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Alteration of Physico-mechanical Properties of Black Tilapia Scale Gelatins using UVA and UVC Irradiation

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Abstract: Fish gelatin is abundant, relatively low cost and biodegradable. However, their inferior mechanical and rheological properties make them less competitive compared to mammalian gelatins. Hence, the effects of Ultraviolet (UV) irradiation on the properties of scale gelatins were analyzed. Gelatins were extracted from black tilapia scale via thermal extraction method. The gelatins were then subjected to Ultraviolet-A (UVA) and Ultraviolet-C (UVC) irradiation for 0.5 to 2.5 h and the changes in gel strength, viscosity, and melting temperature were observed. Results obtained show a marked increase in the gel strength and viscosity of the gelatins. However, the effects on the melting temperature are minimal. Treatment with UVA and UVC improved the gel strength of the gelatins up to 5.12 ± 0.22 N and 4.75 ± 0.09 N, respectively. Further analysis using Fourier Transform Infrared (FTIR) Spectroscopy showed crosslinking formation in the polypeptide chains induced by UV irradiation. UVA was found to be more effective in enhancing the properties of scale gelatins compared to UVC. In general, UV-irradiated scale gelatins showed excellent properties compared to the commercial bovine gelatin. Results indicated the prospects of employing UV treatment in enhancing the properties of fish gelatin.

Keywords: Fish gelatin, fish scale, UV irradiation, biopolymer, gel strength

1. Introduction

Gelatin, a natural polymer, is a unique protein substance with extensive range of applications in food, medical and technical industries [1]. Gelatins that are originally made from pig and bovine hides are used widely all around the world, which accounts 98.5 % of the world gelatin production [2]. These existing gelatins do not meet the requirements of Halal market in Islamic countries [3,4]. Despite religious restrictions, food safety concerns caused by prions from the infected mammalian animals also contributes to the limitations of mammalian derived gelatins [5,6]. Hence, the growing sociocultural and health concerns have led to the increase in emphasizing research on gelatin extraction based on marine lives [7]. In recent times, studies on fish gelatin as substitute to mammalian gelatin are being pursued extensively [8].

Though research and publications regarding the usage of fish gelatins have been increasing recently, its applications are still limited. This is due to the inferior properties of fish gelatins particularly in the gel strength and melting point compared to mammalian gelatins [9]. For the past years, researchers have come out with several methods to improve the properties of fish gelatin. Among the most commonly used methods are enzymatic crosslinking, chemical crosslinking, addition of polysaccharides and salts, and ultraviolet (UV) irradiation [9,10].

UV irradiation is a physical, cost effective, and ecologically friendly technology widely used in preservation and decontamination of food products [11]. In gelatin studies, UV irradiation is used to generate radicals in the amino acids

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which in return lead to stronger crosslinking formation. However, stronger UV doses had been reported to cause chain fragmentation in the gelatin molecules, which may lead to reduction in viscosity [11]. UV irradiation doses can be controlled by manipulating the irradiation time and distance from the samples [9].

Generally, the effects of UV decrease when the wavelength increases. UVC with the shortest wavelength is considered the most dangerous and strongest followed by UVB and UVA [12]. Several studies had been reported to investigate the potential of UVB [9] and UVC [11, 13] in improving the properties of fish gelatins. However, up to date, to our best knowledge, the effects of UVA on fish gelatins remains unexplored.

Therefore, in this study, the ability of different types of UV ray in altering the fish gelatin properties had been analysed. The aim of this study is to compare the effects of UVA and UVC irradiation on the properties of black tilapia (Oreochromis niloticus) scale gelatins. Irradiation time had been manipulated and the changes in gel strength, viscosity, and melting temperature of the gelatins were observed. The effects of UVA and UVC irradiation on functional groups of scale gelatin were also identified using Fourier Transform Infrared (FTIR) Spectroscopy analysis.

2. Materials and Methods

2.1 Sample Preparation

Black tilapia (*O. niloticus*) scale gelatins were extracted using method of [6]. Scales underwent acidic pre-treatment process, and subsequently, gelatins were extracted using thermal extraction method. Gelatin solutions of 6.67 % (w/v) were prepared in a 150 ml beaker by dissolving 7.5 g of the scale gelatin granules in 105 ml of distilled water at 60 ºC for 30 min. The gelatin solutions were cooled at room temperature for 30 min immediately after the heating process prior to UV treatment.

