

EFFECT OF GAMMA IRRADIATION ON MECHANICAL AND THERMAL PROPERTIES OF FISH GELATIN FILM ISOLATED FROM *Lates calcarifer* SCALES

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Received January 14, 2013; Accepted March 14, 2013

ABSTRACT

The objective of this research was to investigate the effect of gamma irradiation on mechanical and thermal properties of fish gelatin films prepared from scales of *Lates calcarifer*. The films were irradiated by gamma rays at varied doses (0–50 kGy). The mechanical and thermal properties of irradiated gelatin films were measured by using colorimeter, Universal Testing Machine, Differential Scanning Calorimetry (DSC), and Fourier Transform Infrared (FTIR) spectrophotometer. The results showed that increasing of irradiation dose up to 50 kGy, the color of irradiated film did not change significantly ($p < 0.05$). The tensile strength of irradiated film was increased with no differences among dose variation but there was no change on elongation at break value ($p < 0.05$). The DSC spectra of irradiated gelatin films showed that irradiation did not affect melting temperature (T_m). In contrast, the glass transition temperature (T_g) of irradiated film has slight tendency to increase with increasing of radiation doses. In general, the FTIR spectra confirmed that gamma irradiation up to 50 kGy affected the mechanical properties of gelatin films.

Keywords: gamma irradiation; fish gelatin film; mechanical property; thermal property; *Lates calcarifer*

ABSTRAK

Penelitian ini bertujuan menginvestigasi efek radiasi gamma terhadap sifat mekanik dan termal film gelatin ikan yang dipreparasi dari sisik kakap putih (*Lates calcarifer*). Film gelatin diiradiasi pada variasi dosis (0–50 kGy) menggunakan sinar gamma. Sifat mekanik dan termal film iradiasi diukur menggunakan kolorimeter, Universal Testing Machine, Differential Scanning Calorimetry (DSC), dan spektrofotometer inframerah (FTIR). Hasil menunjukkan bahwa dengan meningkatnya dosis radiasi hingga 50 kGy tidak menyebabkan perubahan warna film secara signifikan ($p < 0,05$). Tegangan putus film iradiasi meningkat tanpa perbedaan di antara variasi dosis, sebaliknya, tidak terjadi perubahan perpanjangan putus film gelatin ($p < 0,05$). Spektra DSC film gelatin iradiasi menunjukkan bahwa iradiasi tidak memengaruhi T_m (melting temperature) film gelatin. Sebaliknya, T_g (glass transition temperature) film gelatin iradiasi cenderung meningkat dengan meningkatnya dosis iradiasi. Spektra FTIR mengkonfirmasi bahwa iradiasi sinar gamma pada dosis hingga 50 kGy memengaruhi sifat mekanik film gelatin secara umum.

Kata Kunci: iradiasi gamma; film gelatin ikan; sifat mekanik; sifat termal; *Lates calcarifer*

INTRODUCTION

Gelatin film has been the focus of many researches for many years since it is a biodegradable and environmental friendly polymer [1]. In term of biomaterial applications, gelatin possesses attractive properties as it is a partially crystalline polymer and has a relatively low melting point [2-3]. Gelatin has been used as a base material in wide range application because its biosafety has been proved through its long clinical usage as surgical biomaterial [4], wound dressings [5-6], and in drugs delivery system [7-8].

Most research on gelatin film has focused on gelatin derived from mammalian sources such as bovine and porcine. Recently, there has been more interest in using fish as alternative sources of gelatin, due to religious considerations or fear of bovine spongiform encephalopathy [10]. Several studies on fish gelatin have been conducted by using gelatin from bigeye snapper and stripe red snapper [11-13], Baltic cod [14-15], tilapia [16], and tuna [17]. But for application in biomaterial, fish-sourced gelatin is not preferred because it has lower mechanical properties than mammalian-sourced [18]. Therefore, fish gelatin modification is being developed gradually.

