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Absorbent Biopolymer from Crosslinked Plasticized Fish Gelatin

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A moderate absorbency polymer derived from fish gelatin was investigated to enhance its mechanical properties and hydrophilicity through irradiation-induced crosslinking. Tilapia skin gelatin, plasticized with glycerol, was used to examine the effects of varying concentrations of glutaraldehyde (GTA) and different irradiation doses on the biopolymer's performance. The formulations were optimized, then incorporated GTA, and subjected to electron beam irradiation to explore the impact on the swelling capacity and mechanical integrity of the films. Fourier-transform infrared spectroscopy (FTIR) was employed to confirm the integration of crosslinkers and glycerol into the gelatin matrix, facilitating an understanding of the molecular interactions within the biopolymer. The study aims to advance the applications of fish gelatin-based moderate absorbency polymers in environmentally friendly and biodegradable products.

1. Introduction

Biodegradable polymers are engineered materials tailored to serve specific functions for set durations, decomposing into benign substances through natural processes. These polymers, sourced from food waste, animal residues, agricultural byproducts, and renewable substances like starch and cellulose, are increasingly preferred due to their cost-effectiveness and environmental benefits. Utilizing renewable resources significantly enhances the sustainability of raw materials, promotes efficient biodegradation, and reduces carbon dioxide emissions, aiding in global warming mitigation. Microorganisms such as bacteria and fungi break these polymers down into water, carbon dioxide, and methane, with biodegradation rates dependent on the polymer's composition (Samir et al., 2022). There is notable research focused on converting animal waste, particularly fish waste (including skin and bones), into gelatin. This alternative adds economic value to otherwise discarded fish parts and adheres to halal dietary standards, offering a substitute for gelatin derived from non-permissible sources like pigs and cows and reducing the risk of diseases such as Bovine Spongiform Encephalopathy (Siburian et al., 2020).

The development of new materials with enhanced absorbency and sustainability is a key trend in super absorbent polymer (SAP) research. This trend is driven by the need to address the environmental impact of traditional SAPs, which are often non-biodegradable and derived from petroleum-based sources. The focus is on developing bio-based and degradable SAPs, with a particular interest in protein-based materials due to their sustainability and potential for enhanced absorbency (Capezza et al., 2019). This shift towards sustainable materials is reflected in the increasing research on hydrogel, internal curing, and aerogel, considering current advances and future directions in SAP research (Yang et al., 2021). In a related context, SAPs with moderate absorption capacities (9-10 g of liquid per g) are employed across various industries. They help maintain soil

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moisture in agriculture, absorb leaks in packaging, solidify waste in environmental management, and prevent water penetration in cables. These polymers are also used in personal care, cooling apparel, and food transport to improve comfort, provide cooling effects, and prevent spoilage (Yang et al., 2021). The properties of these polymers are largely dictated by their degree of crosslinking and swelling ratio.

Furthermore, chemical crosslinking, such as with glutaraldehyde (GTA), enhances the mechanical properties and water resistance of gelatin films, forming stable bonds between gelatin chain side groups. As per Bigi et al. (2001), using GTA in pigskin gelatin decreases extensibility and increases stress at the break as GTA concentration rises, effectively crosslinking about 60 % of the protein chains. Conversely, physical crosslinking methods like high-energy irradiation (UV, gamma, and electron beams) provide an eco-friendly alternative by generating reactive intermediates that quickly form durable network structures within the polymer chains without harmful chemicals (Chmielewski, Haji-Saied, and Ahmad, 2005). This approach not only boosts the performance of gelatin-based films but also aligns with sustainable manufacturing practices, particularly in producing absorbent biopolymers based on fish gelatin.

