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Effect of Gamma Radiation on Novel Gelatin Extracted from Camel Skin for Pharmaceutical Application

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Key words
Camel skin gelatin, γ-irradiation, Electron paramagnetic resonance, X-Ray diffraction

ABSTRACT

Gelatin is a natural polymer that can be derived from collagen through hydrolysis. It most extensively used in diverse fields, mainly in pharmaceuticals and therapeutic. Herein Gelatin was newly extracted from camel (*Camelus dromedarius*) skin and irradiated with gamma rays from ^{60}Co source. Gelatin was optimized and characterization and functional properties were determined. The structural changes occurring after γ-irradiation at doses from 5 to 30 kGy were reported by physico-chemical techniques such as electron paramagnetic resonance (EPR), Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) for the first time. Results revealed that gelatin yield increased significantly (P<0.05) as the extraction temperature and time increased (19.71%) at 70°C for 12 h. EPR spectroscopy demonstrated that 10 kGy of radiation-induced the decomposition of side groups. New peak positions (Paramagnetic centers) were located at around 3800 G. Moreover, an enhancement of EPR peak (3300 and 3400 G) (g-factor = 2.0) was detected. In addition, free radicals trapped in the crystalline regions were moved toward the boundary regions and the amorphous phases disappeared. XRD patterns showed a new peak at 2θ= 65.8° and a diminution of the relative intensity for the peak of 2θ= 20.54° after 30 kGy. FTIR measurements revealed structural deformations from 5 kGy through chain scission of C–H chains as well as the deformation of the C=O carboxyl groups with increasing γ-radiation doses. The camel skin was thus proved to be a source of gelatin. Irradiation induced a structural deformation with desirable functionalities that make it a potent source of gelatin that could be used in food and biomedical applications.

INTRODUCTION

Gelatin is a water-soluble protein derived from collagen, an abundant connective tissue in bones, skin and animal hides. Most commercial gelatin sources are from mammalians (bovine and mostly porcine). Today, despite its wide applications, the use of this type of gelatin tends to be limited due to the occurrence of several socio-cultural and safety issues (Kittiphattanabawon *et al*., 2010; Sae-Leaw *et al*., 2016). Skin gelatin from different aquatic animals has also been extracted and characterized (Arnesen and Gildberg, 2007; Abdelmalek *et al*., 2016; Jridi *et al*., 2015). However, the use of fish gelatin as supplement or food ingredient is hindered by several factors such as fishy odor, allergic reactions, thermal stability and weak rheological properties (Sae-Leaw and Benjakul, 2015; Binsi *et al*., 2017). Today, there is need to

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explore alternative sources of gelatin which will have no cultural or health concerns. Among these, camel skin could be an excellent candidate. Still, the extraction procedure is yet to be optimized. Several studies reported that the extraction process parameters (temperature, time, and pH) could influence the yield and the length of the polypeptide chains. As a consequence, the biological properties of the resulting gelatin can be greatly affected (Regenstein and Zhou, 2007; Nagarajan *et al*., 2012). Therefore, optimizing the extraction conditions is required to avoid over-hydrolysis for generating the desired product with excellent functionalities. Nevertheless, data is unavailable about the impact of extraction temperature and time on the properties of gelatin from camel skin. Also, extraction methods under strong basic and strong acidic conditions have caused gelatin to leach during the washing steps. To the best of our knowledge, extraction of camel skin gelatin using a combination of strong basic (NaOH) and weak acid (citric acid) solutions has not been reported previously. Being a renewable and biodegradable polymer, camel skin can be considered as a sustainable alternative in food industry in terms of developing materials, enhancing resource efficiency and reducing environmental problems associated with packaging waste. Added to that, the range of novel applications of gelatin in biomedical and pharmaceutical fields can be developed. Gelatin materials for tissue engineering applications must be submitted to sterilization (Hara *et al*., 2010; Ouyang and Bai, 2015). Ionizing radiation such as γ-rays provides physical cross-linking reactions (Dorati *et al*., 2012). It allows avoiding the supplementation of potentially toxic compounds in these polymer networks, which might eventually result in cytotoxic effects. Irradiation causes irreversible molecular alterations due to the breakage of the covalent bonds of the polypeptide chains of gelatin. Therefore, it is important to evaluate radiation effects on gelatin at different doses (5–30 kGy), which are normally used in the sterilization of food and medical products (Diehl, 2002). The application of solid gelatin in food and pharmaceuticals has extensively been investigated (Pan *et al*., 2018). However, the effect of the γ -radiation on the variation of functional and bioactive properties of solid camel skin gelatin has not been explored. As irradiation causes cleavage of various chains and bonds in gelatin molecules, the present study, attempts to investigate radiation effects on gelatin and valorize the camel skin gelatin as an ingredient in food and pharmaceutical products.

MATERIALS AND METHODS

Collection and preparation of camel skin

The skin of camel (*Camelus dromedarius*) was obtained from a slaughtering house in Gabes (Tunisia). Fresh skin was put in polyethylene bags and taken to the laboratory. The skin was washed with cool tap water, cut into small pieces, placed in polyethylene bags and stored at -20°C until use. The storage time was less than 2 months.

Pre-treatment of camel skin

Camel skin was soaked in 0.5 M NaOH with ratio of skin/solution at 1:5 (w/v) for 3 days to remove non-collagenous proteins; the solution was changed every day. The alkaline-treated skin was washed with tap water until neutral pH. The samples were then soaked in 0.1 M citric acid with ratio of 1:5 (w/v) for 1 h. The samples were again washed with tap water until neutral pH.

Extraction of gelatin

The swollen skin was mixed with distilled water at 1:5 (w/v) at different temperatures (50, 60 and 70°C) for various times (3, 6, 9 and 12 h). The mixtures were then filtered using filter paper to remove insoluble materials. The supernatant was freeze-dried and subjected to analyses.

Determination of yield

The yield of gelatin was calculated based on the wet weight of fresh skin as follows:

Yield (%) = [weight of dry gelatin (g)/ weight of initial skin (g)] x 100 …. (1)

Determination of color

Colors of the gelatin samples (66.7 g L^{-1}) were measured by Minolta CM-2006 d spectrophotometer (Konica Minolta Holdings, Inc, Osaka, Japan). L^*, a^* and b^* values, indicating lightness/brightness, redness/greenness and yellowness/blueness, respectively, were recorded. The total difference in color (ΔE^*) was calculated as described previously (Pan *et al*., 2018).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The protein pattern of extracted gelatins was analyzed using SDS-PAGE, following a method adopted previously (Laemmli, 1970). Gelatin samples were dissolved in SDS 5%, heated at 60°C for 20 min and centrifuged at 12000 rpm. The supernatant was mixed in a 1:2 (v/v) ratio with loading buffer (0.5 M Tris-HCl, pH 6.8, glycerol, 10% (w/v) SDS, 0.5% (w/v) bromophenol blue and 5% 2-mercaptoethanol). The mixed solution was heated in water bath (95 °C) for 5 min and loaded into 4% stacking gel and 7.5% resolving gel.