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Gamma irradiation is one of noble tools to improve mechanical properties of gelatin film. By means of radiation, the hydroxyl and superoxide anion radicals are generated from radiolysis of water molecules in medium which can modify the molecular properties of protein films by cross linking reaction [19]. Gamma irradiation provides advantages over other physical and chemical treatment for biomedical application as it has high penetration in medium, do not increase the medium temperature, and do not leave chemical residue [20]. Gamma irradiation is also commonly involved in sterilization of gelatin film and in functionalize gelatin molecule through grafting [21-22].

Several studies about effect of gamma radiation to mechanical properties of gelatin has been published [23-25], but most were concerned with the secondary structure of gelatin molecules in solution or gel state and little is known about film state. Indeed, irradiation of gelatin in film state is sometimes unavoidable such as sterilization of gelatin-based scaffold and grafting of gelatin-based film [26]. So that it is important to study the effect of gamma irradiation to properties of gelatin film in order to figure out the change on structure of gelatin molecule in irradiated film.

Baramundi (*Lates calcarifer*) is one of the commercially important fish species in Indonesia. In local markets, the fish was processed to obtain its meat and scales were dumped without utilization causing environmental pollution and resources waste. *Lates calcarifer* scale was known constituted significant amount of collagen as source of gelatin extraction. For making effective use of *Lates calcarifer* scale as a gelatin resource, it is necessary to study the characteristic of its properties. So, we isolated gelatin from *Lates calcarifer* scales and then prepared film from it by casting at room temperature overnight. The objective of our research was to investigate the effect of gamma irradiation on mechanical and thermal properties of fish gelatin films prepared from scales of *Lates calcarifer*.

EXPERIMENTAL SECTION

Materials

Scales of *Lates calcarifer* was purchased from local market. Distilled water was used for gelatin extraction. All the other reagents were of analytical grade and used without further purification. Distilled water was used for isolation of gelatin.

Instrumentation

Gelatin was extracted from *Lates calcarifer* scales using autoclave, Rayva. The chemical changes of fish gelatin films were analyzed using Fourier Transform

Infrared (FTIR) IR-Prestige-21 spectrometer model 800 series, Shimadzu. Thermal properties of films were determined using DSC-60, Shimadzu. Tensile strength and elongation at break of films were measured using Universal Testing Machine Strograph-R1, Shimadzu. Color differences of irradiated films were measured using Chroma Meter CR-200b, Minolta.

Procedure

Preparation of irradiated gelatin films

Fish gelatin was isolated from scales of *Lates calcarifer* using distilled water at 121 °C and pressure 1–1.5 atm for 20 min. After the process, the prepared gelatin solution was separated from remaining scales and then dried at room temperature for 48 h. Fish gelatin films were prepared by dissolving dried gelatin in distilled water with concentration of 10% (w/v) and homogenized while stirring at 200 rpm for 1 h at room temperature. The gelatin solution was poured into polypropylene mould (13 x 8x 0.1 cm³), and casting at room temperature overnight. The gelatin films were then packaged and sealed in double polypropylene (PP) plastic and irradiated by gamma rays of Co⁶⁰ at the doses from 10 kGy up to 50 kGy.

Color and mechanical test

Colors changes of irradiated gelatin films were measured using a colorimeter (Chroma Meter CR-200b, Minolta). The gelatin film was placed on a white plate and the CIE 1976 color scale was used to measure relative color change: L = 0 (black) to L = 100 (white); a = -80 (greenness) to a = 100 (redness); and b = -80 (blueness) to b = 70 (yellowness).

Mechanical properties of irradiated films such as tensile strength and elongation at break were measured using Universal Testing Machine (Strograph-R1, Shimadzu). Irradiated films were moulded using dumbbell ASTM-0-1822-L for the preparation of the standard size measurement. The crosshead speed was set at 25 mm/min. The resultant data was showed at the recorder. The elongation at break was defined by Eq. 1:

$$\text{Elongation at break} = L_1/L_0 \times 100\% \quad (1)$$

which L_0 is the original length and L_1 is the final length.

