

HARIYANTI-Synthesis of Polyvinyl Alcohol (PVA)-Gelatin Hydrogel from White Snapper (*Lates calcarifer*, Bloch) with Gamma Irradiation and Its Characterizations

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Synthesis of Polyvinyl Alcohol (PVA)-Gelatin Hydrogel from White Snapper (*Lates calcarifer*, Bloch) with Gamma Irradiation and Its Characterizations

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ABSTRACT

The application of nuclear technology in the health sector is increasing. One example is the use of irradiation in production of wound dressings. Research activities have been conducted to study whether polyvinyl alcohol (PVA)-gelatin-based hydrogel from white snapper scales can be processed using gamma irradiation into wound dressings. A series of PVA (10 %) solutions containing gelatin in various concentrations (0-4 %) were treated with 12 freeze-thaw cycles and then irradiated at doses of 10 and 20 kGy. They were subsequently characterized using Fourier transform infrared (FTIR) spectroscopy and scanning electron microscope (SEM). Gel fraction, water absorption, and percentage of hydrogel water evaporation rate were tested gravimetrically, while the elongation at break and the tensile strength of the hydrogels were tested with a universal testing meter. The evaluation showed that the hydrogel gel fraction decreased with increasing gelatin concentration from 0 % to 4 % for both irradiation doses (10 and 20 kGy). The rising gelatin concentration demonstrated that increasing gamma radiation dose improved the hydrogel's water absorption, evaporation rate, tensile strength, and elongation at break. PVA-gelatin hydrogel with irregular pore structure was observed from SEM test results. The FTIR measurement results confirmed the formation of crosslinks in the hydrogel matrix. The PVA-gelatin hydrogel produced through gamma irradiation could be used for wound dressings.

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INTRODUCTION

Gelatin originating from fish scale waste is an alternative ingredient of choice due to its potential for substitution of gelatin extracted from pigs and cows. Gelatin is a natural polymer generated from the hydrolytic degradation of collagen protein, and it has a unique amino acid composition that provides several health benefits [1,2]. Gelatin is a prevalent pharmaceutical and food ingredient and the most abundant ingredient studied in halal research. Religious and cultural convictions,

food fraud prevention, and health concerns drive interest in gelatin source authentication [3]. Currently, many research and development activities have been done to find new gelatin isolation sources.

Characterization activities showed that gelatin from beef, pork, and fish were found at concentrations of 4 %, 6 %, and 8 %, respectively, showing that fish gelatin has lower water vapor permeability compared with beef and pork gelatin. The elongation at break value of fish gelatin was not significantly different from that of pork gelatin. Fish gelatin thickness was not significantly different from beef and pork gelatin thicknesses at 8 % concentration [4].



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White snapper (*Lates calcarifer*) is a species of fish consumed by Indonesians. Its waste can be used as a source for gelatin preparation [5]. Gelatin is non-toxic, biodegradable, and biocompatible in a physiological environment and could accelerate the growth of new cells [12].

Polyvinyl alcohol (PVA) is a synthetic polymer that is non-toxic, non-carcinogenic, biocompatible, resistant to ingredient chemicals, and biodegradable. The characteristic property of PVA is that it can dissolve in hot water at temperatures above 60 °C and increase its solubility by forming a hydrophilic gel solution. However, the mechanical characteristics of PVA hydrogel are inadequate, particularly in its fragility [9]. Therefore, it is necessary to modify it by combining it with synthetic or natural polymers, which not only functions to improve its mechanical properties but also could accelerate its action in wound healing [10,11].

Hydrogel development as biomaterial in the health sector for wound dressings is rapid [12,13]. The purposes are to protect the wound from microbe infection, avoid excessive dehydration, and improve its healing. The hydrogel could be synthesized using the techniques of physics, chemistry, and irradiation [6].

Gamma and electron beams are the most popular tools used for sterilization in health and food preservation fields. At this time, the second type of source energy utilized for the bonding process is polymer/monomer crosslinking and degradation. Gamma and electron beam irradiation are superior to other methods in that, among others, they are fast, they generate no residues, and their irradiation doses can be set following the needs [14].

This study aimed to synthesize a PVA-gelatin hydrogel from white snapper scales by applying gamma irradiation at dose variation of 10 kGy and 20 kGy.

METHODOLOGY

Materials

White snapper scales were obtained from the Center for Research on Product Processing and Biotechnology of Marine and Fisheries, Ministry of Marine Affairs and Fisheries of the Republic of Indonesia, Slipi, Central Jakarta, Indonesia. Polyvinyl alcohol and potassium bromide were purchased from Merck, while distilled water and other chemicals were obtained at pro-analysis quality.