2. Preparation and characterization

Fish gelatin powder (8 g) with a 250-bloom rating was mixed with 100 ml of distilled water using a mechanical stirrer at room temperature. The gelatin powder was purchased from Nichz Ingredient Shop in Shah Alam, Selangor, Malaysia. Subsequently, 30 wt% glycerol was added to the gelatin mixture. Glycerol was purchased from Biotek Abadi Sdn. Bhd., Malaysia. The mixtures were then heated to 50 °C and stirred continuously for 10 min to ensure a homogenous consistency. Following this, the film formulations were crosslinked using Grade II glutaraldehyde (GTA) from Sigma Aldrich, Malaysia, which contains 25 % water. The GTA was added to the gelatin mixture in concentrations of 0.5 %, 1.0 %, 1.5 %, and 2.0 % by weight and mixed for 12 min. Each resulting filmogenic solution, approximately 25 ml, was poured into a clean Petri dish and dried in an oven at 45 °C for 48 h. After drying, the films were carefully peeled from the dishes for further analysis. For the irradiated fish gelatin films, the gelatin solutions excluding GTA were irradiated using an electron beam accelerator, Model EPS 3000 (Cockroft Walton), with doses ranging from 0 to 25 kGy. The accelerator administered these doses at a rate of 5 kGy per pass and an energy of 2.2 MeV.

2.1 Determination of swelling ratio and gel content

This method was proposed by Bigi et al. (2004) with little modification, where the crosslinked gelatin films were weighed at room temperature (approximately 0.5 g). They were then immersed in reverse osmosis (RO) water for 24 h. Wet samples were wiped to remove excess liquid and weighed. The amount of adsorbed water was calculated as;

$$Swelling\ Ratio\ (\%) = \frac{W_w - W_i}{W_i} x100 \quad (1)$$

where Ww and Wi are the weights of the wet and the initial weight before being immersed in RO water. To determine the gel content of the crosslinked films, the swelled samples for GTA crosslinked and irradiated samples were dried in an oven at 60 °C for 24 h and in a vacuum oven at 30 °C for 24 h, respectively. The dried sample was calculated as;

$$Gel\ Content\ (\%) = \frac{W_i - W_d}{W_i} x100 \quad (2)$$

where Wd and Wi are the weights of the dried sample and the initial weight before being immersed in RO water.

2.2 Determination of moisture content

The moisture content was determined using an Infrared Moisture Determination Balance. Approximately 1 cm^2 of cut film was weighed (±0.5 g) and heated at 105 °C for 20 min. After 20 min, the reading of moisture content was recorded.

2.3 Measurement of tensile properties

Tensile strength (Ts) and elongation at break (EB) values were measured using a Universal Tensile Machine (Shimadzu AG-Xplus, 20 kilonewton capacity) from Kyoto, Japan, according to the ASTM D882 method. Film strips measuring 60 mm x 15 mm were prepared using a cutting blade. The gauge length was set at 40 mm, and the film strips were stretched at a rate of 10 mm per min until they fractured. The Ts and EB values were recorded from the average of five samples.

3. Results and discussion

3.1 Determination of swelling ratio and gel content

Figure 1 shows the effects of gel content and swelling ratio on different glutaraldehyde (GTA) contents and various irradiation doses. Gel content gradually increased as the GTA content increased from 0.5 to 2.0 % (w/w) for 8 g, as shown in Figure 1(a). Figure 1(a) shows that the cross-linking fraction can be increased to 57 % and 64 % for 0.5 % and 2.0 % of GTA, respectively. The results from Figure 1(a) show that GTA crosslinking induces a significantly decreased swelling ratio. This might be attributed to the ability to sustain water in the polymer matrix dropping when the crosslinking percentage increased as the crosslinker contents increased. Amadori et al. (2015) also reported the same evidence as this study, which is that the degree of swelling on pig skin gelatin decreases as increasing of GTA content after 6 h of immersion in PBS solution.