2.2 UV Treatment

Samples were exposed to 15 Watt UVA (366 nm; Model Actinic BL, Philips, China) and UVC (254 nm; 15 W, Model G15T8, Philips, China) light source in a laminar flow cabinet (Model AC2-G3, ESCO, Selangor, Malaysia). Distance between the lamp and sample surface is maintained at 30 cm. The exposure times were 0.5, 1.0, 1.5, 2.0, and 2.5 h. Non-irradiated scale gelatin was used as a control. Samples were tested immediately after irradiation process.

2.3 Gel Strength

Gel strength was measured based on British Standard 757: 1975 method (BSI, 1975). The 6.67 % (w/v) gelatin solutions after UV irradiation were chilled in a refrigerator at 4 ºC for 18 h. The gel strength was then determined via Texture Analyser (Model TA-XT Plus, Stable Micro System, Surrey, UK) with a load cell of 5 kg, equipped with a flat-faced cylindrical plunger (12.7 mm diameter). The maximum force (Newton, N) taken for the plunger to penetrate at 4 mm depth into the gelatin gels was recorded as gel strength. Analysis was conducted in triplicate.

2.4 Viscosity

Measured 60 ml of sample irradiated gelatin solution were inserted into Brookfield digital viscometer (LVDV-II+P Brookfield Engineering, U.S.A.) equipped with a No.1 spindle at 40 °C ± 10 °C. The spindle speed was adjusted to 100 rpm to control the torque value to be in between the range of 10 to 100 %. The viscosity was expressed in centipoises (cP). Results were taken in triplicates.

2.5 Melting Temperature

The melting temperature of gelatin was measured using method of [14] with slight modification n. 6.67 % (w/v) of gelatin solutions were prepared and 5 ml of solutions were transferred into a small glass tube (borosilicate tube, 12 mm × 75 mm). The tubes were then covered with parafilm and heated in a water bath at 60 °C for 15 min. After 15 min, the glass tubes were cooled immediately in ice-chilled water and matured at 10 °C for 18 h. A mixture of 75 % chloroform and 25 % dye (food color) was prepared and placed (3 drops) on the surface of the matured gelatin gels. Subsequently, the gels were placed in a water bath at 10 °C and the heating rate of the bath was controlled at the rate of 0.2 – 0.4 °C/min. The temperature of the bath was noted using an electronic digital thermometer (Zeal, England). Temperature at which the dye drops began to move freely down the gel was recorded as the gel melting point. Results were taken in triplicates.

2.6 Fourier Transform Infrared Spectroscopy (FTIR)

The possible interactions of the functional groups in the raw materials and gelatins were examined using FTIR spectrophotometer (Perkin Elmer Spectrum, U.S.A.). All spectra were recorded in the range between 4000 – 600 cm^{-1} with a 4 cm^{-1} resolution. The spectra of all the samples were obtained at 32 scans.

3. Results and Discussion

3.1 Gel Strength

Gel strength is the measurement of gelling power of gelatin and it reflects their quality and price. The gel strength of scale gelatin is found to increase along with the irradiation time for both UVA and UVC treated samples. Prior to UV treatment, the gel strength of scale gelatin is 3.13 ± 0.05 N. UVA irradiation for 0.5 to 2.5 h increases the gel strength of scale gelatins to 3.50 ± 0.07 to 5.12 ± 0.22 N. At the same irradiation time, the gel strength increases to 3.38 ± 0.05 to 4.75 ± 0.09 N when treated with UVC. The changes in the gel strength of scale gelatins after treatment with UVA and UVC for 0.5 to 2.5 h are shown in Fig 1.

The marked increase in gel strength of UVA and UVC-treated scale gelatins can be attributed to the enhanced crosslinking due to UV irradiation. UV irradiation generates radicals at the gelatin amino acids such as tyrosine and phenylalanine. Consequently, during renaturation, the binding of these radicals resulted in cross linking formation. Hence, stronger and more ordered gel network is produced. Fig 2 illustrates the schematic of the effects of UVA and UVC irradiation on scale gelatin molecules and formation of gel network. Similar results had been reported by [9] whom conducted UVB treatment on cold and warm water fish gelatins at different dosage. They reported a significant increase in the gel strength of both gelatins and attributed this to the UVB-induced crosslinking formation.