Extraction of gelatin from fish scales

Extraction of gelatin from fish scales was done according to a previous reference [15]. Briefly, about 200 g of cleaned, dried fish scales were immersed in approximately 300 ml of distilled water in a bottle; then, the bottle was closed and autoclaved. Afterward, the solution was heated at a temperature of 121 °C and pressure of 1 atm for 15 minutes. The extracted solution was poured into a plastic tray with a 0.5 cm thickness, and dried in the air for 2 days to obtain gelatin sheets.

Synthesis PVA-gelatin hydrogel

Five portions of 10 % PVA solution in 150 ml of water were prepared, and then into each solution portion, gelatin powder was added in quantities of 0 g, 1.5 g, 3 g, 4.5 g, and 6 g, respectively. Those portions were mixed with a magnetic stirrer until homogeneous at a temperature of 70-80 °C. Each PVA-gelatin mixture was packed in polypropylene plastic with a size of 20 cm × 20 cm and closed until impermeable by air by using a sealing machine. The mixtures were frozen at -4 °C for 18 hours. Matrix hydrogels were then melted (thawed) at room temperature for 6 hours; this step was repeated two more times. Hydrogel matrices were gamma-irradiated at doses of 10 and 20 kGy [11,14,16].

Determination of gel fraction

This step was performed in accordance with Ref. [14]. Three hydrogel samples of polyvinyl alcohol gelatin with sizes of approximately 1 cm × 1 cm were prepared, and then were dried in the oven at 60 °C for 24 hours and weighed to determine its dry weight (W_a). The dried hydrogel samples were then soaked in distilled water at room temperature for 24 hours. They were weighed until constant weights. To calculate the gel fraction, Eq. (1) is used.

$$Gel\ fraction\ (\%) = \frac{W_b}{W_a} \times 100\ \% \quad (1)$$

W_a = Weight of dry hydrogel before immersion (g)
 W_b = Weight of dry hydrogel after immersion (g)

Determination of water absorption

This step was performed according to a published paper [15,17]. Three samples of polyvinyl alcohol-gelatin hydrogel were prepared with a size of approximately 1 cm × 1 cm and dried in an oven

at 60 °C for 24 hours. The dry hydrogel was then put into a beaker glass containing distilled water. After every 1-hour interval, the hydrogel was removed from the beaker glass, the water on the wet hydrogel surface was wiped out with a tissue, and the wet weight (W_b) was measured. Finally, the hydrogel was dried in the oven at 60 °C for 24 hours and then weighed to obtain dry weight (W_k). Equation (2) is used to calculate the water absorption.

$$\text{Absorbed water (\%)} = \frac{W_b - W_k}{W_k} \times 100 \% \quad (2)$$

W_b = Weight of swollen hydrogel (g)
 W_k = Weight of dry hydrogel (g)

Water evaporation

Three snippets of hydrogel with a size of approximately 1 cm × 1 cm were prepared and weighed. They were then placed on a plastic base with one side open to the air in the room temperature. After one hour, the hydrogel was weighed and left exposed to air. Hydrogel was prepared for every 1-hour interval, and then its wet weight (W_b) was determined. Finally, the hydrogel was dried at 60 °C to ensure the accurate weight. Water lost from hydrogel is a percentage of the total amount of water that fades evenly in Eq. (3).

$$\text{Water lost (\%)} = \frac{W_b - W_t}{W_b - W_d} \times 100 \% \quad (3)$$

W_b = Weight of initial hydrogel (g)
 W_t = Weight of measured of hydrogel (g)
 W_d = Weight of last hydrogel (g)

Determination of tensile strength and elongation at break of PVA-gelatin hydrogel

Tensile strength and elongation at break are essential parameters in this study because they are related to the level of elasticity of the hydrogel produced for wound dressings [18].