(a) (b)

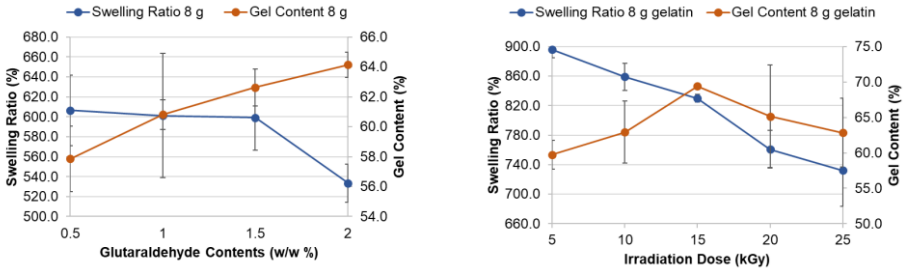

Figure 1: Effect of crosslinking modification on gel content and swelling ratio on the plasticized 8 g of gelatin films; (a) GTA contents (b) irradiation doses

Figure 1(b) shows the effects of irradiation doses (5 to 25 kGy) on gel content and swelling ratio. In the irradiation treatment, plasticized gelatin films must be irradiated in liquid (after gelatin powder is dissolved in water) to induce a crosslinking reaction in a gelatin solution (Haema et al., 2014). The gel content increased rapidly from 5 kGy to 15 kGy, up to 70 % for the gelatin content. After 15 kGy of irradiation dose, the gel content dropped drastically due to the degradation of plasticized gelatin films by irradiation modification. Vieira and Mastro (2002) reported that the viscosities of films decrease in response to an irradiation dose. This might correlate to the degradation stage at this higher dosage of irradiation. In contrast, Haema et al. (2014) and Kimura et al. (2021) reported that gelatin's crosslinking percentage can only achieve around 90 % above 100 kGy irradiation dose. Figure 1(b) also shows the swelling ratio of irradiated plasticized fish gelatin films. The swelling ratio is a measurement of the tendency of the polymer matrix to build up a three-dimensional (3D) structure after being cross-linked (Haema et al.,2014). Furthermore, this 3D network also recognized the capability to sustain water where the low irradiation dose absorbs more water than the higher dose because the crosslinking density is lower. The results show that the swelling ratio decreased rapidly from 5 kGy to 25 kGy. As confirmed, the plasticized gelatin films were successfully irradiated in liquid condition at room temperature. The appropriate crosslinking method for gelatin, particularly focusing on water retention, is crucial to be selected. Irradiation is revealed to be a superior option, capable of achieving high moisture content and effectively inducing crosslinking at lower doses while maintaining a high swelling ratio. This method is considered more sustainable and environmentally friendly as it avoids using chemical additives, thereby enhancing the sustainability of the production process.

3.2 Determination of moisture content

Figure 2(a) shows the moisture content in gelatin samples treated with varying concentrations of GTA, ranging from 0 % to 2 % by weight. The results show a slightly increasing moisture content with increasing concentrations of GTA, especially noticeable beyond 0.5 % glutaraldehyde content. As the GTA concentration increases from 0.5 % to 2 %, moisture content increases. The error bars indicate some variability in the moisture content measurements but are not excessively large (standard deviation around 2), suggesting that the data points are relatively consistent. Liu et al. (2017) reported different results from this study, where crosslinked gelatin films with transglutaminase had lower moisture content than those without crosslinking because of the decrease in water retention due to cross-linking. However, the crosslinking of the plasticized gelatin film by GTA increased the moisture content due to the by-product water from the crosslinking reaction between gelatin and GTA, which then caused a hydrophilic surface on the films (Ai et al., 2002). This is proven by Fourier transform infrared spectroscopy analysis in Figure 3(a). From Figure 3(a), it can be seen qualitatively that the peak ~3,200

cm^{-1} has more intense and sharp peaks with the addition of GTA compared to without GTA (observed from the number of transmittance changes). At this peak, it explains the stretching of the -OH groups of the absorbed water molecules in this region. Furthermore, bending -OH groups of the absorbed water molecules can also be seen qualitatively at the peak ~1,600 cm^{-1}, where the peak is more intense and sharper by increasing GTA content from 0 to 2 % (w/w). Gelatin has been crosslinked by GTA can be seen at the peaks imine (~1650 cm-1), amide II (~1550 cm-1) and aliphatic bend alkyl (~1440 cm-1), there are some changes in intensities especially by increasing GTA contents up to 1% (w/w).

