Physicochemical Properties of Milkfish Gelatin-Natural Starch Composite

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Abstract

BACKGROUND: Halal gelatin sourced from fish can be improved in quality through mixing with other polymers so that it can be an alternative as food, pharmaceutical, and cosmetic ingredient.

AIM: The purpose of this study was to determine the characteristics of milkfish scale gelatin after the formation of a composite with corn, potato, and cassava starch to be used as a pharmaceutical and food excipient.

METHODS: The gelatin composite of milkfish scales with corn, potato, and cassava starch was made by casting method, using a ratio of gelatin and starch (4.5:0.5). Characteristic assessment includes organoleptic, viscosity, swelling index, FT-IR spectroscopy, and calorimetry. Data analysis used a non-parametric one-way ANOVA statistical method (p < 0.05).

RESULTS: The composites produced from mixing milkfish gelatin with corn starch, potato, and cassava showed hygroscopic properties, increased viscosity values, and decreased swelling index in milkfish gelatin-corn starch composite (7.89 cP and 25.0%), with potato starch composite (8.36 cP and 21.0%), and cassava starch composite (8.64 cP and 12.7%), compared to milkfish gelatin (0.11 cP and 75%) at p < 0.05. The behavior of the composite FT-IR spectrum follows the milkfish gelatin spectrum pattern with a shift in wavenumber in the typical bands (Amide I, Amide II, Amide III) in the gelatin spectrum. There was a shift of glass transition temperature to higher values in milkfish gelatin-corn and potato starch composites; melting temperature increased in milkfish gelatin-corn and cassava starch composites, and all composites showed a decrease in melting enthalpy.

CONCLUSION: The spectral pattern of the composite follows the typical spectral pattern of milkfish gelatin and all-natural starch composites showed increased viscosity, water retention, and thermal stability compared to milkfish gelatin. Milkfish gelatin with corn and potato starch composites may be used as pharmaceutical and food excipients.

Introduction

Fish gelatin is currently a concern for research as an alternative to mammalian (pork and bovine) gelatin which has limited use due to religious, cultural, and health reasons [1, 2, 3, 4]. Al-Nimry et al. [4] and Huang et al. [5] reported that fish gelatin has the potential to be an option in cosmetic, food, biomedical, and pharmaceutical applications. Large global water areas with abundant fish production and by-products of the fish processing industry are sources of gelatin raw materials that can increase economic value and are environmentally friendly [4, 6, 7, 8]. Several researchers continue to develop methods of producing gelatin from fish waste, namely, skin [8], scales [10], fins, and fish bones [11, 12] to produce optimum yield and quality of gelatin.

Compared to mammalian gelatin, fish gelatin is generally of lower quality. The low quality of fish gelatin such as gel strength, melting temperature, thermal and rheological stability, and mechanical properties mainly influenced by its proline and hydroxyproline content limits its extensive application [2, 4, 9, 14, 15]. To be able to replace the function of mammalian gelatin in the food, cosmetic, and pharmaceutical industries, including edible films, many researchers have attempted to improve the quality of fish gelatin by modifying it [4, 6, 14]. Modification of fish gelatin has been carried out by various methods and involves cross-linking reactions, nucleophilic substitution, electrostatic interactions, esterification, and complex formation with other compounds or polymers such as lactose, sucrose, polysaccharides, and cellulose [15, 16, 17, 18, 19, 20]. The combination of different modified methods is stated as a new strategy to improve the quality of fish gelatin [1, 14, 19].

Hydrogen bonds can occur in gelatin and several types of other polymers either directly between the –CO group and the glycine residue hydrogen of two adjacent main chains, or between the -NH group of one polymer chain and the –CO group of another chain, or...
through a water molecule bridging the −CO and −OH groups of hydroxyproline, or a water molecule bridging the −OH group of the two hydroxyproline residues. In these interactions, the number and types of hydrogen bonds that enter the gelatin structure have not been clearly defined [21].