They were measured using an Instron universal testing meter. Matrix hydrogel wound dressing samples resulting from radiation standard size dumbbells were tested by ASTM with a tensile speed of 30 mm/min at 32 °C. A layer of agar-agar film with a thickness of 0.06-0.08 mm and a length of 5 cm was printed in standard dumbbell form, for a total of five samples for each composition. Both ends are clamped on the Instron machine, with one end moving and the other stationary. The sample was drawn until the matrix of gelatin hydrogel breaks. The equation to calculate the elongation at break is as follows in Eq. (4).

$$E (\%) = \frac{L_b}{L_o} \times 100 \% \quad (4)$$

E = elongation at break (%)
 L_b = the final length of sample
 L_o = the original length of sample

The tensile strength is calculated by the following Eq. (5).

$$\text{Tensile strength} = \frac{F}{A} \quad (5)$$

F = load achieved at break (Kg)
 A = cross-sectional area of material (cm²)

FTIR spectrum measurement of PVA-gelatin hydrogel

The spectra of irradiated PVA, gelatin and PVA-gelatin hydrogel were measured using a Shimadzu IRPrestige-21 FTIR at 4000-400 cm⁻¹. The sample in the form of powder was mixed with a specific amount of KBr and then crushed homogeneously. Then, it was placed into the attenuated total reflection (ATR) instrument, and their spectra were obtained using the IRPrestige-21 FTIR.

RESULTS AND DISCUSSION

Effect of gelatin concentration and irradiation dose on gel fraction

The effect of gamma irradiation dose on PVA-gelatin hydrogel gel fraction is shown in Fig. 1. The gel fraction decreased as the gelatin concentration increased. At 10 kGy irradiation dose, the gel fraction decreased from approximately 68 % to approximately 48 %, while at 20 kGy irradiation dose the gel fraction decreased from approximately 80 % to approximately 48 %. Due to the formation of peroxide compounds, the gel fraction of PVA-gelatin hydrogels did not reach 100 % as a by-product of the reaction of monomers/polymers with water-soluble oxygen and unreacted monomers/polymers. [19,20] and the possibility of gelatin not being completely crosslinked with increasing gelatin concentration [21,22].

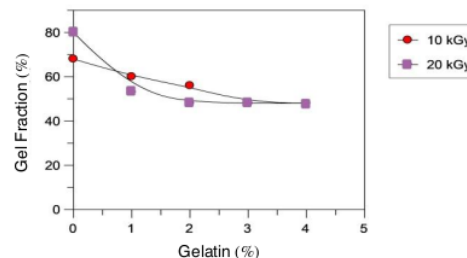


Fig. 1. Effect of gelatin concentration and variations in gamma irradiation dose on the PVA-gelatin hydrogel fraction.

Effect of gelatin concentration on water absorption

Figures 2 and 3 show the relatively high water absorption ability at doses of 10 and 20 kGy gamma-irradiated PVA-gelatin hydrogels. At a dose of 10 kGy, the water absorption increases with measurement duration and reaches a maximum value at 1 % gelatin concentration. Maximum water absorption occurs after 8 hours, and the hydrogel's high water absorption capacity reaches 357 %. Meanwhile, at the 20 kGy irradiation dose, the water absorption increases with measurement duration and reaches a maximum value at 3 % gelatin concentration. Maximum water absorption occurs after 8 hours, and the hydrogel's high water absorption capacity reaches 388 %. The hydrophilic OH-, COOH-, and NH- groups that bind to water contribute here. The water absorption by the hydrogel remains constant because it is already immersed in water or in a condition of equilibrium [14].

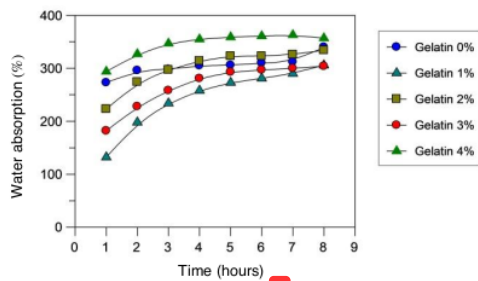


Fig. 2. Relationship between immersion time and water absorption of PVA gelatin hydrogel at irradiation dose of 10 kGy.

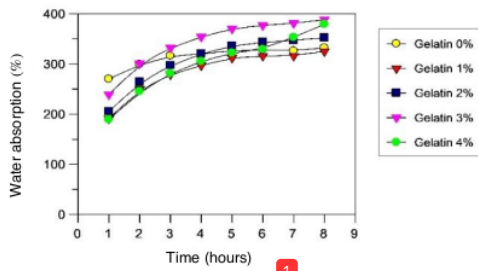


Fig. 3. Relationship between immersion time and water absorption of PVA gelatin hydrogel at irradiation dose of 20 kGy.

Saarai et al. (2011) estimated that the ability of hydrogel to absorb water used for wound dressing applications is between 200 % and 500 % [23]. This research results show that the PVA-gelatin hydrogel attains a percentage of water absorption value that

meets the requirements for a wound dressing application [24].