Determination of functional properties

Emulsifying properties

Emulsion activity index (EAI) and emulsion stability index (ESI) of gelatin samples were determined, following the conditions adopted previously (Pearce and Kinsella 1978). Soybean oil (2 ml) and gelatin solution (1, 2 and 3%, 6 ml (w/v)) were homogenized at a speed of 20,000 rpm for 1 min. Emulsions were diluted 100- fold with 0.1% (w/v) SDS. The absorbance at 500 nm of the resulting dispersion was measured at 0 min and 10 min using a spectrophotometer. All determinations are means of at least three measurements.

EAI and ESI were calculated by the following formula:

$$EAI\ (m^2/g) = (2 \times 2.3 \times A \times DF)/ løC \quad \ldots (2)$$

$$ESI = A0x\ \Delta t/\Delta A \quad \ldots (3)$$

Where $A = A_{500}$, DF = dilution factor, l = path length of cuvette (m), ø = oil volume fraction (0.25), C= protein concentration (g/m^3), $\Delta A = A0 - A10$ and $\Delta t = 10$ min.

Foaming properties

Foam expansion (FE) and foam stability (FS) of gelatin solutions were determined (Shahidi *et al*., 1995). Gelatin solutions (1, 2 and 3%, w/v) were homogenized using a homogenizer (model system polytron PT 1200 E, KI-11030031 PT-DA 07/2EC-E107) at 13,000 rpm for 1 min and then transferred into 100 ml cylinders. The sample was allowed to stand for 0, 15, 30 and 60 min. FE and FS were then calculated using the following equations:

$$FE\ (\%) = (V_T/V_0) \times 100 \quad \ldots (4)$$

$$FS\ (\%) = (V_t/V_T) \times 100 \quad \ldots (5)$$

Where V_T is total volume after whipping; V_0 is the original volume before whipping and V_t is total volume after leaving at room temperature for different times (15, 30 and 60 min). All determinations are means of at least three measurements.

Viscosity

The denaturation temperature (Td) was measured following the methods previously described (Zhao *et al*., 2018). Briefly, dried gelatin was dissolved in distilled water to obtain a concentration of 0.6% (w/v). Gelatin samples were heated at different temperatures (from 4 °C to 40°C) with a heating rate of 4°C. The relative viscosity of the prepared solution was contrasted with that obtained at 4°C. The temperature at which the relative viscosity was 50% was defined as the Td of the sample.

Setting time for gelatin gel

Setting time for gelatin was determined at 4 °C and room temperature (25–26 °C) as described previously (Muyonga *et al*., 2004). Gelatin solution (6.67%, w/v) (2 ml) was transferred to a thin wall (12 mm - 75 mm) test tubes (PYREX®, Corning, NY, USA) and preheated at 60°C for 10 min, followed by incubation in an ice bath (4°C) and at room temperature (25–26 °C). An aluminum needle with a diameter of 0.1 cm was inserted into the gelatin sample every 10 s until the time it could not come off. This time is considered as the setting time. The measurement was carried out in three determinations.

Physico-chemical characterization

Gamma irradiation

The irradiations of gelatin were performed at the Tunisian Cobalt-60 gamma irradiation facility with energies of 1.173 and 1.332 MeV at a dose rate of 36 Gy/min. The dose rate was determined using Fricke dosimeter chemical standard dosimeter. The traceability to Aerial, the Secondary Standard Dosimetry Laboratory (SSDL), was established using the Alanine/EPR dosimetry system. Gelatin was placed in a polystyrene phantom to ensure electronic equilibrium and was irradiated at room temperature (293–298 K) with a dose range from 5 kGy to 30 kGy.

Electron paramagnetic resonance spectroscopy (EPR)

EPR spectra of the gelatin samples were recorded at room temperature on a Bruker ER-200D spectrometer operating at 9.8 GHz X-Band frequencies with modulation amplitude of 0,2 mT, modulation frequency of 100 khz, sweep width of 210 mT and microwave power of 63 mW.

X-ray diffraction (XRD)

The XRD analysis of gelatin was conducted using Brucker D8 advance with Cu-Kα radiation of wavelength λ = 1.541 Å in 2θ values in the range of 15–90°. The results obtained by X-ray measurement were analyzed with the X'Pert High Score Plus program.

Fourier transform infrared (FTIR)

FTIR spectroscopy was used to study the structural and chemical properties of un-irradiated and irradiated gelatin. The measurement was recorded by Vertex 70 infrared spectrometer from 400 to 4000 cm^{-1} at a spectral resolution of 2 cm^{-1} and 32 scans (Jebahi *et al*., 2012).

Statistical analyses

The obtained data set was performed to determine the effects of determination the effect of gamma radiation on novel gelatin extracted from camel skin with the analysis of variance (ANOVA) test. For this purpose, Levene homogeneity test and Kolmogorov-Smirnov normality test were applied to test the homogeneity of variances and normality assumptions, which are necessary conditions

for performing ANOVA. For this purpose, Levene homogeneity test and Kolmogorov-Smirnov normality test were applied to test the homogeneity of variances and normality assumptions, which are necessary conditions for performing ANOVA. As a result of these analyzes, it was determined statistically that the data set was distributed homogeneously and provided the assumption of normality ($p>0.05$). In the light of this information, as a result of the ANOVA test, it was determined that there was a statistical difference between the groups ($p<0.05$). In the light of this information, as a result of the ANOVA test, it was determined that there was a statistical difference between the groups ($p<0.05$).

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee.

RESULTS

Gelatin yield

Yield of gelatin from camel skin extracted under various temperatures (50°C, 60°C and 70°C) and times (3h, 6h, 9h and 12 h) are shown in Figure 1. Extraction yields with different conditions ranged from 3.7% to 19.26% on a dry weight basis. Increased yield was observed with increasing extraction temperatures and times and the highest yield (19.26%) was recorded for gelatin extracted at 70 °C for 12h ($P < 0.05$).

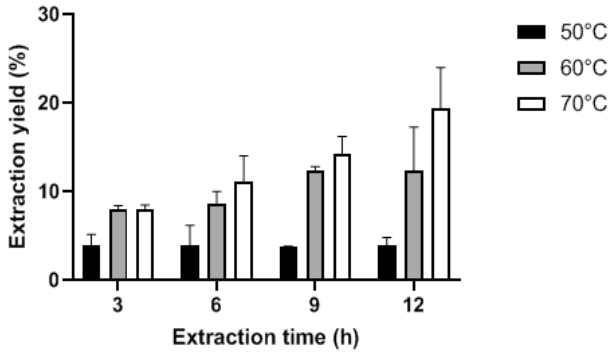

Fig. 1. Extraction yield (%, dry weight basis) of gelatin from the skin of *Camelus dromedarius* extracted at different temperatures for various times.