The tensile strength was defined by Eq. 2:

$$\text{Tensile strength} = F/A \quad (2)$$

where F is the amount force of exerted while stretching a sample until the samples fails (kg), and A is the area of the samples (cm²).

Gel fraction of gelatin film was measured by immersing the film in a water bath for 24 h at room temperature. The swollen gelatin gel was then placed in a 60 °C oven for 2 days to completely dry the samples. The gel fraction was defined by Eq. 3:

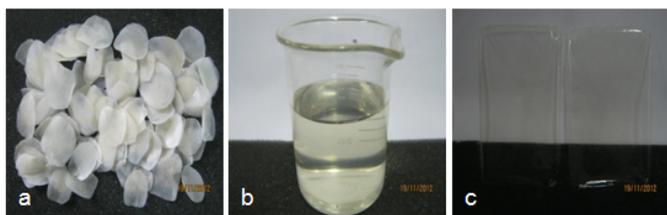


Fig 1. Raw material for extraction and gelatin product: (a) scales of *Lates calcarifer*, (b) gelatin solution, and (c) gelatin films

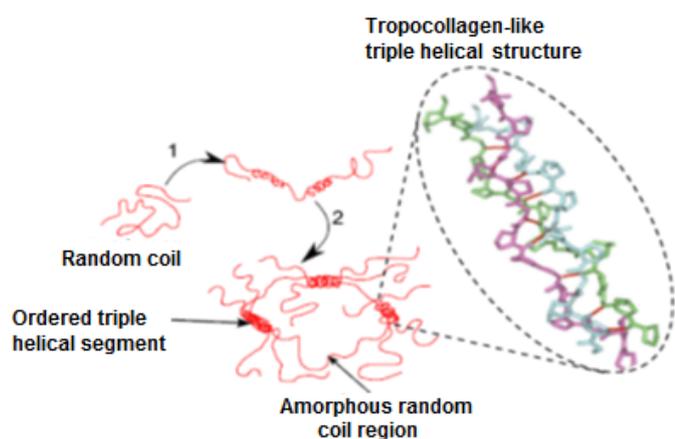


Fig 2. Schematic representation of the partial renaturation of gelatin by physical cross links during film formation

Gel fraction = $(W_d/W_i) \times 100\%$ (3)
 which W_d is the oven-dried temperature, and W_i is the initial weight of the gelatin film.

DSC characterization

A thermal analysis instrument (DSC-60, Shimadzu) was used to determine the thermal properties of irradiated films. The amount of gelatin films to measure DSC spectra was 5.0 ± 0.1 mg). Each sample was heated from 23°C to 200°C in air at a rate $10^\circ\text{C}/\text{min}$.

FTIR characterization

FTIR was used to characterize the presence of specific chemical groups in the irradiated gelatin films. FT-IR spectra were obtained over the range of $4000\text{--}400\text{ cm}^{-1}$. Samples were milled and mixed with dried KBr powder placed in a sampling cup, 20 scans were required at 2 cm^{-1} resolution with subtraction of the KBr background. FT-IR spectra were also obtained for pure gelatin for comparison with the irradiated gelatin films.

Statistical analysis

All the statistical analysis was performed by One-Way ANOVA and least significant difference (LSD) test with significance level defined at $p < 0.05$.

RESULT AND DISCUSSION

Fish Gelatin Film

As shown in Fig. 1, isolation of gelatin from *Lates calcarifer* scales using distilled water as solvent produced a transparent, viscous, slightly yellowish solution and exhibited mild fishy odor. Unlike mammalian gelatin, extracted gelatin was in sol phase at room temperature. Relatively lower gelling temperature of fish gelatin compared to mammalian gelatin was related to lower amounts of amino acids proline and hydroxyproline in fish gelatin molecule [27].