Fish gelatin has a molecular structure with hydroxyl, carbonyl, and amide groups derived from its constituent amino acids, namely, glycine-proline-hydroxyproline. Compounding fish gelatin with several other compounds and polymers has been carried out with results that can be utilized because of its rheological properties and gel stability as packaging and scaffolding materials. Fish gelatin composites with gum arabic, xanthan, tragacanth, and sodium alginate can improve thermal stability, swelling strength and slow down gel melting, cross-linking of fish gelatin with microcrystalline cellulose using a genipin cross-linker [15], binding with citric acid, fructose, and ascorbic acid, starch oxidized, and aloe vera produces good properties as a packaging material [2], [6], [16], [18], [19], [20], [21], [22]. Fish gelatin cross-linked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) or transglutaminase (TGase) has shown a much better helical structure return and a significantly higher melting temperature of fish gelatin film than pork gelatin [22]. Cross-linking with TGase also affects the rheology of gelatin [23].

In this paper, we will describe the characteristics of the composite as a result of mixing milkfish scale gelatin (FMG) with several types of starch, namely, corn (MS), cassava (CS), and potato (SS). Starch with a structure that provides substitution/binding sites contains different levels of amylose and amylopectin for each species. The amylose content of CS is about 17.9–23.6% [24], SS 24.95%, and MS 25.60% [25]. The viscosity of these three types of starch is very different at a concentration of 5%, where cassava starch has the highest viscosity [25]. The use of starch with different amylose and amylopectin contents is expected to provide an overview of the properties that can be studied from its modification with milkfish scale gelatin.

**Methods**

**Material**

Milkfish scale gelatin (FMG) was obtained from the collection of the Department of Pharmacy, Faculty of Medicine and Health Sciences, UIN Alauddin Makassar, Indonesia, distilled water, corn starch (MS), potato starch (SS), and cassava starch (CS) were obtained from PT Brataco, a local distributor in South Sulawesi, Indonesia.

**Procedure**

Gelatin-natural starch composite preparation

Composite FMG with MS, CS, and SS was prepared according to the method of preparation by Chuaynukul et al. [26] with some modifications. FMG and starch in a ratio (4.5:0.5) were dissolved in deionized distilled water at 90°C; the volume of the mixture was 175 mL and 125 mL, respectively. When the temperature of each mixture was at 65–70°C, the gelatin solution was poured into each starch solution and stirred for 30 min (250 rpm) followed by 30 min (750 rpm). The mixture was dried in an oven (Memmert) at 80°C for 48 h. The mixture was dried in an oven (Memmert) at 80°C for 48 h, then the FMG composite with corn, potato, and cassava starch was called GM, GS, and GC, respectively.

**Characterization**

Organoleptic test (color and texture)

FMG composite films with corn starch (GM), potato (GS), and cassava (GC) were visually observed for color and texture, as well as surface morphology using a stereomicroscope (Euromex 1466).

Viscosity measurement

The viscosity of the raw materials (FMG, MS, CS, and SS) with concentrations equivalent to their constituents in the formation of composites and GM, GS, and GC composites was measured at room temperature using a viscometer Ostwald CFRC-100 Cannon-Fenske Routine Viscometer, size 100, calibrated. Measurements were carried out in three replications.

Swelling index measurement

The swelling index was measured by adopting the ASTM-D5890 test method [27] by measuring the volume of expansion of 2 g of raw materials and composites in water for 12 h at room temperature. Measurements were carried out in three replications.

Fourier-transform infrared (FTIR) spectroscopy

The FTIR spectra of the composite films were recorded using a Thermo Fisher Scientific Nicolet iS10 FTIR Spectrometer. The spectrum was obtained in the range of wave numbers 4000–400 cm\(^{-1}\) at room temperature. Spectral data analysis using the OriginPro 2018 program.

Differential scanning calorimetry (DSC)

The sample (20.0 mg) was heated with a heat flow rate of 25°C/min from 25 to 425°C using a
DSC 4000 (PerkinElmer) apparatus with heat flow and temperature calibrated using zinc and indium and nitrogen gas cooling at 20 ml/min. Measurements were carried out in three replications.