Effect of gelatin concentration on the water evaporation

Determining the water evaporation is very important for determining the amount of water trapped in the PVA-gelatin hydrogel that is due to the application of the PVA-gelatin hydrogel as a dressing for wounds from wet conditions to dry conditions at the end of its application. It is known that a higher water absorption rate can prevent wound exudate accumulation and a lower evaporation rate can avoid frequent dressing changes [25].

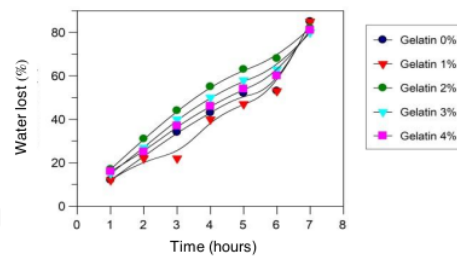


Fig. 4. Relationship between hydrogel water evaporation rate and evaporation time with variations in gelatin concentration at irradiation dose of 10 kGy.

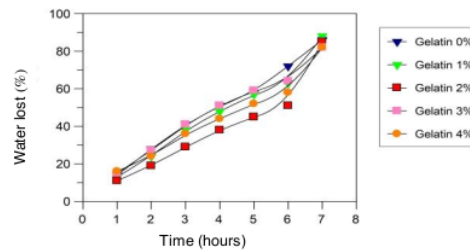


Fig. 5. Relationship between hydrogel water evaporation rate and evaporation time with variations in gelatin concentration at irradiation doses of 20 kGy.

Figures 4 and 5 show that as the water evaporation rate increased for up to 7 hours and the gelatin concentration increased up to 4 %, the evaporation rate of hydrogel water at 7 hours at a dose of 10 kGy gamma irradiation (80 % - 85 %) was lower than the evaporation rate of hydrogen water at a dose of 20 kGy gamma irradiation (82 %-88 %). The increase in the water evaporation rate is caused by an increase in the gamma irradiation dose, and the hydrogel's cross-density increases. Since the rate of water evaporation from the hydrogel matrix is reduced, it may be utilized as a wound dressing for up to 7 hours.

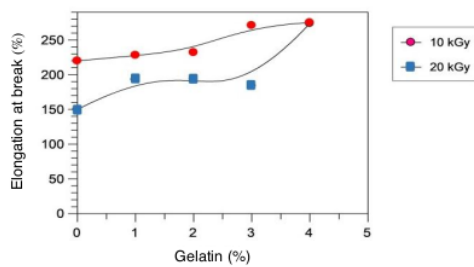


Fig. 6. Effect of gelatin concentration and variation of irradiation dose on the elongation at break of the 10 kGy and 20 kGy PVA-gelatin hydrogels.

The effect of gelatin concentration on the elongation at break of hydrogels resulting from gamma irradiation at doses of 10 kGy and 20 kGy is presented in Fig. 6. Irradiation dose and gelatin concentration influence the elongation at break of hydrogels. The elongation at break increased by 184 % at the dose of 10 kGy, and 271 % at the dose of 20 kGy. These results were produced by a gelatin concentration of 3 %. Increasing the irradiation dose led to increased crosslinking, which causes the hydrogel to be flexible and not brittle. Thus, it is safe and comfortable when applied, and does not cause damage to the skin when removed from the wound site.

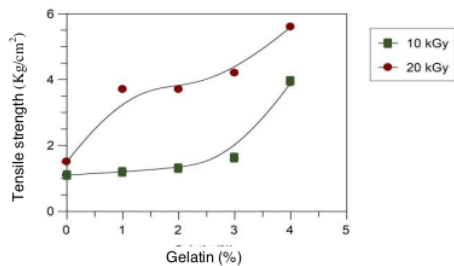


Fig. 7. Effect of gelatin concentration and variations in gamma irradiation dose on the tensile strength of the 10 kGy and 20 kGy PVA-gelatin hydrogels.