It is noteworthy that scale gelatins treated with UVA has higher gel strength compared to UVC. For example, scale gelatins irradiated with UVA for 0.5 to 2.5 h experienced an increase in the gel strength from 11.96 to 63.54 %. Meanwhile, at the same irradiation time, UVC increases the gel strength from 7.97 to 51.77 %, only. This can be linked to the stronger dosage of UVC which encourage molecule degradation, while forming new crosslinks simultaneously. The simultaneous process of chain fragmentation and crosslinking that occurs limits the increase of gel strength in the UVC-treated samples as can be seen in Fig 2. However, crosslinking appears to dominate over the chain degradation, which is proved by the increase in gel strength as the irradiation time increases. Results obtained is in accordance to [11], whom stated that the gel strength of a commercial tilapia fish gelatin increases because the UV-induced crosslinking appears to dominate over the effect of chain degradation.

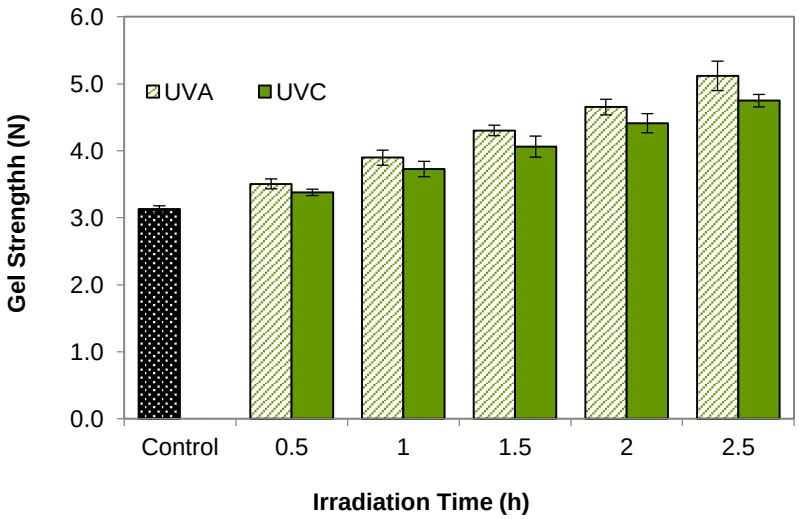

Fig. 1 - Gel strength of scale gelatins treated with UVA and UVC at different time

3.2 Viscosity

UV irradiation on scale gelatins increases the viscosity level as can be seen in Fig 3. The viscosity of initial non-irradiated scale gelatin is 10.80 ± 0.42 cP. The value increases to 11.37 ± 0.03 to 12.35 ± 0.17 cP, when irradiated with UVA at 0.5 to 2.5 h. Likewise, treatment with UVC at the same irradiation time also increases the viscosity level from 10.89 ± 0.01 to 11.84 ± 0.53 cP. Fig. 3 shows the viscosity level of scale gelatins treated with UVA and UVC at different time. The increase in the viscosity can be attributed to the irradiation-induced crosslinking formation. UV irradiation caused crosslinking of the polypeptide chains, leading to the formation of higher molecular weight components exhibiting thicker solutions. Otoni et al. (2012) whom conducted UVB treatment on cold and warm water fish gelatins had reported similar findings. They stated that the fish gelatin samples demonstrated an increase in viscosity after UV irradiation and correlated this incident with cross linking formation in gelatin molecules.