Analysis

Differences in viscosity, raw material expansion index, glass transition temperature, melting temperature, and melting enthalpy of the composites were analyzed using the non-parametric one-way ANOVA statistical method using SPSS IBM 25 at p < 0.05 [28], [29], [30], [31], [32], [33].

Results

Organoleptic

The form of milkfish scales gelatin (FMG), and GM, GS, and GC composites are shown in Figure 1.

Surface texture

Optical observation of the surface texture of the composite film is shown in Figure 2.

Viscosity and swelling index

The value of viscosity and swelling index of FMG and its composites with natural starch and their differences at p<0.05 are presented in Table 2.

FT-IR spectra

FT-IR spectra of FMG, natural starch, and composites are shown in Figure 3.

DSC thermogram

The thermal behavior of FMG and composites is shown on the DSC thermogram in Figure 4.

Discussion

The form of milkfish scale gelatin (FMG) and its composites with corn starch (GM), potato (GS), and cassava (GC) are shown in Figure 1. The organoleptic characteristics data are visually presented in Table 1. The brownish color of the FMG-starch complex is caused by the Maillard reaction on heating starch. The same thing was also reported by Alvarez-Ramirez et al. [30] who found that there was a decrease in the clarity of the room with each increase in temperature and heating time. The surface of the FMG complex film is sticky, hygroscopic, and brittle. This indicates that the FMG complex can still absorb moisture. Fragile FMG complexes do occur in films formed without plasticizers. A brittle film is needed in this study to facilitate size reduction for further observations.

The surface of the film observed with a ×4.5 stereomicroscope showed the peculiarities of the structure for each composite according to the type of starch used. The surface of GS and GC appears smoother and wavy which may be caused by bubbles (rather large) on the internal part of the film, the particles still appear to be inhomogeneous. In contrast to the GM film, the small bubbles on the internal part of the film make the surface appear rougher and the bubbles are arranged more homogeneously. Nazmi et al. [31] reported that the gelatin mixed film with CMC showed a rough and wavy surface due to the formation of holes in the film. The holes with shorter crack structures are thought to be the effect of the formation of hydrogen bonds between gelatin and CMC as well as inter- and intra-molecular bonds of film-forming components.

The composite viscosity showed a significant difference (p < 0.05) with the respective raw materials and FMG at the concentrations used for composite formation, the highest viscosity values in GC and GS as presented in Table 2. The viscosity values of each composite also do not show the cumulative value of the viscosity of the constituent raw materials. Changes in viscosity due to mixing FMG with each of these starches can give clues to the interaction between the two which were further confirmed by analysis of the vibrational shift of the groups through the typical spectrum shown on FT-IR and changes in thermal properties through DSC. Gelatin viscosity was reported to increase after mixing with cellulose nanofiber [32], a natural gum [18].

Figure 1: Milkfish scales gelatin (a) and milkfish scales gelatin composite with natural starch: GM (b), GS (c), and GC (d)
Table 1: Organoleptic characteristics of FMG and FMG composites with natural starch

<table>
<thead>
<tr>
<th>Sample</th>
<th>Color</th>
<th>Odor</th>
<th>Hygroscopicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMG</td>
<td>Yellowish</td>
<td>A little fishy</td>
<td>Hygroscopic</td>
</tr>
<tr>
<td>GM</td>
<td>Brownish-yellow</td>
<td>Odorless</td>
<td>Hygroscopic</td>
</tr>
<tr>
<td>GS</td>
<td>Brownish</td>
<td>Odorless</td>
<td>Hygroscopic</td>
</tr>
<tr>
<td>GC</td>
<td>Brownish</td>
<td>Odorless</td>
<td>Hygroscopic</td>
</tr>
</tbody>
</table>