Gelatin film was obtained by casting from gelatin aqueous solution at room temperature, as shown in Fig. 1. Gelatin films were easy to handle and its thickness was 0.2549 ± 0.0624 mm. During film formation, gelatin molecule was partially renaturated as schematically shown in Fig. 2 [28].

The gelatin oligomers are strongly intertwined, forming extended physical cross links resulting from the partial reversion of polypeptide chains into ordered triple helical segments, whose structure is similar to that of tropocollagen [28]. The helical structure is obtained if only the solvent is evaporated at room temperature [29-30].

The Effect Irradiation Dose on Color Gelatin Films

In visual observation, gelatin films remain clear and transparent after irradiated at doses up to 50 kGy. Colorimetry measurement also indicated no significant ($p < 0.05$) changes on color values for gelatin films with increasing irradiation dose up to 50 kGy as shown in Table 1.

In contrast, several reports showed that gamma irradiation treatment increases yellowness of gelatin film as showed by higher b^* of Hunter color value [31-33]. Research by Inamura et al. [33] concluded that yellowness of pigskin gelatin film was significantly increase after irradiated at 10 and 40 kGy compared to control. Yellowness shift in the irradiated protein samples is usually due to the disruption of the ordered structure of protein molecules and also by interaction between rendered amino group ($-\text{NH}_2$) derived from hydrolysis process and carbonyl group in gelatin via Maillard reaction [31-32].

Lee et al. [31] stated that color change of gelatin films under radiation is general, but is not universal. In this research, color of gelatin film relatively resistant against radiation. We can assume that this condition related to intrinsic characteristic of gelatin but the underlying reason is not yet clear.

Table 1. The dose effect of gamma irradiation on color change of gelatin films

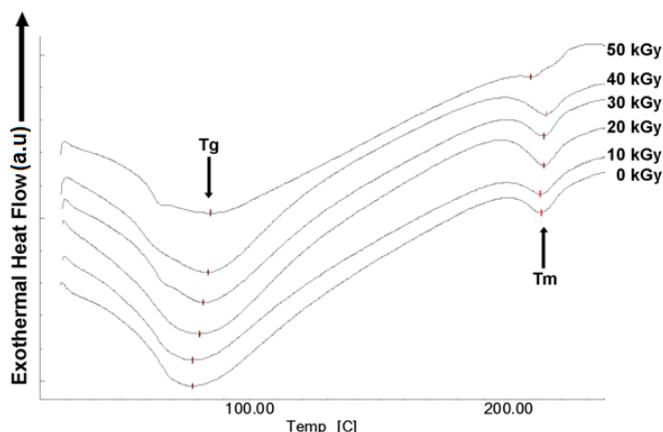
Irradiation dosage (kGy)	Hunter color values		
	L*	a*	b*
0	61.9 ± 0.2 ^a	46.8 ± 0.5 ^a	-36.7 ± 0.9 ^a
10	61.7 ± 0.2 ^a	46.6 ± 0.3 ^a	-36.2 ± 0.5 ^a
20	61.8 ± 0.4 ^a	46.8 ± 0.4 ^a	-36.5 ± 0.9 ^a
30	61.8 ± 0.2 ^a	46.5 ± 0.3 ^a	-36.2 ± 0.5 ^a
40	61.8 ± 0.1 ^a	46.5 ± 0.3 ^a	-36.1 ± 0.5 ^a
50	61.8 ± 0.2 ^a	46.4 ± 0.4 ^a	-35.9 ± 0.6 ^a

Same letter (^a) within the same column did not differ significantly ($p < 0.05$; $n = 10$)

Table 2. Mechanical properties of fish gelatin films irradiated at different irradiation doses.

