatin solution indicates the chemical treatment used in the extraction stage (Alfaro *et al*., 2015). The higher the acid concentration, the more acid cations are trapped in ossein so that the measured pH becomes lower (Winarti *et al*., 2021). Previous studies showed similar trend in results, where there was a decrease in the pH value as the concentration of acids increased. A study reported that variations in increasing concentrations of hydrochloric acid, sulfuric acid and acetic acid used to soak beef bones resulted in a decrease in the pH value of gelatin (Fatimah *et al*., 2023). Likewise, with the results reported on gourami (*Ospheronemus Gouramy Lac*) bone gelatin, when collagen was soaked with HCl during the demineralization process, large amounts of unreacted HCl were purportedly absorbed into the collagen. This absorbed HCl was trapped in the collagen fibril network, making neutralization difficult during washing (Rahmi Hafsari *et al*., 2020). This phenomenon is thought to have an impact on the acidity level in the current extraction process. pH values of gelatin from bones of milkfish in this study are ranged from 5.06 to 5.90. pH value is one of the important factors affecting gel strength, gelling and melting temperatures, and viscosity which is generally used to characterize commercial gelatin, and to determine in what fields gelatin can be applied. For many applications, high viscosity gelatin is preferred and appreciated at a higher price, and this condition is achieved in gelatin with an acidic pH (Lin *et al*., 2017). Gelatin with a low pH is used in the food industry, while neutral pH gelatin finds applications in meat products, pharmaceuticals, chromatography, and paints (Fatimah *et al*., 2023).

The recommended maximum moisture and ash content for gelatin are 15% and 2%, respectively (Gelatine Manufacturers of Europe, 2022). Figure 4a shows the water content values of milkfish bone gelatin which vary with increasing concentration and soaking time using sulfuric acid. The lowest water content of gelatin from this study was 14%. This value is the only one that complies with the required commercial gelatin standard, and is obtained by immersing in 3% of sulfuric acid for 36 h. In the use of gelatin for applications in the food sector, the value of water content has an important role since it affects the texture and determines the shelf life against damage caused by microbes (Fatimah et al., 2023). Previous studies resulted different effects of acid concentration and soaking time on the water content of the gelatin produced. A decrease in gelatin water content due to longer soaking time was found (Yessirita & Prima Putra, 2023), and the using of acid concentrations from 1 to 5% resulted in various gelatin water contents, with the lowest value obtained at a concentration of 3 % and the highest value at an acid concentration of 5 % (Rahmi Hafsari et al., 2020).

The ash content of gelatin products from this study is in the range of 3.1 to 9.1% (Figure 4b), and all of them are above the maximum value of the required standard. The gelatin produced from this study exhibits a comparatively elevated ash content, potentially attributed to suboptimal demineralization processes. This could also stem from inadequate filtration, allowing other minerals to remain mixed in the gelatin solution prior to the drying phase (Fatimah *et al*., 2023). High ash content in gelatin products from shortfin scad heads also reported from a previous study, which probably resulted from a combination of high mineral content and low acid concentration used (Kuang & Mohtar, 2018). The relatively high ash content (>2%) suggested that the extracted gelation is of poor quality. However, the lowest ash content from this study which is closest to the standard is gelatin with 3% sulfuric acid pretreatment and 36 h of soaking time. This research shows that soaking in a sulfuric acid solution for a longer time leads to a reduction in the ash content of gelatin derived from milkfish. This aligns with the findings of Yessirita & Prima Putra (2023), who also observed that the ash content of tuna gelatin decreased with prolonged soaking. Additionally, it has been clarified that the ash content is influenced by the demineralization process in gelatin processing, whereby greater dissolution of minerals leads to lower ash content. When immersed in an acid solution, a reaction takes place between the acid and calcium phosphate, resulting in the formation of soluble calcium salts, consequently reducing the ash content of the resulting gelatin.



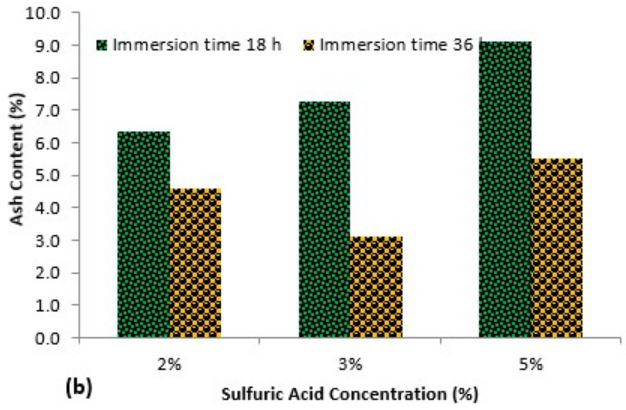


Figure 4. The water content and ash content of gelatin.

D. Appearance and thickness of gelatin film with green tea extract

The thickness and color of biopolymer-based films are critical parameters in food packaging and coating applications. They significantly impact the quality of the applied food and, ultimately, influence consumer decisions (De Carli *et al*., 2022). The physical appearances and thickness values of the gelatin films at the addition of different amounts of green tea extract (1, 1.5, and 2 %) (w/w) are shown in Figure 5 and Figure 6, respectively. The overall appearance of the gelatin film is transparent, although it changes to yellowish brown as more green tea extract is added (Figure 5). The color change due to the addition of green tea extract may be caused by the chlorophyll content and carotenoids which have the property of giving yellow-green pigmentation (Jamróz *et al*., 2019).