The high hydrophilic nature of gelatin makes it able to absorb more water around it. The addition of natural starch decreased the hydrophilicity of gelatin, where after being in an aqueous environment for 12 h, all composites (Table 2) experienced water absorption inhibition which was significantly different from FMG (p < 0.05). Natural starch does not swell in the presence of water at room temperature, because it does not absorb and does not dissolve in water. The hydrophobicity of the composites increased compared to FMG by about 50% for GM and GS, while about 72% for GC. A similar condition was reported by Li et al. [33] that there was an increase in the hydrophobicity of gelatin after mixing with chitin, and the water absorption of the chitin-gelatin composite was based on capillary action. The limited water binding of GC then results in a more viscous gel than GM and GS. Pasaribu et al. [34] reported that the decrease in swelling properties of the hydrogel as a result of cross-linking maleoyl chitosan with oxidized alginate was due to the limitation of polymer chain mobility due to the increase in the high cross-link density. This swelling index is thought to be related to the presence of pores in the composite that provides space for water absorption and retention. The decrease in the swelling ability of chitin whiskers and gelatin nanocomposites was due to the strong hydrogen bonding interaction between the two polymers and the increase in cross-link density [35]. The high water holding ability of G-semi-IPN indicates that the gel can be used as a functional material to reduce syneresis [36]. This condition can be used to entrap water-soluble drugs in a drug delivery system design. The viscosity and swelling index of this composite are in the same direction, where when the water absorption ability decreases (FMG > GM > GS > GC), the viscosity value increases (GC > GS > GM > FMG).

Table 2: Viscosity and swelling index of milkfish scale gelatin, natural starch, and composite gelatin with natural starch

<table>
<thead>
<tr>
<th>Material</th>
<th>Viscosities (cP)</th>
<th>Swelling index (%) 12 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMG</td>
<td>0.11 ± 0.00</td>
<td>75.0 ± 0.25</td>
</tr>
<tr>
<td>SS</td>
<td>0.13 ± 0.00</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>MS</td>
<td>0.63 ± 0.00</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>CS</td>
<td>0.41 ± 0.00</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>GM</td>
<td>7.89 ± 0.24</td>
<td>29.0 ± 0.00</td>
</tr>
<tr>
<td>GS</td>
<td>8.36 ± 0.32</td>
<td>21.0 ± 0.00</td>
</tr>
<tr>
<td>GC</td>
<td>8.64 ± 0.50</td>
<td>12.7 ± 0.03</td>
</tr>
</tbody>
</table>

Signs a,b,c,d show a significant difference for each value in the same column at P<0.05

The characterization of the structure of FMG and the results of their mixing with corn starch, potato starch, and cassava starch were carried out through analysis of the resulting FT-IR spectra, as shown in Figure 3. The typical peaks of FMG and their shifts due to mixing with these starches are presented in Table 3. Spectra milkfish scale gelatin (FMG) showed a typical pattern for gelatin with peaks and bands at wavenumbers for Amide A (3274.4 cm$^{-1}$), Amide B (2936.9 cm$^{-1}$), Amide I (1632.7 cm$^{-1}$), Amide II (1532.7 cm$^{-1}$), and Amide III (1079.9 cm$^{-1}$ and 1031.0 cm$^{-1}$). Similar spectral patterns were shown in the gelatin structure observed by Sow et al. [37]. Hassan et al. [28] reported a spectrum of fish gelatin with the main peaks observed at 3310-3270 cm$^{-1}$ (Amide A), 1700–1600 cm$^{-1}$ (Amide I), 1550–1400 cm$^{-1}$ (Amide II), and 1240–670 cm$^{-1}$ (Amide III). The Amide B band of gelatin was observed by Staroszczyk et al. [22] at wavenumbers 3075–3068 cm$^{-1}$ as NH vibrations and 2930–2944 cm$^{-1}$ as CH$_2$ asym vibrations. Amide A band is a joint contribution of O-H strain and small N-H vibrations, Amide I band is due to −COO− and −NH$_3$ vibrations, while the Amide II band is due to N-H bonds, C-N and C-C vibrations [29], [32]. There was a widening of the...
band and a slight shift in the adsorption of Amide A of gelatin composite GS and GC to a lower wavenumber at 3271.0 cm\(^{-1}\) and 3273.7 cm\(^{-1}\), respectively, indicating the occurrence of hydrogen bonds between the carboxyl groups in gelatin and the hydroxyl groups in starch in aqueous media [23], [32]. The hydroxyl groups in starch are indicated in typical bands at 3300 cm\(^{-1}\) (MS), 3288 cm\(^{-1}\) (SS), and 3284 cm\(^{-1}\) (CS). The spectrum in the 3000–3600 cm\(^{-1}\) region is a marker for the presence of stretching O-H groups [38]. Amide II band shifts in GM and GS to 1527.1 cm\(^{-1}\) and 1528.5 cm\(^{-1}\), respectively, indicate the involvement of the –NH groups of gelatin in hydrogen bonding [33], [37]. The peak intensity in this region was decreased for all composites compared to FMG. This decrease in intensity can be a sign that the –NH group on the gelatin molecule has been converted to N-H (SNH). This decrease in intensity is thought to occur due to the conformational change of FMG into composites in the presence of starch molecules [22]. The spectrum pattern shown by the FMG composite with natural starch leads to a typical gelatin pattern compared to the respective starch spectrum patterns. The same thing happened in cross-linking of oxidized polysaccharides with gelatin [39], interactions of gelatin with sodium alginate [30], and composites of gelatin with esterified starch [40]. The conditions that appear in the shift in wavenumber in the typical bands and changes in the intensity of the absorption peaks indicate the interaction between the gelatin and natural starch molecules, forming GM, GS, and GC composites with different characters from FMG.