Radiation dose (kGy)	Elongation at Break (%)	Tensile Strength (kg/cm ²)	Gel Fraction (%)
0	10 ± 0.1 ^a	737.66 ± 28.28 ^a	1.43 ± 0.63 ^a
10	10 ± 0.1 ^a	805.94 ± 56.62 ^b	3.80 ± 1.26 ^{ab}
20	10 ± 0.1 ^a	813.84 ± 23.94 ^b	5.04 ± 1.76 ^b
30	10 ± 0.1 ^a	829.25 ± 26.48 ^b	5.22 ± 1.86 ^b
40	10 ± 0.1 ^a	831.74 ± 40.21 ^b	5.75 ± 1.12 ^b
50	10 ± 0.1 ^a	825.34 ± 39.84 ^b	6.27 ± 2.22 ^b

Same letter (^a) within the same column did not differ significantly ($p < 0.05$; $n = 5$)

**Fig 3.** DSC curves of fish gelatin films irradiated at different irradiation doses

Effect of Irradiation Dose on Mechanical Properties of Gelatin Films

Effect of gamma radiation on tensile strength and elongation at break of the films at doses up to 50 kGy are shown in Table 2. Elongation at break of gelatin films did not affected gamma irradiation ($p < 0.05$). Brittleness of gelatin film at glassy state is well understood as gelatin molecular structure become rigid if water content is low. Interestingly, it is observed that gamma irradiation was significantly improved tensile strength of gelatin film ($p < 0.05$). Tensile strength and gel fraction of irradiated gelatin film increased significantly compared to control. But, the improvement seems not correlated to increase of radiation doses as tensile strength and gel fraction was not significantly difference among irradiation dose variation.

This result was in good agreement with reports by Jo et al. [23], Lacroix et al. [34], and Ressouany et al. [35] which concluded that mechanical properties of protein films can be increased by gamma irradiation. Jo et al. [23] reported the significant increased on tensile strength of gelatin-based film after irradiated at 10 kGy. Lacroix et al. [34] indicated that an irradiation of 32 kGy increased the puncture strength significantly in the whey protein isolate, caseinate blend or soy protein isolate film. Ressouany et al. [35] explained that gamma irradiation generates free radicals that induce chemically cross linking reaction among protein molecules at doses higher than 16 kGy.

Chemical cross links among gelatin molecules strengthen and stabilized 3D network structure of molecule gelatin formed by physical cross links during film formation [36]. Chemical cross links was proven by the increase of gel fraction of irradiated gelatin film and caused change on mechanical behavior of gelatin film such as increasing tensile strength of the film [37-39]. But not like result of Jo et al. [23], degradation of gelatin film in this research not yet occur at irradiation doses up to 50 kGy as no significant decrease on tensile strength of gelatin film.

DSC Characterization

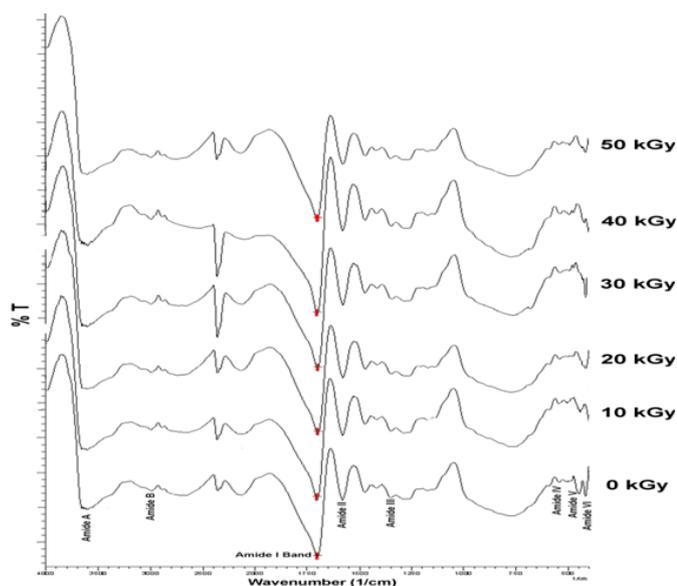
The DSC curve of fish gelatin films exhibited an endothermic peak as shown in Fig. 3. The endothermic peak is typical of thermo-reversible material [2,40] and is considered as melting temperature (T_m). Endothermic peaks of collagenous material are generally associated with the helix-coil transition of collagen, the rupture of hydrogen bonds and a