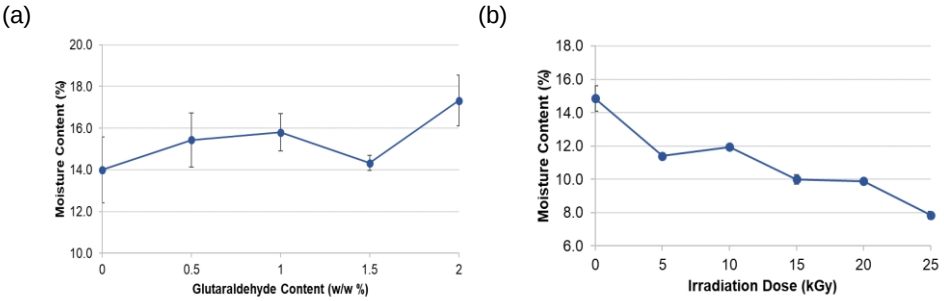

Figure 2: Effect of crosslinking modification on moisture content on the plasticized 8 g of gelatin films; (a) GTA contents (b) irradiation doses

Figure 2(b) illustrates how irradiation doses ranging from 0 to 25 kGy affect the moisture content in plasticized gelatin. It is observed that the moisture content in the 8 g gelatin samples decreases as the irradiation dose increases. According to the gel content results, the crosslinking density is observed to increase from 0 to 15 kGy, which consequently reduces the moisture content of the gelatin films. A further reduction in moisture content is noted after the irradiation dose exceeds 15 kGy, attributed to both the crosslinking and degradation of the gelatin structure. The degradation of gelatin films at higher doses is corroborated by FTIR analysis, as detailed in Figure 3(b). This can be seen at the peak 2,900 cm^{-1} (aliphatic group, CH_2) with some decrease of wavenumber (attributed to the lower bonding energy by irradiation modification at 25 kGy.

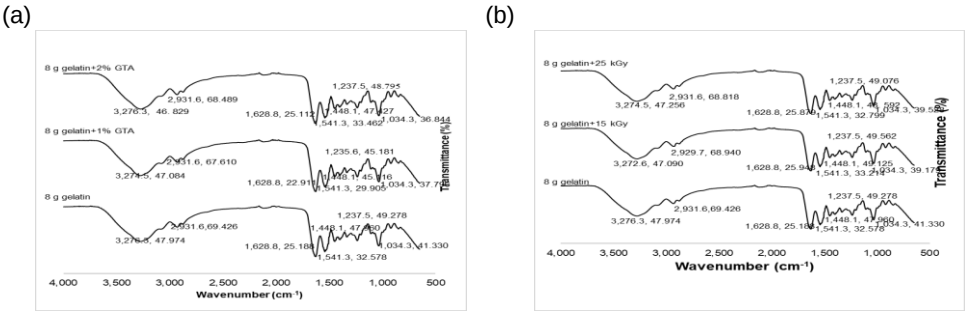

Figure 3: FTIR spectrum on the effect of crosslinking modification on plasticized 8 g gelatin films; (a) GTA contents (b)irradiation doses cm^{-1}

3.3 Tensile properties

Figures 4(a) and 5(a) are depicted as displaying the changes in tensile strength (Ts) and elongation at break (EB) of gelatin crosslinked with varying GTA contents. It is observed from Figure 4(a) that the tensile strength increases up to a GTA content of 1.5 %. This increase in Ts is attributed to the enhanced crosslinking percentage, which strengthens the fish gelatin film, even at lower GTA concentrations. These enhancements in mechanical properties have been similarly noted in previous studies that involved the use of GTA with unplasticized bovine gelatin (Bigi et al., 2001) and genipin with bovine gelatin (Bigi et al., 2002). However, a 2 % (w/w) GTA concentration is shown to reduce the Ts, likely due to the gelatin film reaching excessive brittleness at this level of GTA content. Martucci et al. (2012) also indicated that testing GTA contents higher than 1 % was impractical due to the brittle nature of the films. The EB increased with the GTA-induced crosslinking, and no significant changes were observed in the increased GTA content. A slight reduction is observed at a 2 % (w/w) GTA concentration due to the excessive brittleness.