The thermal properties of FMG and gelatin complexes with natural starch GM, GS, and GC were observed through the thermogram of the DSC results in Figure 4. The thermogram shows the thermal properties of FMG and its composites with natural starch, which illustrates the transition temperature shift in the two endodermic conditions with different temperatures due to heating. The glass transition temperature (T\(_g\)) is first melting transition temperature and the maximum transition temperature (T\(_m\)) is the maximum temperature for the endothermic melting peak.

The DSC results for FMG and their composites with natural starch showing a shift in the glass transition temperature (T\(_g\)) of GM, GS, and GC composites toward FMG are summarized in Table 4. The glass transition temperature values for GC composites decreased by 11.51°C, while in GS and GM increased by 4.59°C and 28.38°C, respectively. This glass transition temperature shift had shown the properties of each composite.

### Table 3: Characteristics of FT-IR spectra of milkfish scales gelatin (FMG) and GM, GS, and GC composite

<table>
<thead>
<tr>
<th>Region</th>
<th>Peak position (cm(^{-1}))</th>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amide A</td>
<td>3271.4 3275.5 3277.1 3273.7</td>
<td>νOH, νNH</td>
<td>[28]</td>
</tr>
<tr>
<td>Amide B</td>
<td>2936.9 2934.9 2937.6 2930.6</td>
<td>νCH, asym</td>
<td>[22]</td>
</tr>
<tr>
<td>Amide I</td>
<td>1632.7 1631.9 1629.5 1635.9</td>
<td>νC=O, νNH</td>
<td>[32], [33]</td>
</tr>
<tr>
<td>Amide II</td>
<td>1532.7 1527.1 1528.5 1544.2</td>
<td>νNH, νC-N, νC-C</td>
<td>[32], [33]</td>
</tr>
<tr>
<td>Amide III</td>
<td>1238.9 1238.9 1240.0 1242.4</td>
<td>νC-N, νNH</td>
<td>[22]</td>
</tr>
<tr>
<td>1077.9 1079.8 1080.4 1077.6</td>
<td>νC-O</td>
<td>[22]</td>
<td></td>
</tr>
<tr>
<td>1031.0 1029.4 1031.1 996.5</td>
<td>νC-O</td>
<td>[22]</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4: Thermal characteristics of milkfish scales gelatin (FMG) and GM, GS, and GC composite**