SDS–PAGE

The SDS-PAGE pattern of camel skin gelatin for different extraction temperatures (50°C, 60°C and 70°C) and times (3h, 6h, 9h and 12h) are presented in Figure 2. All samples showed typical electrophoresis profiles of gelatin that consisted of α-chain (α1 and α2) and β-chain as the major components with the small amount of γ-chain. In this study, no severe degradation in protein patterns at higher temperature was observed.

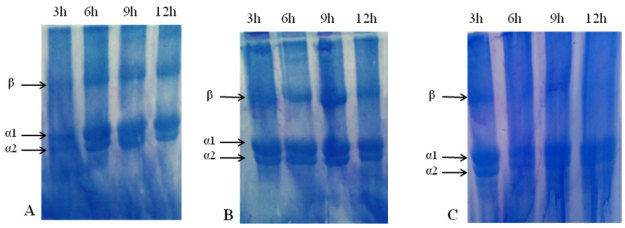

Fig. 2. SDS-PAGE patterns of gelatin extracted from camel skin at 50°C (a), 60°C (b) and 70°C (c) for various times (3h, 6h, 9h and 12h).

Color

Color of gelatin from camel skin with different extraction conditions, expressed in terms of L*(lightness), a*(redness) and b* (yellowness), are shown in Table I. The lightness of gelatin gel decreased as extraction temperature and time increased ($P < 0.05$). However, increases in a^* and ΔE^* values were found when the extraction time increased ($P<0.05$). The increases in a^* and ΔE^* values were in accordance with the increased yield.

Table I. Color of gelatins from the skin of *Camelus dromedarius* at different temperatures for various times.

Extraction conditions		Color value			
		L*	a*	b*	Δ E
50°C	3h	55.12 ± 0.40^{j}	-0.53 ± 0.33^{k}	-0.06 ± 0.21^{k}	39.94 ± 0.11^{a}
	6h	66.05 ± 1.35^{e}	-0.32 ± 1.22^{g}	4.49 ± 1.44^{g}	29.26 ± 1.18^{h}
	9h	57 ± 1.55^{i}	-0.27 ± 0.25^{e}	3.85 ± 0.11^{e}	38.19 ± 0.20^{c}
	12h	68.19 ± 1.62^{b}	-0.18 ± 1.36^{c}	3.39 ± 1.27^{c}	27.01 ± 1.25^{k}
60°C	3h	68.72 ± 1.22^{a}	-0.28 ± 1.33^{f}	4.03 ± 1.19^{f}	26.55 ± 1.59^{l}
	6h	66.99 ± 0.48^{d}	-0.27 ± 0.46^{e}	3.55 ± 0.28^{e}	28.21 ± 0.36^{i}
	9h	62.14 ± 1.56^{h}	-0.16 ± 1.10^{b}	7.27 ± 1.55^{b}	33.58 ± 1.12^{d}
	12h	56.9 ± 1.41^{i}	-0.02 ± 1.22^{a}	5.81 ± 1.17^{a}	38.5 ± 1.14^{b}
70°C	3h	67.34 ± 0.23^{c}	-0.39 ± 0.30^{h}	5.57 ± 0.17^{h}	28.15 ± 0.27^{j}
	6h	65.5 ± 1.34^{f}	-0.44 ± 1.51^{i}	5.09 ± 1.51^{i}	29.89 ± 1.28^{g}
	9h	65.44 ± 1.44^{f}	-0.22 ± 1.40^{d}	5.22 ± 1.31^{d}	29.97 ± 1.22^{f}
	12h	63.69 ± 1.20^{g}	-0.49 ± 1.45^{j}	6.99 ± 1.16^{j}	32 ± 1.33^{e}

Values are presented as mean ± SD (n = 3). Different superscripts within the same column.

Emulsifying properties

Emulsifying properties are used to enhance the protein performances. Emulsifying activity index (EAI) and emulsion stability index (ESI) of camel skin gelatin extracted under different conditions and at different

concentrations are shown in Table II. EAI of all gelatin samples decreased with the increase of gelatin (1%, 2% and 3%) (P<0.05). The EAI of camel skin gelatin was significantly (P < 0.05) affected by the extraction temperature and the lowest EAI values were observed at 70°C. In general, ESI values significantly decreased with increasing temperature (P < 0.05). The best results were obtained for samples extracted at 50°C.

Foaming properties

Foam expansion (FE) and foam stability (FS) at 15, 30 and 60 min after whipping were determined to evaluate the foam capacity and foam stability of camel skin gelatin. FE and FS of camel skin gelatin at various concentrations (1, 2, and 3 g/100 mL) extracted under various conditions are shown in Table II. Foam expansion values increased with increasing gelatin concentration (P<0.05). In addition, FE increases with increasing extraction temperature. However, slight decreases in FE were observed in gelatin extracted at 70°C for 12 h. Results show also that the highest FS value was found when using 60°C and 70°C at different concentrations.

Setting time for gel formation of gelatin extracted with different conditions

Setting time required for gel formation at 4 °C and room temperature (25 °C) were depicted in Figure 3. The setting time at 4 °C of camel skin gelatin increased when the extraction temperature and time increased (P < 0.05). Gelling time at 4°C ranged from 1.43 min to 2.15 min without significant difference. At 25°C, camel skin gelatin extracted under different temperatures (50°C, 60°C and 70°C) and for different times (3h, 6h, 9h and 12h) were able to set within 16 min.

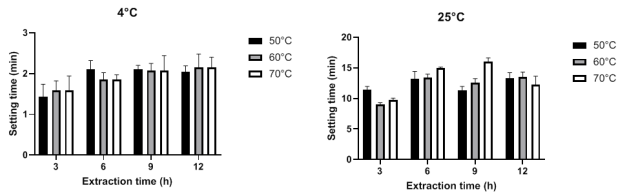

Fig. 3. Setting time of gels of gelatin from the skin camel at 4 °C and room temperature.

Viscosity and denaturation temperature

The relative viscosity of all gelatin solutions is shown in Figure 4. The denaturation values of all gelatin samples, with different extraction conditions, were ranged from 32°C to 40°C.

Table II. Emulsion activity index (EAI) and emulsion stability index (ESI) of camel skin gelatin at different concentrations. Values are given as mean ± SD from triplicate determinations. Different superscripts in the same column indicate significant differences (p < 0.05). Different uppercase letters in the same column within the same gelatin sample indicate significant differences (P < 0.05). Different lowercase letters in the same column within the same concentration and at the same extraction time indicate significant differences (P < 0.05).

