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Study the Properties of Cross Linked Gelatin Films with Glutaraldehyde

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Abstract: Mechanical and swelling properties as well as fine structure of gelatin film cross linked using glutaraldehyde (GTA) have been studied to verify the influence of GTA concentration on the stability of film. Gelatin has been extracted from cow's bone by heat/pressure method that it has 9.6% moisture content, 2.4% ash and the viscosity of a 6.67% solution at 25°C was 1.4mPas. Gelatin films were prepared from solutions by the casting technique, as follows: 10 wt% gelatin, 4.5 wt% glycerin and GTA with the concentration between 0.01 and 0.2 wt%. The result indicated that increasing GTA concentration in gelatin film causes an increase in the stress at break and a decrease in the swelling percentage of gelatin film, but the strain at break increased slightly, as a result of GTA cross linking. The results of FTIR spectroscopy showed adding GTA to gelatin films leads to induce cross linking between gelatin and GTA that improves the mechanical properties. SEM photographs displayed a smooth and void free surface on the gelatin films as increasing GTA concentration.

Key words: Gelatin · glutaraldehyde · mechanical properties · FTIR · SEM

INTRODUCTION

In recent years have been seen an explosion in the level of research devoted to the development of new biodegradable materials, essentially due to the desire to protect the environment [1]. Among other biopolymers, gelatin has been extensively studied due to its low cost, biodegradability, biocompatibility and non-immunogenic. Gelatin is obtained by thermal denaturation or physical and chemical degradation of collagen, the most widespread protein in the body occurring in most connective tissues as skin, tendon and bone [2, 3]. The main sources of gelatin are pigskin, cow hide and fish bone/skin that there are four processes for gelatin extraction: Acid, Alkaline, Enzymatic and Heat/Pressure process. Among all of these processes, heat/pressure process is done at a short time and no chemicals, but the extracted gelatin has low bloom index [4-8]. Food, pharmaceutical and photographic industries are the main users of gelatin. Also it uses in the biomedical field as hard and soft capsules, microspheres, sealants for vascular prostheses, wound dressing, adsorbent pads for surgical use and implantable devices [2, 3, 9].

Although gelatin has some advantages, but there are some problems with the use of gelatin as a material: it dissolves in aqueous solution and has poor mechanical properties, which limits its possible applications as a biomaterial especially for long-term applications [2, 3]. On the other hand, gelatin contains the large number of functional side groups that it readily undergoes chemical crosslinking.Thus, submitting crosslinking to gelatin materials causes to improve the mechanical stability and decrease the solubility [2, 3, 10]. Chemical crosslinking usually is performed using crosslinking agents such as formaldehyde, glyoxal [11], glutaraldehyde [3] and genipin [2] as well as polyepoxy compounds [12] and transglutaminase [11], were used to produce modified gelatin films. Glutaraldehyde (GTA) is by far the most widely used chemical because it is inexpensive, easily available and its aqueous solutions can effectively stabilize collagenous tissues in a relatively short period [3].

In this research work, at first cow's bone gelatin (CBG) has been extracted by heat/pressure method and its characteristics have been investigated. Then the effect of GTA concentration has been studied on mechanical

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properties, swelling and fine structure of the prepared gelatin films.

MATERIALS AND METHODS

Gelatin extraction: The Holstein cow's bone was crushed and washed with water. For gelatin extraction using heat/pressure method [8], the crushed bones were treated in a high-pressure tank along with water at a ratio of 1:3 (bone: water) at 104°C and 1.2 bar for 5-6 cs. The gelatin solution was then filtered using a multi-layered gauze (cloths filter) to remove all solid materials. This solution was first defatted by phase separation method then the remaining gelatin solution was boiled to achieve a concentrate gelatin solution. The solution was dried at room temperature for 48 h and milled. The produced powder (CBG) was used for the experimental work.

Determination of moisture content: Ceramic beaker was dried at 104°C for 24 h. The beaker was cooled down in a vacuum incubator and precise weight was recorded. Approximately 1gr of CBG was put in the ceramic beaker and dried at 104°C for 24 h, cooled down in a vacuum incubator [13]. Then the exact weight was recorded and the moisture content in bone gelatin was calculated.