<table>
<thead>
<tr>
<th>Material</th>
<th>T(_g) (°C)</th>
<th>T(_m) (°C)</th>
<th>ΔH (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMG</td>
<td>49.39 ± 0.01</td>
<td>333.87 ± 0.02</td>
<td>72.31 ± 0.01</td>
</tr>
<tr>
<td>GM</td>
<td>77.77 ± 0.01</td>
<td>342.54 ± 0.03</td>
<td>25.76 ± 0.01</td>
</tr>
<tr>
<td>GS</td>
<td>53.98 ± 0.00</td>
<td>330.5 ± 0.00</td>
<td>12.05 ± 0.00</td>
</tr>
<tr>
<td>GC</td>
<td>37.88 ± 0.04</td>
<td>337.0 ± 0.00</td>
<td>22.44 ± 0.00</td>
</tr>
</tbody>
</table>

*Signs a,b,c,d show a significant difference for each value in the same column at P<0.05*

Different thermal behavior was shown by the GM and GS composites, where they showed a shift of T\(_g\) to higher values. This property is associated with the possibility of cross-linking between the gelatin and starch polymers which have affected the thermal properties of each composite. The increase in T\(_g\) of GM, which was higher than GS, could indicate that the degree of cross-linking that occurred in FMG with corn starch was higher than its bond with potato starch. This behavior was also shown in the results of mixing chicken skin gelatin with CMC where there
was an increase in the $T_m$ value compared to chicken skin gelatin, the researchers stated that the increased glass transition value indicated that a cross-linking reaction had occurred due to the addition of CMC to the gelatin and increased the thermal stability of the mixture. Zhao et al. [43] reported that the thermal properties of the gelatin film were affected by the increase in the $T_g$ value of the modified gelatin with the addition of amino acids. The glass transition temperature shift was also shown in the kudzu starch complex coacervate modified gelatin – octenyl succinate anhydride, and it is suspected that there has been an electrostatic interaction between polymers [28].

There was a shift in the melting transition temperature ($T_m$) of GM and GC to a higher value and a shift in the melting enthalpy of endoderm ($\Delta H_m$) to a lower value than FMG. This also strengthens the possibility of cross-linking and increases the thermal stability of GM and GC composites. Greater thermal stability and increased degree of cross-linking were indicated by a decrease in the $\Delta H_m$ value of chemically and enzymatically modified gelatin. A decrease in the $\Delta H_m$ value due to increased cross-linking caused by other polymers being joined results in a reduction in the number of endothermically broken hydrogen bonds and simultaneously an increase in the number of exothermically broken covalent cross-links [44].

In contrast to the glass transition value, the melting transition value for GS composites experienced a significant decrease in value compared to FMG. Modification of FMG with potato starch was found to reduce the value of $T_m$ due to the evolution of the remaining water that makes gelatin plastic due to the interaction of these molecules. The same thing also happened to a mixture of chicken skin gelatin with CMC which showed an increase in the $T_g$ value but a decrease in the $T_m$ value [30]. In GM, GC, and GS composites, which showed a decreased $\Delta H_m$ value, it could be interpreted that the composite formed cross-links with a greater number of covalent bonds than hydrogen bonds. The opposite occurred in the codfish gelatin cross-linked film with EDC and TGase where the $H$ value of fusion increased and it was stated by the researcher that in cross-linking, there was a higher number of hydrogen bonds than covalent cross-linking [21]. From the thermal behavior of the results of this study, it can be stated that the GM and GC composites are not only an ordinary mixture between polymers but also are formed through covalent cross-linking which successively increases the degree of cross-linking which is indicated by an increase in $T_g$, $T_m$, and a decrease in $\Delta H_m$ compared to FMG. GS composites were also cross-linked with increased $T_m$ but underwent residual water evolution which caused the $T_m$ and $\Delta H_m$ values to decrease compared to FMG.

**Conclusion**

Composites produced from mixing FMG with natural starch can improve the gelatin characteristics of milkfish scales by increasing viscosity by limiting water absorption and increasing thermal stability. Composites are thought to occur through cross-linking, either hydrogen bonds or covalent bonds between starch and gelatin molecules.

**Acknowledgment**

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