Furthermore, the effect of gelatin concentration on the tensile strength of the 10-kGy and 20-kGy gamma-irradiated hydrogels is shown in Fig. 7. It can be seen that with increasing gelatin concentration from 0 % to 4 %, the hydrogel tensile strength tends to increase. At an irradiation dose of 10 kGy, there was an increase in the tensile stress of the hydrogel from 1.1 kg/cm² to 3.9 kg/cm² while at an irradiation dose of 20 kGy, there was an increase in the tensile stress of the hydrogel from 1.5 kg/cm² to 5.6 kg/cm². At concentration of 0 % gelatin and at 20 kGy, the tensile strength of the hydrogel (1.5 kg/cm²) was greater than at an irradiated dose of 10 kGy with a gelatin concentration of 0 %

(1.1 kg/cm²). At concentration of gelatin 3 % and at 20 kGy, the tensile stress of the hydrogel at break is 4.2 kg/cm² and at dose of 10 kGy is 1.6 kg/cm². The increasing tensile strength at break of the hydrogel at a dose of 20 kGy continued to increase as the gelatin concentration was increased.

FTIR spectrum analysis

FTIR spectrum of PVA powder, gelatin powder, and PVA-gelatin hydrogel produced at 20 kGy irradiation is presented in Fig. 8. It can be seen that the FTIR PVA spectrum consists of peak bands in the wavelength region of 3433.29 cm⁻¹ (OH) and 2924.09 cm⁻¹ (CH stretch). The FTIR spectrum of gelatin consists of three peak bands in the wavelength region of 3473.80 cm⁻¹, 2924.09 cm⁻¹, and 1737.86 cm⁻¹ which shows the functional groups of NH, CH stretching, C=O stretching (ketone), respectively.

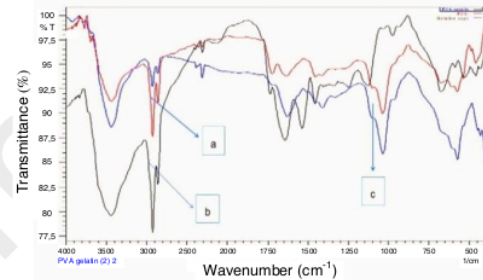


Fig. 8. FTIR spectrum of (a) PVA, (b) gelatin, and (c) PVA-gelatin hydrogel.

Based on the interpenetrating polymer network (IPN) shown in Fig. 9, it can be explained that the spectrum produced by gamma-irradiated PVA-gelatin hydrogel at wave numbers in the 3400-3500 cm⁻¹ range has a smaller spectrum area than PVA spectrum and larger than gelatin spectrum. The spectrum area changes because gelatin penetrates the PVA network structure and forms an interpenetrating structure network [26,27].

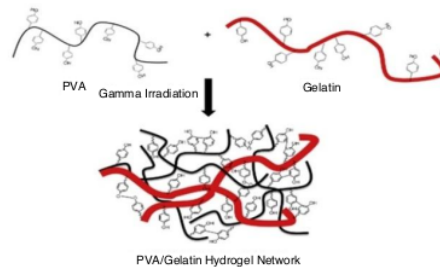


Fig. 9. IPN PVA-g hydrogel structure.

SEM analysis

SEM analysis of gamma-irradiated hydrogel PVA-gelatin photomicrograph with a magnification of 500× is shown in Fig. 10. It is known that the surface morphology of the hydrogel is porous and fibrous, forming holes with a diameter of 18.75 to 25 μm. The method of freeze-drying might result in irregular pores. The pores in the hydrogel network allow water to pass through. It shows the presence of crosslinks in the white snapper scales' polyvinyl alcohol-gelatin hydrogel structure [28].

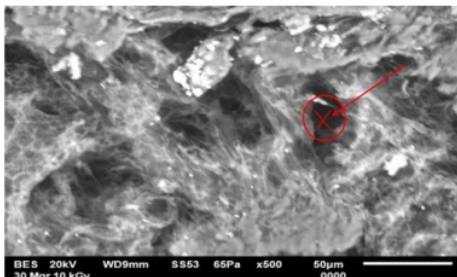


Fig. 10. SEM photomicrograph of PVA-gelatin hydrogel after gamma irradiation of 10 kGy at 500× magnification

CONCLUSION

The irradiation dose and gelatin concentration influence the PVA-gelatin hydrogel of white snapper scales. Increasing the gelatin content in the PVA-gelatin hydrogel significantly increases water absorption, water evaporation, tensile strength, and elongation at break of the hydrogel increases. The opposite trend occurs in the gel fraction, which decreases with an increase in gelatin concentration. The hydrogel of PVA-gelatin from white snapper scales is best for wound dressing at a gelatin concentration of 4 % and an irradiation dose of 20 kGy.

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AUTHOR CONTRIBUTION

We state that all authors contributed equally as primary contributors to this paper. The paper's final version was read and approved by all authors.

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