Extraction conditions	Concentration (g/100 ml)	Emulsion activity index (EAI) (m^2/g)	Emulsion stability index (ESI) (min)
50°C-3h	1	15,62±0.5aA	176,67±0.4aA
	2	6,26±0.45aB	97,14±0.31aB
	3	4,3±0.37abC	58,42±0.22aC
50°C-6h	1	13,26±0.3aA	90,00±4.99aA
	2	7,36±0.22aB	88,89±0.2aA
	3	6,07±0.32aC	80,00±5.1aB
50°C-9h	1	12,15±0.01aA	44,00±3.2aB
	2	7,16±0.13aB	90,97±3.4bA
	3	4,97±0.56aC	50,63±2.1aB
50°C-12h	1	12,89±0.3bA	21,88±1.1cC
	2	7,81±0.36aB	50,78±0.41aA
	3	5,52±0.25aC	27,27±0.36bB
60°C-3h	1	11,96±0.12bA	26,96±1.5bB
	2	5,71±0.03aB	23,64±2.1bC
	3	4,79±0.15aC	41,18±0.5bA
60°C-6h	1	12,34±0.46aA	29,13±0.9bB
	2	6,72±0.26aB	27,04±0.5bC
	3	5,46±0.11aC	42,38±0.31bA
60°C-9h	1	11,28±0.51abA	23,72±0.3cC
	2	5,46±0.05bB	61,13±1.5cA
	3	5,01±0.12aB	38,86±0.41cB
60°C-12h	1	12,16±0.11cA	38,15±0.2aA
	2	5,52±0.21cB	31,58±0.61bB
	3	4,67±0.15bC	33,04±1.1aB
70°C-3h	1	7,92±0.31cA	9,25±0.7cC
	2	4,14±0.07bB	13,24±0.45cB
	3	3,93±0.21bB	18,82±0.11cA
70°C-6h	1	11,23±0.33bA	20,33±0.33cC
	2	4,97±0.15bB	24,55±0.1cA
	3	4,17±0.16bC	21,25±0.2cB
70°C-9h	1	10,43±0.21bA	33,69±0.7bB
	2	6,23±0.54abB	169,00±7.1aA
	3	4,81±0.44aC	43,16±0.1bB
70°C-12h	1	15,10±0.07aA	27,92±2.2bA
	2	6,17±.11bB	28,53±0.2cA
	3	4,77±0.1bC	28,53±0.15bA

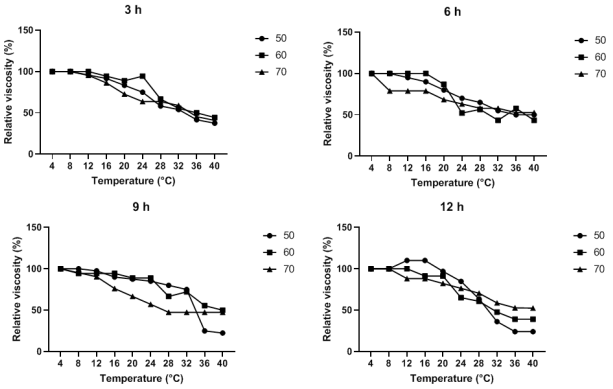

Fig. 4. Relative viscosity (%) change of gelatin extracted from *Camelus dromedarius* at different temperatures (50°C, 60°C and 70°C) for various times (3h, 6h, 9h and 12h). All data are presented as the mean ± SD of triplicate results.

FTIR analysis

The chemical shift of amide A, I and II for non-irradiated and γ-irradiated gelatin is shown in Figure 5. The absorption for hydrogen bonded N–H associated to amid A amide at 3.362 cm^{-1} was shifted to higher wave number 3.430 cm^{-1} (very acute) and to 3.332 cm^{-1} (very broad) for 5 and 20 kGy dose, respectively. The results also showed that the absorption band at 1.656 cm^{-1} of C=O stretching (amide I) for non-irradiated gelatin has also shifted to 1646 cm^{-1} (broad) for 5 kGy dose and then to higher wave number at 1.650 cm^{-1} (very cute) for 20 kGy dose. Furthermore, the absorption for N–H bending (amide II) coupled with C–N stretching showed no similar trend to that of amide I throughout the studied irradiation ranges. The increase of radiation doses induces the shift of amide A, I and II wave number gradually due to the degradation of protein molecules. However, the shift of absorption of amide A, I and II peaks after 20 kGy dose with increased intensity is associated with crosslinking and due to the formation of hydrogen bond between the amino acid residues of the chains. The frequency of the nitrile (C≡N) stretching vibration was appeared after irradiation in the range 2.100–2.400 $cm^{-1}$and increased at 15 kGy.

EPR analysis

Figure 6 illustrated the EPR spectra of un-irradiated and γ-irradiated gelatin. It is clearly seen that in the case of slightly irradiation (10 kGy), two new peaks were signaled due to the existence of free radicals in gelatin system at around of 3300 G and 3400 G in the vicinity of g=2. Moreover, the amplitude of the EPR spectrum of the first paramagnetic center was one and a half times greater than the non-irradiated one. Doses from 5 to 30 kGy were showed the disappearance of the new peaks. More interesting spectrum EPR demonstrated the augmentation of the first and the second paramagnetic center resulted in the increase in free radical concentration after irradiation with Co gamma rays the spectrum changed notably as a function of doses. In fact, the increase of gamma radiation induces increase of paramagnetic center intensities.

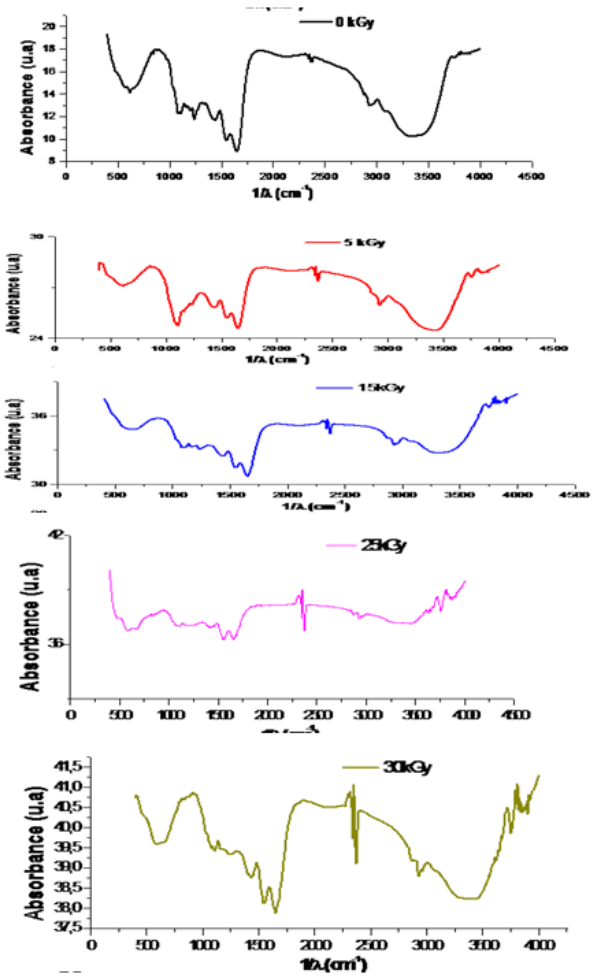

Fig. 5. FTIR spectra of camel skin gelatin variation of peak wave numbers (cm^{-1}) of functional groups of gelatin with different γ-radiation doses 5 kGy, 15 kGy, 20, 25 and 30 kGy with control samples (0 kGy).