(a) (b)

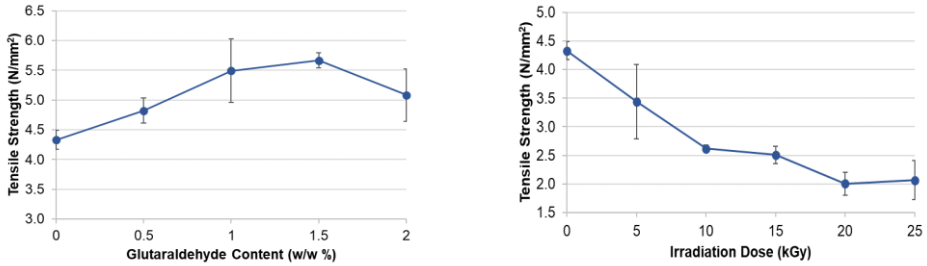

Figure 4: Effect of crosslinking modification on tensile strength on the plastisized 8 g of gelatin films; (a) GTA contents (b) irradiation doses

Figures 4(b) and 5(b) display the changes in tensile strength (Ts) and elongation at break (EB) of gelatin films crosslinked at various irradiation doses. A decline in both Ts and EB was observed due to the irradiation process. Nasreen et al. (2016) noted that exposure to gamma radiation can lead to cross-linking and chain scission in polymers. Specifically, gamma radiation tends to break down polysaccharides or other organic polymers by cleaving glycosidic linkages, particularly in polysaccharide compounds. Gelatin, a protein-based natural biopolymer, is also susceptible to degradation under gamma radiation (Nasreen et al., 2016). Although irradiation-induced crosslinking can somewhat enhance the mechanical strength of plasticized gelatin films, it typically degrades the structure of natural polymers such as polysaccharides and gelatin.

(a) (b)

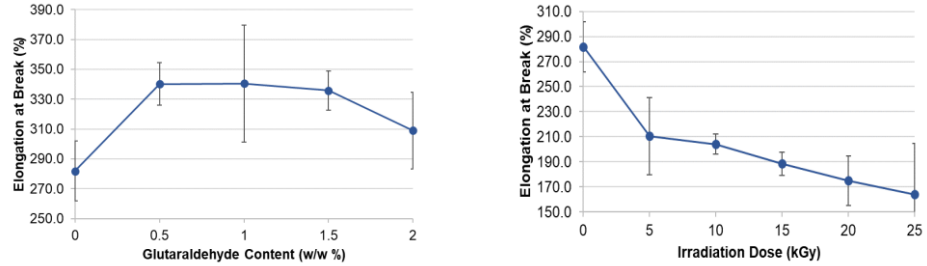

Figure 5: Effect of crosslinking modification on elongation at break on the plasticized 8 g of gelatin films; (a) GTA contents (b) irradiation doses

4. Conclusions

The results show that the gel content for crosslinked GTA in plasticized gelatin was up to 64 % for 2 % GTA by weight. However, the irradiation modification showed up to 70 % only at 15 kGy irradiation dose and then dropped at 20 and 25 kGy due to degradation of biomaterials. The swelling ratio decreased as the crosslinker contents (GTA and irradiation) increased, this was probably due to the water retention decreasing as the 3D network structure was formed by crosslinking modification. Moreover, the moisture content for crosslinked GTA in films increased because the reaction of GTA and gelatin tends to produce water as a by-product. In contrast, irradiation-induced crosslinking made the moisture content of plasticized films drop, which was attributed to the crosslinking and degradation during the modification. The Ts and EB for incorporation of GTA have increased as the GTA contents increased. This shows that this GTA successfully crosslinked the plasticized gelatin films. However, irradiation modification shows a decreasing trend for Ts and EB of plasticized gelatin films. This was probably due to the degradation in bio-film during the irradiation-induced crosslinking.

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