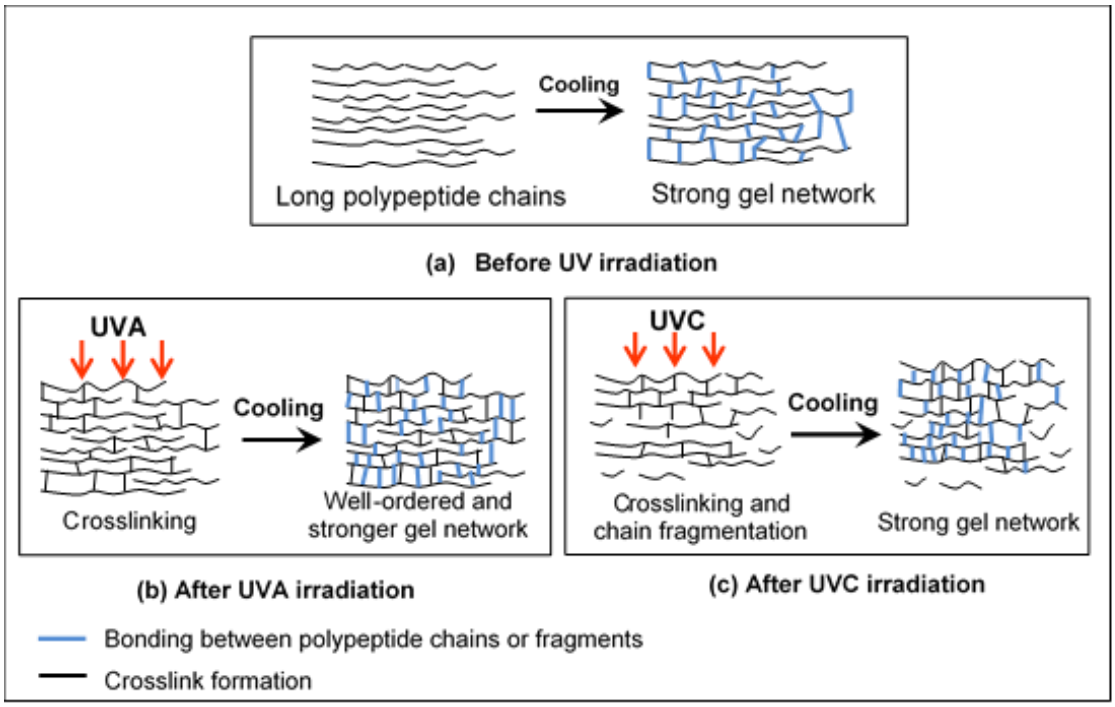

Fig. 2 - Effects of UVA and UVC irradiation on scale gelatin molecules and formation of gel network

It is also interesting to note that the UVA-treated samples possess slightly higher viscosity level than that of UVC-treated. The viscosity of scale gelatins treated with UVA for 0.5 to 2.5 h had an increase up to 5.28 to 14.38 %, respectively. Meanwhile, samples treated with UVC for the same period, only experienced an increase from 0.86 to 9.60 %, respectively. Though UVC possess stronger dosage compared to UVA, the minimal increase in viscosity might be due to the simultaneous occurrence of crosslinking and chain fragmentations during irradiation. Similar to the results obtained from gel strength analysis of scale gelatins in section 3.1, irradiation with UVC not only causes crosslinking, but also fragmentations of the polypeptide chains (Fig 2). Consequently, the chain fragmentations prevent the viscosity of the samples to increase further. According to[11], crosslinking formation or molecular degradation might occur during UV irradiation subject to the protein nature and irradiation dosage. They also added that UV irradiation induced degradation might occur simultaneously with formation of new crosslinks.