XRD pattern

Diffraction pattern of non-irradiated gelatins were obtained and compared with that of irradiated ones (Fig. 7). XRD pattern of gelatin powder showed amorphous morphology with a characteristic broad hump in the range of 15-30 • 2θ. Moreover, a sharp peak with low intensity were located at 2θ = ~7° (not shown). At the dose of 5 kGy, a modification of crystallinity was detected. But the most

important modification can be occurred at high doses (25 and 30 kGy), a new sharp peak 2θ = 25.54° was appeared. As showed in Table III, peaks at 2θ= 29.027 characteristic of amorphous phase signaled an important relative intensity 83.27% and minimum of area and FMWM (677) (Table IV). However, after irradiation, the relative intensities were decreased because the crystallinity was increased, and the amorphous regions phases were disappeared.

Table III. Foaming properties of camel skin gelatin at different concentrations. Values are given as mean ± SD from triplicate determinations. Different uppercase letters in the same column within the same gelatin sample indicate significant differences (P < 0.05). Different lowercase letters in the same column within the same concentration and for the same extraction time indicate significant differences (P < 0.05).

Extraction conditions	Concentration (g/100 ml)	Foam expansion (FE) (%)	Foam stability (FS) (%)		
			t=15 min	t= 30 min	t= 60 min
50-3h	1	40±0.4bB	36±0.1cC	36±0.15cC	36±0.12cC
	2	60±1.1cA	60±0.5cA	56±0.3cA	56±0.22bA
	3	60±3.5cA	52±2.5cB	52±1.7cB	52±1.15cB
50-6h	1	60±0.5cC	50±2.5bC	50±1bC	44±3.2bC
	2	70±1.5cB	62±0.5bB	60±0.3cB	60±0.35cB
	3	96±1.2aA	96±2.15aA	96±0.2aA	94±0.5aA
50-9h	1	60±0.5aC	56±0.5cC	52±1.3cC	52±2.8bC
	2	90±1.1aB	84±4bB	80±1.5bB	70±5.1aB
	3	96±3.2bA	96±1.5aA	90±2.4aA	80±2.5bA
50-12h	1	96±4bA	96±2.5aA	80±1.7bA	80±1aA
	2	96±5.5bA	96±4.5aA	80±5.2bA	80±4.7bA
	3	98±7.2aA	80±5.5bB	80±3.5bA	76±2.2bA
60-3h	1	100±0.5aA	100±0.3aAB	98±1.1aAB	94±2.5aA
	2	110±0.5aA	104±0.2aA	102±0.5aA	98±0.8aA
	3	100±4.5bA	96±3bB	96±2.5bB	95±1.2bA
60-6h	1	100±0.5bB	96±0.3aB	96±0.4aB	94±0.2aB
	2	110±0.7bA	100±0.22aA	100±0.35aA	100±0.1aA
	3	90±0.5aC	90±0.3aC	80±0.4bC	80±0.5bC
60 °C 9h	1	100±3.7aA	78±1.5bA	70±0.5bB	52±2.5bB
	2	90±3.5aAB	80±2.8bA	60±3.5cC	50±3bB
	3	80±8.5bB	78±1.5bA	78±2bA	70±2.5cA
60°C 12h	1	120±6.5aA	80±2.5bC	80±2.5bB	70±1.2bB
	2	110±4aAB	100±1.5aA	100±1aA	90±0.5aA
	3	96±7.5aB	90±0.2abB	60±1.2cC	52±0.5cC
70°C 3h	1	100±0.5aB	98±0.7bB	96±0.3bB	80±1.5bB
	2	100±0.5bB	98±1bB	96±0.5bB	60±2.5bC
	3	120±1.5aA	120±0.8aA	114±1.8aA	104±2.5aA
70-6h	1	110±0.5aAB	100±1aA	98±1aA	90±1.5aA
	2	120±2.5aA	100±1.5aA	98±0.5bA	92±1bA
	3	100±7aB	96±3.5aA	90±4.5aA	78±2bB
70-9h	1	100±1aB	96±2aA	94±1.5aA	90±2aA
	2	104±3aB	96±2.5aA	90±1.5aA	52±1.2bB
	3	120±4.5aA	96±1.8aA	90±2.2aA	90±1.5aA
70°C 12h	1	100±0.5bA	98±1.5aA	90±2aB	60±2.5cB
	2	100±2abA	98±2aA	92±2.5aB	90±1aA
	3	104±1.5aA	98±0.5aA	98±0.5aA	90±0.5aA

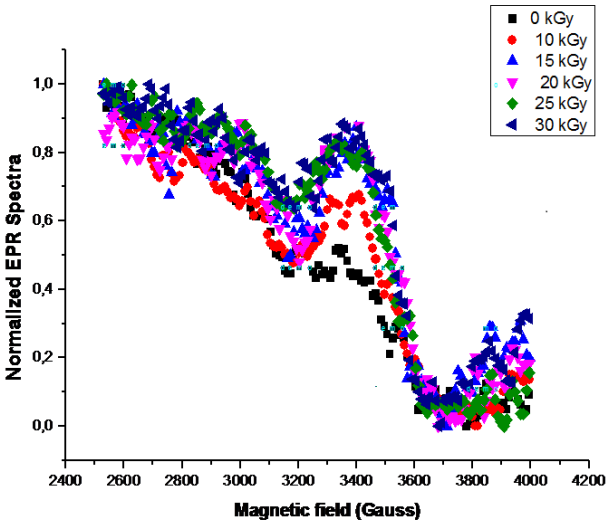

Fig. 6. EPR spectrum of unirradiated and irradiated camel skin gelatin with different γ-radiation doses 5 kGy, 15 kGy, 25 kGy and 30 kGy in comparison with control samples (0 kGy).

Table IV. Relative intensity observed at peak 2θ= 29,027.

Treatment dose (Kgy)	0	5	10	15	20	25	30
Relative intensity (%)	83.27	62.19	41.60	59.95	64.04	52.25	69.73

Table V. Determination of peak position, area and FWHM of XRD data.