Determination of ash: The ash content of CBG was determined by pyrolysis [13] of 1gr gelatin at 900°C to reach a constant weight.

Determination of pH: The pH of 1% and 2% gelatin solutions was determined by a pH-meter (Horiba-N12) at 25.6°C.

Determination of viscosity: The viscosity of a 6.67% gelatin solution [14, 15] at 25°C was determined with rotary viscometer (Especialidades, Medieas, MYR S.L., Type V2-R, Spain) at speed of 10 and 30 rpm.

Amino acid analysis: Amino acid analysis was conducted using Pico.Tag method [14]. This method involves three steps: 1) Hydrolysis of the gelatin to yield free amino acids, 2) Pre-column derivatization of the sample that at this step gelatin is first hydrolyzed with HCl and then derivatized with phenylisothiocyanate to produce phenylthiocarbamyl amino acids. 3) Analysis by reverse phase HPLC (Waters 1525).

Analyzed amino acids were detected by dual λ absorbance detector (Model: Waters 2487).

Atomic absorption analysis: The amount of the Cu, Ca, Zn, Al, Pb, Cr in CBG were quantified on a atomic absorption spectrophotometer (Varian-Spectr AA-200). Standard curves were prepared from absorption at 4 different concentrations.

Film preparation: Film-forming solutions were prepared by dissolving 10 gr of CBG and 4.5 gr glycerin in 100 ml distilled water. Then from 0.01 to 0.2 gr glutaraldehyde was added to the solutions. The solutions were poured on to Plexiglas plates (15×20 cm) and dried at room temperature for 24-48h. The gelatin films were stored in 28% relative humidity and 25°C conditions for 3 days before testing.

Mechanical properties: The stress and strain at break of gelatin films, average of five determinations, were determined using an Instron Testing Machine Micro250 (SLD) according to method ASTM D882-91 [16]. A speed of 50 mm/min was used with an initial grip separation of 10 cm. The thickness of the samples was determined using a Thickness Gauge (JEWELS Co.).

Swelling: Gelatin films were weighed in air-dried condition. They were then immersed in distilled water for different periods of time. Wet samples were wiped with filter paper to remove excess liquid and weighed again. The amount of adsorbed water was calculated as:

$$W\ (\%) = 100\ (W_w - W_d)/W_d$$

Where W_w and W_d are the weights of the wet and air-dried samples [2, 3].

Fourier transform infrared spectroscopy: FTIR spectra were obtained from very thin gelatin films and potassium bromide (KBr) discs containing the gelatin powder. All spectra were obtained using a Bomem-MB-100 infrared spectrophotometer over a range of 4000-500 cm^{-1} with a resolution of 4 cm^{-1}.

Scanning electron microscopy: The morphology of the gelatin films was determined by observation of the samples on a scanning electron microscope (SEM) (LEO440i) with an acceleration voltage of 10 kV. The gelatin films were mounted on stubs using double side sticky tab and coated with a thin layer of gold to avoid charging in the microscope.

RESULTS AND DISCUSSION

In order to produce gelatin from cow's bone by heat/pressure method the required time for treatment and concentrate steps is less than 10 h and drying at room temperature takes 48 h. The duration of drying can be reduced by warm air method. At the used methods for producing gelatin [7, 14, 15, 17], the time is necessary for producing gelatin of bone is too long. Also most of them need chemicals during the processing. The method used in this research for producing gelatin of cow's bone, was done with short time and no chemicals, but in the severe conditions.

The results of the moisture content, ash content, pH solution and viscosity of CBG are shown in Table 1.

Moisture content of CBG is 9.6% that is in the range of 9-12.9% moisture content reported [11, 14, 18] for other gelatin.CBG contains 2.4% ash which allowed to use in food industry. It is lower than the recommended maximum of 2.6% [7, 19] and much less than the amount of ash reported for fish bone (3-11%) [14].