Table 3. Glass transition temperature (T_g) and melting temperature (T_m) of fish gelatin film irradiated at different irradiation doses

Radiation dose (kGy)	T _g (°C)	T _m (°C)
0	59.23	214.02
10	61.58	214.90
20	64.12	213.18
30	65.19	211.88
40	66.55	212.16
50	65.83	213.19

Table 4. Characteristic infrared bands of peptide linkage

Designation	Approximate frequency (cm ⁻¹)	Description
Amide A	3300 - 3270	NH stretching
Amide B	3100 - 3030	NH stretching
Amide I	1600 - 1690	C=O stretching
Amide II	1480 - 1575	CN stretching, NH bending
Amide III	1229 - 1301	CN stretching, NH bending
Amide IV	625 - 767	OCN bending
Amide V	640 - 800	Out-of-plane NH bending
Amide VI	573 - 606	Out-of-plane C=O bending

**Fig 4.** FTIR spectra of fish gelatin films irradiated at different irradiation doses

rearrangement of the triple helix into a random configuration [41-42].

Irradiated and control gelatin films showed glass transition temperature (T_g) at around 60 °C with broaden peak, as shown in Fig. 3. The T_g of gelatin film which lower than 150 °C indicated the gelatin films were not perfectly dehydrated [43]. But, moisture content of gelatin film is considered difficult to be controlled as gelatin film is easily dehydrated by water vapor in air. Increasing water content of gelatin film decreases the intensity, shift to lower temperature and broadens of the T_g peak because water has a plasticizer effect to gelatin film [44]. Sobral et al. [40] also reported that gelatin has

glass transition temperature at 49 °C when they conditioned at 75% of relative humidity.

As shown in Table 3, T_g of irradiated film showed slight tendency to increase in relation to radiation doses. This result also indicated that gamma irradiation induced cross linking reaction in molecules of gelatin film. Plazek and Ngai [45] noted that one possible way to increase T_g is by increasing the cross link density. Reutner et al. [46] by using X-ray and calorimetry study proved that collagen-like structure of gelatin was rebuilt by involving water as part of its formed crystals. Natively, water molecules absorbed into gelatin are found to be only partially crystallizable. The fraction of crystallizable water increases as increases of cross linking density. The evaporation of water and specifically of the crystallizable water giving rise to the T_g of gelatin film [46-48]. Several researches also reported that increased in T_g observed with the increase in cross linking of gelatin by chemical cross linker [48-50].

The T_g of irradiated film was spread between 211.88 °C and 214.90 °C with no correlation with radiation doses, as shown in Table 3,. This value was very close to the T_m of control film at 214.02 °C. Previous studies generally found that cross linking did not change the melting point of gelatin film [14,42,51].

FTIR Characterization

The FTIR spectra of gelatin extracted from *Lates calcarifer* scales using distilled water at 121 °C and pressure 1–1.5 atm for 20 min are shown in Fig. 4. It reveals that the FTIR spectra of gelatin films control (0 kGy) shows a typical spectrum for fish gelatin with three major peak regions marked as 1 (3600–2700 cm⁻¹),