Dose (kGy)	Peak position	Area	FWHM
0	29,027	1040,386	1,350
5	28,986	677,794	0,879
10	29,518	869,157	0,388
15	29,272	915,892	0,675
20	29,231	1148,621	1,391
25	29,292	1574,817	0,573
30	29,292	1341,835	1,534

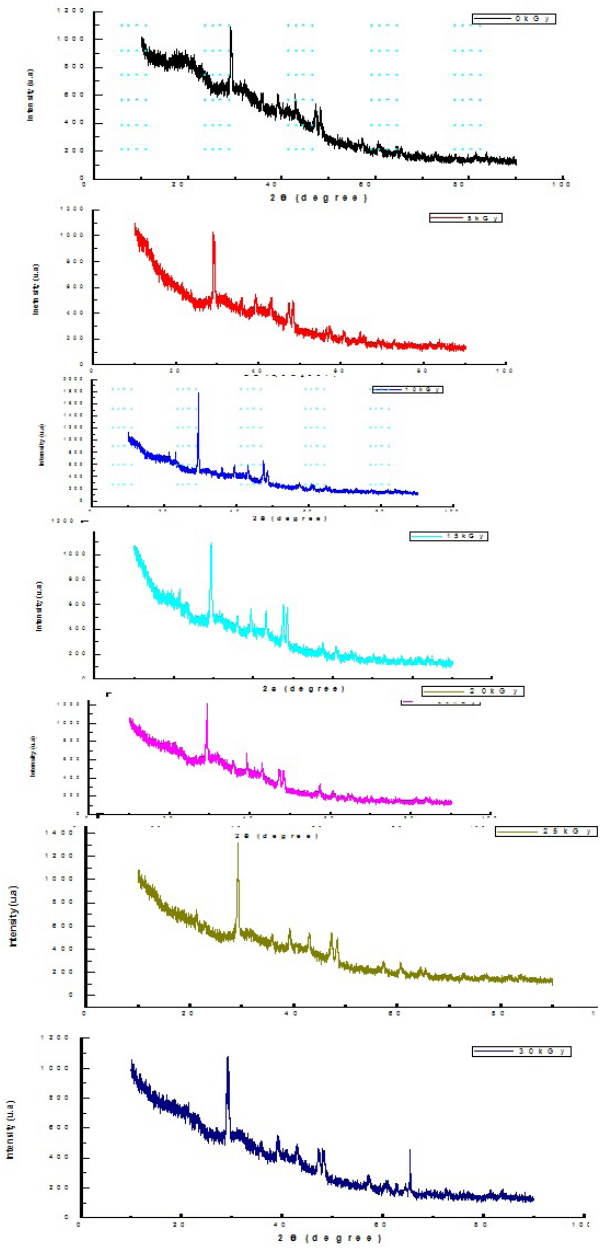

Fig. 7. X-ray diffractograms of camel skin gelatin with different γ-radiation doses: 5 kGy, 15 kGy, 20, 25 and 30 kGy with control samples (0 kGy).

DISCUSSION

In this study, gelatin was extracted from camel skin wastes, as an alternative source. Gelatin yield is influenced by the extraction condition. Herein gelatin yield is in agreement with previous works Kaewruang *et al*. (2013) reported that an increase in water extraction temperature from 45 to 75 °C caused an increase in yield of gelatin from the skin of unicorn leatherjacket. This result was higher than yields of cuttlefish gelatin Balti *et al*. (2011) and chicken skin gelatin Sarbon *et al*. (2013) reported as 7.84% and 2.16%, respectively. In this study, no severe degradation in protein patterns at higher temperature was observed, suggesting a high thermal stability of gelatin of camel skin. With increasing extraction temperature and time, covalent bonds stabilizing the triple helix of

native mother collagen can be destroyed a consequence, more free α chains and β chains were released from the skin complex leading to higher yield. The color parameter showed a congruence with pervious work that reported the highest L^* for gelatin extracted for short time (3h) (Mad-Ali *et al*., 2016). The decrease of the L value may be due to the occurrence of a non-enzymatic browning reaction, especially Maillard reaction, during extraction at higher temperature for longer time. Concerning emulsifying properties, several authors have reported the decrease of emulsifying capacity with increasing protein concentration. Moreover, foams with higher protein concentrations were denser and more stable, resulting in an increase in the thickness at the interface (Zayas, 1997). Camel skin gelatin extracted at 60 and 70°C had a better foaming ability compared with gelatin extracted at 50°C, though the difference was not statistically significant (p>0.05). In general, viscosity of each sample was decreased continuously with increasing the temperature from 4 to 40°C. Td defined as the temperature at which the relative viscosity was 50%. At this temperature, the gelatin molecules dissociates into random coils (Zhang *et al*., 2007). Lower Td is one of the most drawbacks limiting the use of gelatin molecule for food and pharmaceutical applications. Furthermore, these values were higher than those for the fish skins (Zhao *et al*., 2018; Wang *et al*., 2019). As a result, the triple helix structure was converted into one, two or three random chain gelatin molecules. Thereby, relative viscosity was directly correlated with the thermal stability of the gelatin gel. Considering the importance of thermal stability of gelatin in both food and biomedical applications, camel skin gelatin could be an excellent alternative to other gelatin sources. After extraction and irradiation, FTIR spectroscopy has been used to monitor the structural change in the functional polymer groups. Un-irradiated gelatin contained Amide I, II, III, A and B. These peaks were present with the irradiated ones but at modified intensities. Gelatin lost its triple helix structure when the amide I was affected (Derkach *et al*., 2019). The stretching vibrations of C=O and C-N groups frequency is found in the range between 1600 and 1700 cm^{-1}. At 5 kGy irradiation, the absorption band at 1,656 cm^{-1} of C=O stretching (I) has shifted at 1.644 cm^{-1} (broad) and then at 30 kGy, wave number moved to 1.657 cm^{-1} (broader). This value is close to un-irradiated gelatin which proves that gelatin undergoes denaturation then crosslinking at higher doses. For amide III, the recorded peak indicates a blend of N-H deformation and C-N stretching vibrations. Bands were present in the 1320-1295 cm^{-1} (largely affected with 5 kGy but returned to the normal structure at 30 kGy suggesting the same interpretation with that of amide I. Both amide A (3323 cm^{-1}) and amide B band (3057 cm^{-1}) were not affected by the action of radiation. Also, the alkyl C–H stretching bands of the amino acid residues at 2969, 2927 and 2859 cm^{-1} remained unaffected. However, the changes occurred only at 20 kGy and then the normal aspect was retrieved at 30 kGy. Moreover, the frequency of the nitrile (C≡N) stretching containing unnatural amino vibration in the range 2.100–2.400 cm^{-1} was accentually apparent at 20 kGy (Ma *et al*., 2015). With low dose ionizing radiation (5 kGy), irradiated camel gelatin acquired a highly disordered molecular structure that affected the triple helix and α helix structure. This denaturation was caused by hydrogen bond rupture that is responsible for the stabilization protein conformation. A high dose of gamma radiation (25 kGy) makes cross linking the major phenomenon that dominates chain scission mechanisms. A typical XRD pattern was obtained from pure and un-irradiated powder of gelatin. This spectrum is archetypal of an incompletely crystalline biomaterial with a large peak positioned at 2θ = ~19°. A second small peak placed in the region of 2θ = ~7° was related to gelatin like triple helix structure (Badii *et al*., 2014). The intensity of the peak was related to the content of triple helix. The latter molecule confers strict amino acid order that necessitates the recurrence of a (Gly-X-Y) (n) with a high quantity of amino acid in the gelatin (Ramshaw *et al*., 1998). However, it is clear that sharper peaks (increased crystal size) were obtained from the γ-irradiated gelatin sample at 2θ= ~10°, 15° and 20°. XRD showed a significant alteration in the arrangement of molecules in the crystal lattice. On the other hand, EPR spectroscopy permits to determine the paramagnetic centers. In fact, gelatin spectra recorded at least two components at 3300 and 3300 G. Signal intensities of irradiated and unirradiated samples of the camel gelatin powder were shown. This first band was allocated to the comparatively stable radical at the end of the CH2–CH2~chain • α showing interaction with two protons. The species found in the paramagnetic field were determined as anions radical with an unpaired electron positioned on the carboxyl carbon (Silva *et al*., 2008). It is seen that the hyperfine constants are slighter than anion radicals of other amino acids (Aydin, 2010). At around 3300 G (g-factor = 2.0), the peak is related to glycine residue, appeared~•CH as a minor product. The other compound of homolytic scission is ~ (O) C• radical that demonstrated an important singlet (Aydin, 2010). The methylene group is capable to offer hydrogen atoms to both radicals •~ which leads to the ~CH formation. At higher radiation doses (25 and 30 kGy), the free radicals were increased. In fact, spectrum EPR demonstrated the augmentation of the free radical concentration after irradiation with Co gamma rays and the spectrum changed notably as a function of doses. Macromolecules generate a