The pH of the gelatin solution (Table 1) is almost neutral, but the pH of the gelatin was extracted by acid method is between 4.5 and 6 and the range of pH for Alkaline gelatin is 5 to 7 [7]. These differences are probably concerned to the kind of gelatin producing process.

Gel strenght, determined by the bloom value and viscosity are the important properties of gelatin, that viscosity increases with increasing bloom strength. The viscosity and gel strength is influenced by raw material, amino acid composition and production process [9, 14, 15]. Increasing extraction temperature for young fish bones from 50 to 70°C was caused to decrease the bloom value from 155 gr to 0 [20]. The viscosity of 6.67% CBG solution was 1.4mPa.s.. Because the gelatin was extracted at high temperature, also according to the amino acid composition of CBG (Table 2) the amount of proline (9.4 g/100 g gelatin) is lower than other gelatins [11, 14] and it has been recognized that gelatin with high levels of imino acids tends to have higher gel strength [14, 13].

The CBG is found to contain proline at level between those of mammalian gelatins and those of coldwater fish species. However, the researcher reported that the proline content of gelatins has a strong influence on their functional properties [13-15].

The results of Atomic Absorption analysis (Table 3) showed the existence of Zn, Cu, Al, Pb, Ca and a little Cr in CBG (Table 3). The amount of calcium (0.34 mg g^{-1}) is higher than 0.29 mg g^{-1} reported for fish gelatin [13] that it is resulted from mineral part of bone and used tap water(hard water) for gelatin production. The amount of lead is higher than 1.5 mg kg^{-1} reported by Food Chemical Codex [7]. This is probably as a result of high air-pollution.

Aldehydes (glutaraldehyde and formaldehyde) crosslinking influence the stress at break and the extensibility of films based on high bloom gelatin [3, 11]. In gelatin films increasing the bloom index from 80 to 270 gr causes to increasing the stress at break from 1.3 to 5.1 MPa [9]. The results of mechanical properties of the CBG films show that the stress at break increases (Fig. 1) and the strain at break increases slightly (Fig. 2) on increasing GTA concentration.

Table 1: The characteristics of cow's bone gelatins

Characteristics	Cow's bone gelatin
Moisture content (%)	9.6
pH (solution 1%)	7.01
pH (solution 2%)	6.77
Ash (%)	2.4
Viscosity (mPa.s.)	1.4

Table 2: Amino acid composition of cow's bone and bovine hide gelatins

	gAA/100 g gelatin	
Amino Acids (AA)	Cow's bone gelatin	Bovine hide gelatin [10]
Aspartic acid	11.47	7.46
Threonine	3.15	2.11
Serine	2.94	3.62
Glutamic acid	15.56	11.28
Proline	9.40	12.52
Glycine	17.24	32.63
Alanine	6.67	10.88
Valine	2.09	2.18
Methionine	0.78	0.42
Isoleucine	1.15	1.44
Leucine	2.27	3.00
Tyrosine	0.66	0.40
Phenylanine	3.15	1.99
Lysine	3.78	3.46
Histidine	0.67	0.77
Arginine	2.38	9.90