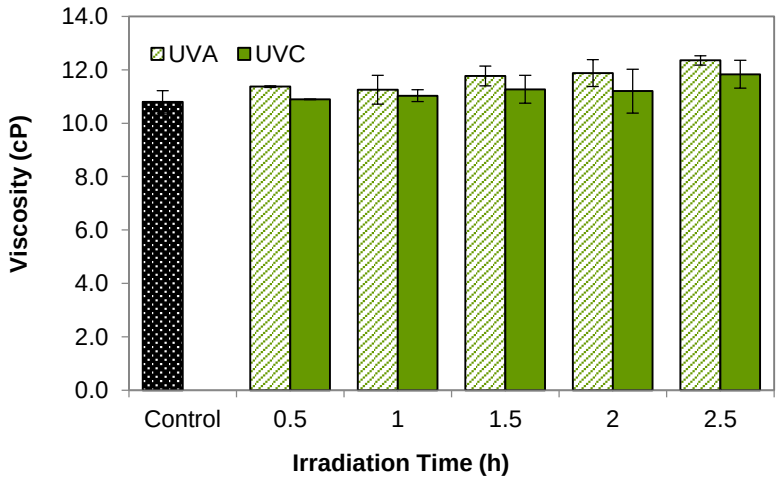

Fig. 3 - Viscosity of scale gelatins treated with UVA and UVC at different time

3.3 Melting Temperature

It is surprising to note that UV irradiation did not give any significant effect on the melting temperatures of scale gelatins. Fig 3 shows the changes in melting temperature of scale gelatins after UV irradiation at 0.5 to 2.5 h. Referring Fig 4, slight increment in the melting temperature can be observed along with the irradiation time. Initially, the melting temperature of non-irradiated scale gelatin is 26.0 ± 0.5 °C. Irradiation with UVA for 0.5 h increases the melting temperature to 26.5 ± 0.4 °C. Further increasing the irradiation time to 2.5 h, increases the melting temperature to 26.8 ± 0.2 °C. Similarly, treatment with UVC for 0.5 to 2.5 h also increases the melting temperature from 26.3 ± 0.5 to 26.7 ± 0.5 °C, respectively. Though slight increment can be observed in all the samples irradiated at different time, the effects are too minimal (< 4 %) and therefore can be neglected.

Despite giving a significant impact on the gel strength and viscosity of the samples, UV irradiation is incapable to alter the melting temperature significantly. This is because the crosslinking that occur was not extensive enough to affect the melting temperature of the samples. Hence, it is presumed that further increasing the UV irradiation time able to upsurge the melting temperature in scale gelatin samples. Longer irradiation time might be able to create UV-induced reinforcement in the gel network, thus higher energy will be needed to melt the samples. Researcher [9] also found that UVB irradiation had little effect on the melting temperature of cold and warm water fish gelatins. They stated that crosslinking of the polypeptide chains was not extensive enough to affect the melting properties of the gelatin.

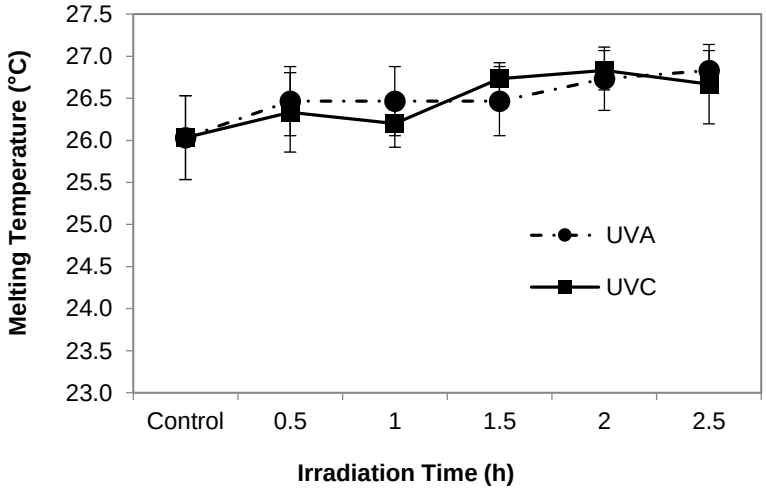

Fig. 4 - Melting temperature of scale gelatins treated with UVA and UVC at different time

3.4 Fourier Transform Infrared (FTIR) Spectroscopy

The changes in functional groups of scale gelatins irradiated with UVA and UVC for various time periods were determined via FTIR analysis. The infrared spectra of control and UV irradiated scale gelatin samples are depicted in Fig 5. Meanwhile, the regions detected, peak wavenumbers, as well as their denotations for UVA and UVC irradiated scale gelatins are detailed in Table 1 and Table 2, respectively.

Results obtained revealed identical pattern of amide A and amide B band in both UVA and UVC treated scale gelatins. No major changes observed in the peak wavenumbers after UV irradiation, indicating insignificant changes to have occurred in the functional groups. This may explain the melting temperature of the UV irradiated samples, where no changes occurred. The UV irradiation dosage is presumed to be not strong enough to modify the functional groups in the gelatin molecules. In general, the presence of amide A and B bands refer to the N-H stretching vibrations and C-H stretching vibrations in the samples [15]. Similar results were reported by [11], whom found minor changes in the FTIR spectra of UVC irradiated tilapia skin gelatin. They associated this situation to the insignificant changes occurred in the functional groups of gelatins.