three-dimensional hydrogen network actively contributing in the transfer of protons to make possible the conversion/recombination of radicals (Przybytniak *et al*., 2019). These results provide important data for studying the feasibility of gelatin for biological and medical applications in the future. In fact, these results prove that gamma irradiation is an effective sterilization technique that should be taken into account because its application can alter the chemical structure of the pharmaceutical, skin allograft or food product.

CONCLUSION

Large quantities of gelatin are found in camel skin waste. It underwent chain scission when irradiated and sterilized. FTIR spectroscopy revealed that cross linking phenomenon started to dominate over degradation/denaturation process with the increase of irradiation. It is due to the formation of hydrogen bonds between the amino acid residues of the chains. The effect of radiation over gelatin caused modification in the arrangement of molecules in the crystal lattice was signaled with XRD analysis. A new sharp peak $2\theta = 25.54°$ appeared at high doses (25 and 30 kGy). The spectrum EPR demonstrated the augmentation of the free radical concentration after irradiation with Co gamma rays and the spectrum changed notably as a function of doses. The radicals found in camel skin gelatin might initiate (i) damages in backbone substituent (• CH_2CH_2~) which affect the structure of the peptide, (ii) strand breaks at glycine residues (~CH_2•~). and (iii) release of hydrogen atom from glycine (~CH•~).

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdelmalek, B.E., Gómez-Estaca, J., Sila, A., Martinez-Alvarez, O., Gómez-Guillén, M.C., Chaabouni-Ellouz, S., Ayadi, M.A., and Bougatef, A., 2016. Characteristics and functional properties of gelatin extracted from squid (*Loligo vulgaris*) skin. *LWT Fd. Sci. Technol*., **65**: 924-931. https://doi.org/10.1016/j.lwt.2015.09.024

Arnesen. J.A., and Gildberg, A., 2007. Extraction and characterisation of gelatin from Atlantic salmon (*Salmo salar*) skin. *Bioresour. Technol*., **98**: 53-57. https://doi.org/10.1016/j.biortech.2005.11.021

Aydin, M., 2010. Study of the structure of free radicals in gamma: Irradiated amino acid derivatives. *Braz. J. Phys*., **40**: 429-431. https://doi.org/10.1590/S0103-97332010000400013

Badii, F., MacNaughtan, W., Mitchell, J.R., and Farhat, A., 2014. The effect of drying temperature on physical properties of thin gelatin films. *Drying Technol*., **32**: 30-38. https://doi.org/10.1080/07373937.2013.808206

Balti, R., Jridi, M., Sila, A., Souissi, N., Nedjar-Arroume, N., Guillochon, D., and Nasri, M., 2011. Extraction and functional properties of gelatin from the skin of cuttlefish (*Sepia officinalis*) using smooth hound crude acid protease-aided process. *Fd. Hydrocolloid*, **25**: 943-950. https://doi.org/10.1016/j.foodhyd.2010.09.005

Binsi, P.K., Nayak, N., Sarkar, P.C., Joshy, C.G., Ninan, G., and Ravishankar, C.N., 2017. Gelation and thermal characteristics of microwave extracted fish gelatin–natural gum composite gels. *J. Fd. Sci. Technol*., **54**: 518-530. https://doi.org/10.1007/s13197-017-2496-9

Derkach, S.R., Kuchina, Y.A., Baryshnikov, A.V., Kolotova, D.S., and Voron'ko, N.G., 2019. Tailoring cod gelatin structure and physical properties with acid and alkaline extraction. *Polymers*, **11**: 1724. https://doi.org/10.3390/polym11101724

Diehl, J.F., 2002. Food irradiation past, present and future. *Radiat. Phys. Chem*., **63**: 211-215. https://doi.org/10.1016/S0969-806X(01)00622-3

Dorati, R., Colonna, C., Tomasi, C., Bruni, G., Genta, I., Modena, T., and Conti, B., 2012. Long-term effect of gamma irradiation on the functional properties and cytocompatibility of multiblock co-polymer films. *J. Biomater. Sci. Polym. Ed*., **23**: 2223-2240. https://doi.org/10.1163/156856211X613915

Hara, M., Koshimizu, N., Yoshida, M., Haug, I.J., Ulset, A.S.T., and Christensen, B.E., 2010. Cross-linking and depolymerisation of γ-irradiated fish gelatin and porcine gelatin studied by SEC-MALLS and SDS-PAGE: A comparative study. *J. Biomat. Sci. Polym. Ed*., **21**: 877-892. https://doi.org/10.1163/156856209X449452

Jebahi, S., Oudadesse, H., Bui, X.V., Keskes, H., Reba, T., el Feki, A., and Feki, H., 2012. Repair of bone defect using bioglass-chitosan as a pharmaceutical drug: An experimental study in an ovariectomised rat model. *Afr. J. Pharm. Pharmacol*., **6**: 1276-1287. https://doi.org/10.5897/AJPP12.214