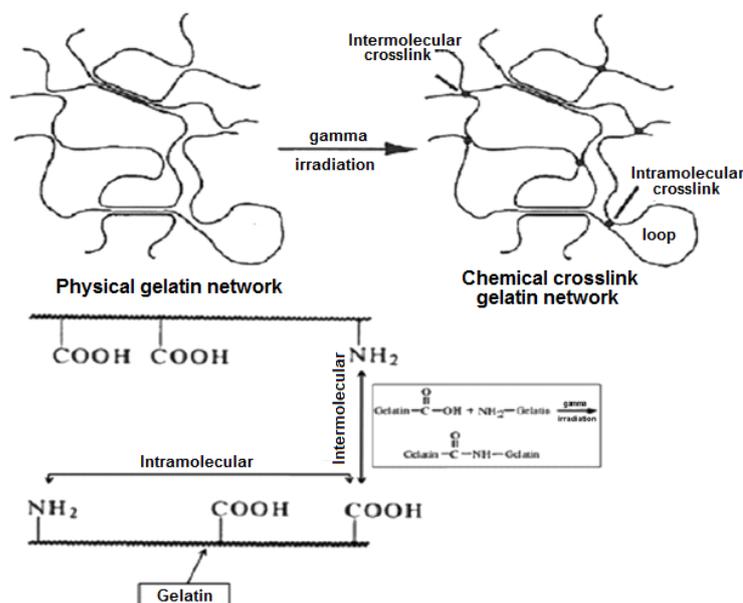


Fig 5. Illustrations of the effect of cross linking on gelatin network structure

2 ($1900\text{--}900\text{ cm}^{-1}$), and 3 ($900\text{--}400\text{ cm}^{-1}$) related to vibration of the peptide linkage. The regions are assigned to the bonds: Amide A and B; Amide I, II, and III; and Amide IV, V, and VI [52-54]. The characteristic IR bands of gelatin are listed in Table 4.

The spectrum of irradiated gelatin film at doses up to 50 kGy exhibited similar peaks with control, as shown in Fig. 4. Three major regions were showed similar shape and wave numbers at the peak. But it is observed that the intensity of transmittance at amide I band that was lower than control film.

The amide I are the most prominent vibrational bands of the protein backbone as it derived mainly (approximately 80%) from the C=O stretch vibrations of the peptide linkage [52,55-57]. Lower intensity of transmittance at amide I band of irradiated gelatin film indicated that gamma irradiation induced cross linking reaction in molecules of gelatin film through formation peptide linkage. As amide I band is the most sensitive spectral region to small variations in molecular geometry and hydrogen bonding patterns, lower intensity of transmittance may also related to formation secondary structure such as α -helix and β -sheet of collagen-like gelatin molecular. Gelatin network structure after chemically cross linked was illustrated in Fig. 5 [58].

FTIR spectra of irradiated gelatin films indicated that carboxyl groups (-COOH) and amine groups (-NH₂) in gelatin molecules are functional groups that involved in formation of peptide bond. Reaction between carboxyl and amine groups is induced by gamma radiation through indirect ionizing interaction. We hypothesized that radiolysis of water forms free radical that ionized carboxyl groups in gelatin molecules. The ionized

carboxyl group is a very strong base which quickly forms corresponding amide when amine is present.

CONCLUSION

In summary, transparent gelatin film has been prepared from fisheries waste in the form of *Lates calcarifer* scales. Gamma irradiation at doses up to 50 kGy significantly ($p < 0.05$) improved the tensile strength and gel fraction of fish gelatin film without significant ($p, 0.05$) change in Hunter color values. An increase of Tg of irradiated film related to radiation dose variation was also observed. FTIR spectra confirmed that improvement of mechanical and thermal properties was caused by cross-linking reaction that generated peptide linkage among gelatin molecules in irradiated film. We hypothesized the reaction mechanism involving radiolysis of water by gamma irradiation that generates free radicals which that caused ionization of carboxyl groups in gelatin molecule. The ionized carboxyl group is then reacted to amine group forms corresponding amide.

ACKNOWLEDGEMENT

Author gratefully acknowledge to colleagues at irradiation facilities PATIR-BATAN which has been providing the samples irradiation.

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