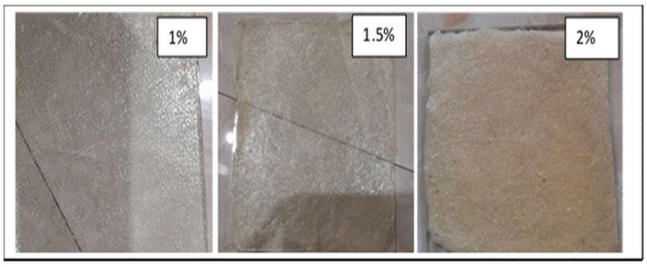


Figure 5. Gelatin film products with various green tea extract

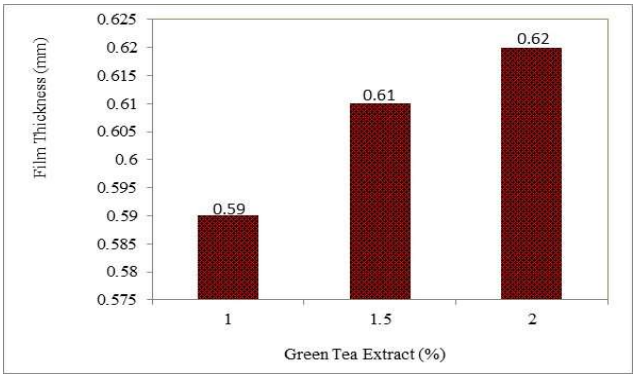


Figure 6. Thickness value of gelatin films.

The film thickness obtained was between 0.59 mm to 0.62 mm, where the thinnest and thickest samples are at a green tea concentration of 1% and 2%, respectively (the average of three times data collection). This indicates that an increase in the green tea extract concentration in the film solution is directly proportional to the thickness of the gelatin film. A similar trend was observed in previous studies, which reported that incorporation of extracts or essential oils into the biopolymer film matrix led to a decrease in film transparency along with an increase in film thickness (De Carli *et al*., 2022; Jamróz *et al*., 2019; Zhang *et al*., 2020). It was also reported that the density of furcellaran/gelatin film increased with an increased amount of tea extracts added to the film matrix (Jamróz *et al*., 2019).

D. *Tensile strength and elongation of gelatin film with green tea extract*

The mechanical performance and the effect of green tea extract content of the gelatin films were evaluated by tensile and elongation tests (Figure 7). The incorporation of more green tea extract into the gelatin matrix resulted to an increase in the tensile strength, but on the other hand, it decreased the elongation. Tensile strength values sequentially from the largest to the smallest are obtained by adding 2 %, 1 %, and 1.5 % green tea extract. Gelatin film containing 1.5 % green tea extract results in a decrease in tensile strength of 2.7 % compared to gelatin with a concentration of 1 % green tea extract, but there is an increase of 19.6 % when using 2 % concentration of green tea extract. However, some published studies exhibited that the addition of green tea extract had a different effect on the tensile strength and film elongation produced. Sadeghi et al., (2022) reported that the incorporation of green tea extract into the film matrix of polycaprolactone and polylactic acid resulted in better mechanical properties (an increase in tensile strength and elongation at break), while different results were previously reported by Martins et al. (2018), where the addition of green tea extract caused an increase in molecular mobility thereby reducing the tensile strength of the PLA film.

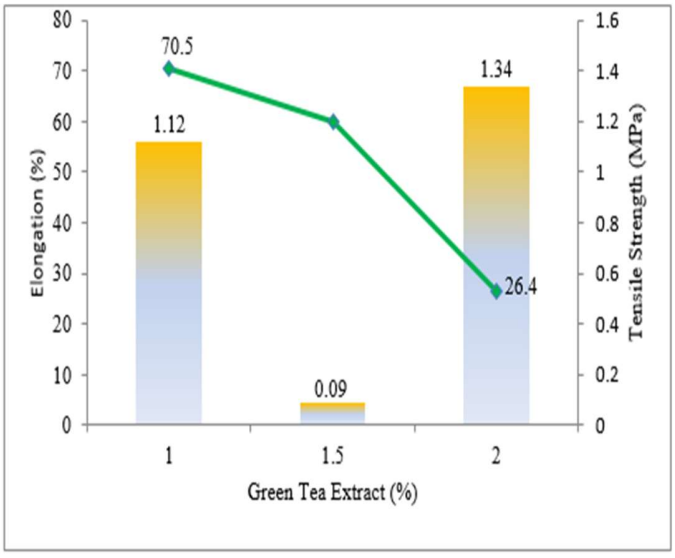


Figure 7. Effect of green tea extract on the film mechanical

IV. CONCLUSION

The study established that both the immersion time and concentration of sulfuric acid during the pretreatment stage has a significant effect on the properties of gelatin extracted from milkfish bone. Key parameters such as pH, water content, ash content and yield were influenced by these variables. Specifically, Soaking using 3% sulfuric acid for 36 h produced gelatin with the best characteristics, with a pH of 5.51, water content 14%, and ash content 3.1%. The best film produced with 1% green tea extract has 0.59 mm thickness, 1.12 MPa tensile strength, and 70.5 % elongation. These findings suggest that the processing conditions and additive concentrations can be tailored to enhance both the mechanical and physical properties of gelatin-based films. This offers the potential for diverse applications in food packaging and biomedical fields. Further works could explore the use of sulphuric acid at different concentration levels and immersion times to optimise gelatin yield as well as the expected qualities. Other natural additives such as plant extracts could also be investigated on the mechanical and biodegradability of geltin.

AUTHOR CONTRIBUTIONS

D. R. Barleany: Conceptualization, Methodology, Writing – original draft. **D. K. Sari:** Data curation, Project administration **R. S. D. Lestari:** Supervision. **H. Alwan:** Visualization, Software. **M. Yulvianti**: Writing – review & editing. **A. N. Afifah:** Investigation, Resources. **N. Nisrina**: Formal analysis. **A. Gunawan**: Validation.

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