Jridi, M., Nasri, R., Salem, R.B.S.B., Lassoued, I., Barkia, A., Nasri, M., and Souissi, N., 2015. Chemical and biophysical properties of gelatins extracted from the skin of octopus (*Octopus vulgaris*). *LWT Fd. Sci. Technol*., **60**: 881-889. https://doi.org/10.1016/j.lwt.2014.10.057

Kaewruang, P., Benjakul, S., and Prodpran, T.,

2013. Molecular and functional properties of gelatin from the skin of unicorn leatherjacket as affected by extracting temperatures. *Fd. Chem*., **138**: 1431-1437. https://doi.org/10.1016/j.foodchem.2012.09.114

Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., and Shahidi, F., 2010. Comparative study on characteristics of gelatin from the skins of brownbanded bamboo shark and blacktip shark as affected by extraction conditions. *Fd. Hydrocolloid*, **24**: 164-171. https://doi.org/10.1016/j.foodhyd.2009.09.001

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature*, **227**: 680-685. https://doi.org/10.1038/227680a0

Ma, J., Pazos, I.M., Zhang, W., Culik, R.M., and Gai, F., 2015. Site-specific infrared probes of proteins. *Annu. Rev. Phys. Chem*., **66**: 357-377. https://doi.org/10.1146/annurev-physchem-040214-121802

Mad-Ali, S., Benjakul, S., Prodpran, T., and Maqsood, S., 2016. Characteristics and gel properties of gelatin from goat skin as affected by pretreatments using sodium sulfate and hydrogen peroxide. *J. Sci. Fd. Agric*., **96**: 2193-2203. https://doi.org/10.1002/jsfa.7336

Muyonga, J.H., Cole, C.G.B., and Duodu, K.G., 2004. Extraction and physicochemical characterisation of Nile perch (*Lates niloticus*) skin and bone gelatin. *Fd. Hydrocoll.*, **18**: 581-592. https://doi.org/10.1016/j.foodhyd.2003.08.009

Nagarajan, M., Benjakul, S., Prodpran, T., Songtipya, P., and Kishimura, H., 2012. Characteristics and functional properties of gelatin from splendid squid (*Loligo formosana*) skin as affected by extraction temperatures. *Fd. Hydrocoll.*, **29**: 389-397. https://doi.org/10.1016/j.foodhyd.2012.04.001

Ouyang, Z., and Bai, S., 2015. Preparation and *in-vitro* biocompatibility of gelatin/SA/HYA composite scaffold for tissue engineering. *Polym. Polym. Compos*., **23**: 503-508. https://doi.org/10.1177/096739111502300709

Pan, J., Li, Q., Jia, H., Xia, L., Jin, W., Shang, M., Xu, C., and Dong, X., 2018. Physiochemical and functional properties of tiger puffer (*Takifugu rubripes*) skin gelatin as affected by extraction conditions. *Int. J. Biol. Macromol*., **109**: 1045-1053. https://doi.org/10.1016/j.ijbiomac.2017.11.080

Pearce, K.N., and Kinsella, J.E., 1978. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *J. Agric. Fd. Chem*., **26**: 716-723. https://doi.org/10.1021/jf60217a041

Przybytniak, G., Sadło, J., Dąbrowska, M., and Zimek, Z., 2019. Radicals initiated by gamma rays in selected amino acids and collagen. *Nukleonika*, **64**: 11-17. https://doi.org/10.2478/nuka-2019-0002

Ramshaw, J.A., Shah, N.K., and Brodsky, B., 1998. Gly-XY tripeptide frequencies in collagen: A context for host–guest triple-helical peptides. *J. Struct. Biol*., **122**: 86-91. https://doi.org/10.1006/jsbi.1998.3977

Regenstein, J.M., and Zhou, P., 2007. *Collagen and gelatin from marine by-products*. *Maximising the value of marine by-products.* Wood Head Publishing, pp. 279-303. https://doi.org/10.1533/9781845692087.2.279

Sae-Leaw, T., and Benjakul, S., 2015. Physico-chemical properties and fishy odour of gelatin from seabass (*Lates calcarifer*) skin stored in ice. *Fd. Biosci*., **10**: 59-68. https://doi.org/10.1016/j.fbio.2015.02.002

Sae-Leaw, T., Benjakul, S., and O'Brien, N.M., 2016. Effects of defatting and tannic acid incorporation during extraction on properties and fishy odour of gelatin from seabass skin. *LWT Fd. Sci. Technol*., **65**: 661-667. https://doi.org/10.1016/j.lwt.2015.08.060

Sarbon, N.M., Badii, F., and Howell, N.K., 2013. Preparation and characterization of chicken skin gelatin as an alternative tomammalian gelatin. *Fd. Hydrocoll.*, **30**: 143-151. https://doi.org/10.1016/j.foodhyd.2012.05.009

Shahidi, F., Han, X.Q., and Synowiecki, J., 1995. Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). *Fd. Chem*., **53**: 285-293. https://doi.org/10.1016/0308-8146(95)93934-J

Silva, P., Albano, C., and Perera, R., 2008. Use of electron paramagnetic resonance to evaluate the behavior of free radicals in irradiated polyolefins. *Rev. Latinoam. Metal. Mat*., **28**: 79-90.

Wang, S.S., Yu, Y., Sun, Y., Liu, N., and Zhou, D.Q., 2019. Comparison of physicochemical characteristics and fibril formation ability of collagens extracted from the skin of farmed river puffer (*Takifugu obscurus*) and tiger puffer (*Takifugu rubripes*). *Mar. Drugs*, **17**: 462. https://doi.org/10.3390/md17080462

Zayas, J.F., 1997. *Solubility of proteins functionality of proteins in food*. Springer-Verlag/ Harwood Academic Publishers, Berlin/UK. pp. 6e22, 22e27. https://doi.org/10.1007/978-3-642-59116-7_2

Zhang, Y., Liu, W., Li, G., Shi, B., Miao, Y., and Wu, X., 2007. Isolation and partial characterization of pepsin-soluble collagen from the skin of

grass carp (*Ctenopharyngodon idella*). *Fd. Chem*., **103**: 906-912. https://doi.org/10.1016/j.foodchem.2006.09.053

Zhao, H., Wang, Q., Liu, C., Shang, Y., Wen, F., Wang, F., Liu, W., Xiao, W., and Li, W., 2018. A role for the respiratory chain in regulating meiosis initiation in *Saccharomyces cerevisiae*. *Genetics*, **208**: 1181-1194. https://doi.org/10.1534/genetics.118.300689

Zhao, W.H., Chi, C.F., Zhao, Y.Q., and Wang, B., 2018. Preparation, physicochemical and antioxidant properties of acid-and pepsin-soluble collagens from the swim bladders of miiuy croaker (*Miichthys miiuy*). *Mar. Drugs*, **16**:161. https://doi.org/10.3390/md16050161