Even though the FTIR spectra of scale gelatins shows insignificant changes, on closer observation, slight changes can be observed in the amide I bands of scale gelatins. Amide I (1700-1600 cm^{-1}) absorption band is a useful peak in FTIR analysis of the secondary structure of gelatin. The amide I band of scale gelatins treated with UVA for 0.5, 1.5, and 2.5 h are found to be 1632 cm^{-1}, 1632 cm^{-1}, and 1630 cm^{-1}, respectively. Meanwhile, the peak wavenumber of amide I bands were at 1632 cm^{-1} (0.5 h), 1631 cm^{-1} (1.5 h), and 1631 cm^{-1} (2.5 h) for UVC treated samples. These changes might be possibly due to the crosslinking formation in the polypeptide chains induced by UV irradiation.

Moreover, it can be seen that the frequencies of amide I band decreases along with the irradiation time in both UVA and UVC treated scale gelatins. This indicates the formation of crosslinks in the samples [11]. Additionally, the FTIR spectra of amide II and III bands associated with the bending vibration of N-H groups and stretching vibration of C-N groups were also detected in scale gelatin samples [16]. Slight changes in amide II and III were also observed in both UVA and UVC treated scale gelatins when the irradiation time increases. These changes may be caused by the crosslink

of the gelatin molecules due to radicals formation. This is in align to the increase on the gel strength and viscosity of the samples after UV irradiation.

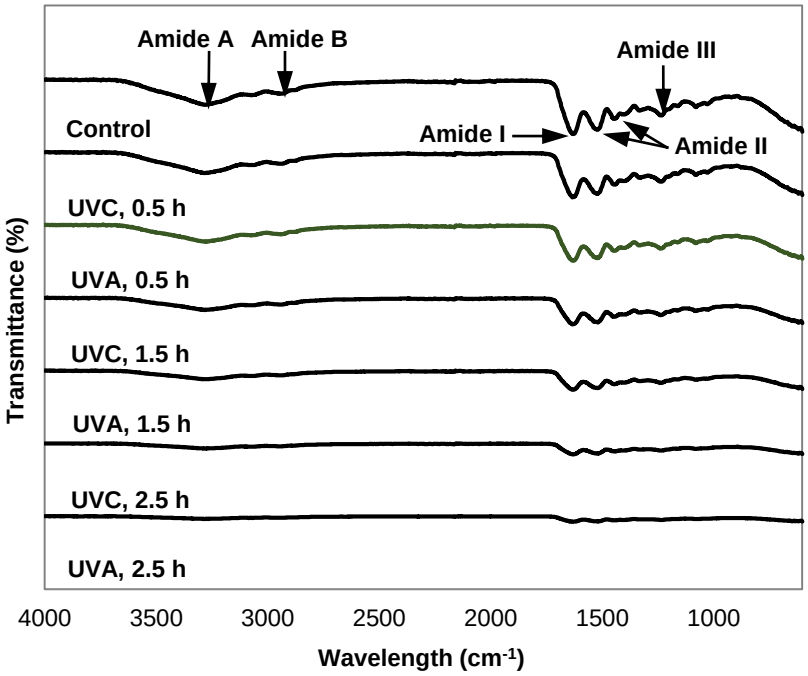

Fig. 5 - FTIR spectra of scale gelatins treated with UVA and UVC at different time

4. Conclusion

Results obtained indicate UV irradiation is capable to modify the properties of fish gelatins. UV irradiation on scale gelatins induce crosslinking formations in the samples, thus, increases the gel strength and viscosity. However, it could not enhance the melting temperature of the samples. Hence, further work is necessary to investigate in depth on the impact of UV irradiation on the melting temperature of gelatins using longer irradiation time. In addition, results obtained clearly show that UVA able to increase the gel strength and viscosity of scale gelatins better than UVC. UVC, being the strongest type of UV ray causes chain fragmentation to occur simultaneously with crosslinking in the gelatin molecules. Consequently, the chain fragmentations prevent the properties of gelatins to hike further.

Acknowledgement

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