Table 3: Metals in 1gr cow's bone gelatin

metal	Ca	Cu	Zn	Al	Pb	Cr
mg	0.34	0.003	0.033	0.28	0.32	<0.004

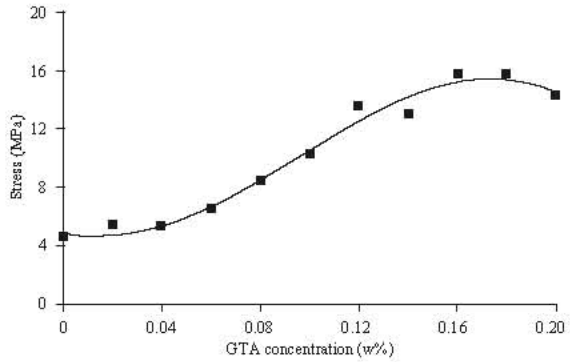

Fig. 1: Effect of glutaraldehyde on the stress at break of gelatin films

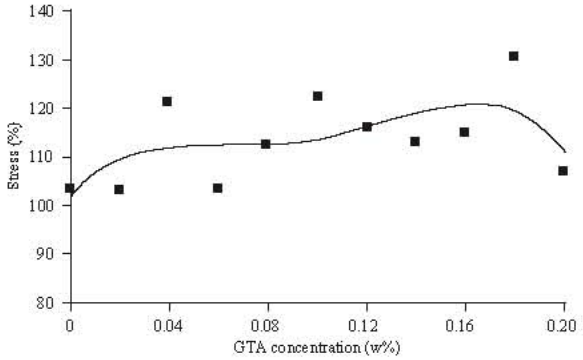

Fig. 2: Effect of glutaraldehyde on the strain at break of gelatin films

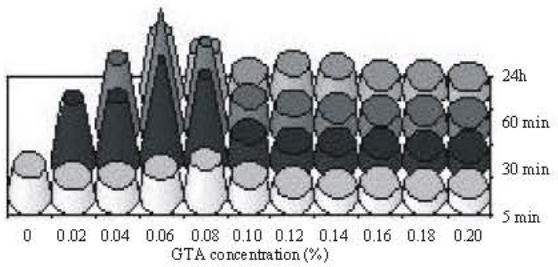

Fig. 3: Swelling (W%) of gelatin films after different times of storage in distilled water as a function of GTA concentration (the height of cones show swelling percentage)

It is evident that the main variations in stress at break of gelatin films are between 0.05 and 0.18%wt GTA concentrations, but a significant increasing in stress at break is at 0.16%wt GTA concentration. The tensile strength of CBG film containing 0.16% GTA is about 3.5 times with respect to uncrosslinked gelatin. Although the CBG is a gelatin with low quality (viscosity = 0), the tensile strength of crosslinked gelatin films is higher than reported gelatin films [3, 9].

Table 4: The effect of GTA and time on swelling (%w) of gelatin films

GTA (wt%)	5 min	30 min	60 min	24 h
0	388.98	nd	nd	cs
0.02	250.76	800.65	nd	cs
0.04	276.60	809.33	866.403	nd
0.06	357.82	1266.32	1485.38	768.5731
0.08	476.06	1081.12	1051.13	559.5377
0.1	263.01	368.99	377.7	247.6847
0.12	169.62	261.72	285.18	357.93
0.14	158.09	237.57	237.03	315.55
0.16	169.17	214.91	217.37	220.88
0.18	155.95	196.44	215.89	223.74
0.2	159.28	183.32	185.68	202.20

cs = compeletly solved nd = cannot determined

As a concequence, the tensile strength of CBG films can be improved just with 0.16% GTA and simultaneously the strain at break increases slightly as well as the films are flexible and easily handled.

Table 4 and Fig. 3 show the swelling variations of gelatin films as a function of GTA concentration following different time of storage in distilled water. The swelling percentage of uncrosslinked gelatin film is about 390% after 5min. swelling measurements at longer times are hindered by the solubility of the film which begins to dissolve in water. In general, increasing GTA concentration provokes to decrease the swelling percentage and increase the time of films solubility. There isn't a significant variation when GTA concentration has increased more than 0.16 (%w) and the swelling percentage reaches to a minimum value. It confirms the results of mechanical properties, because at 0.16% GTA concentration is observed the stress at break is maximum and the swelling percentage is minimum, it's probably due to the maximum crosslinking between GTA and gelatin at this GTA concentration.

The FTIR spectra exhibited by bone gelatin powder differ from those exhibited by bone gelatin films (Fig. 4) specially in the amide I (about 1650 cm^{-1}), amide II (about 1550 cm^{-1}) and amide III (about 1240 cm^{-1}) regions. Compared to the spectra for bone gelatin powder, the gelatin films contain GTA show higher intensity amide I and amide II bands. It means the extent of order in GTA gelatin films maybe higher than that in gelatin powder. The intensity of the amide III band has been associated with triple helical structure [20] that the intensity of amide III band for the GTA gelatin films is higher than that for the gelatin powder. It seems that the GTA gelatin films have more intermolecular associations, as a result of GTA crosslinking.

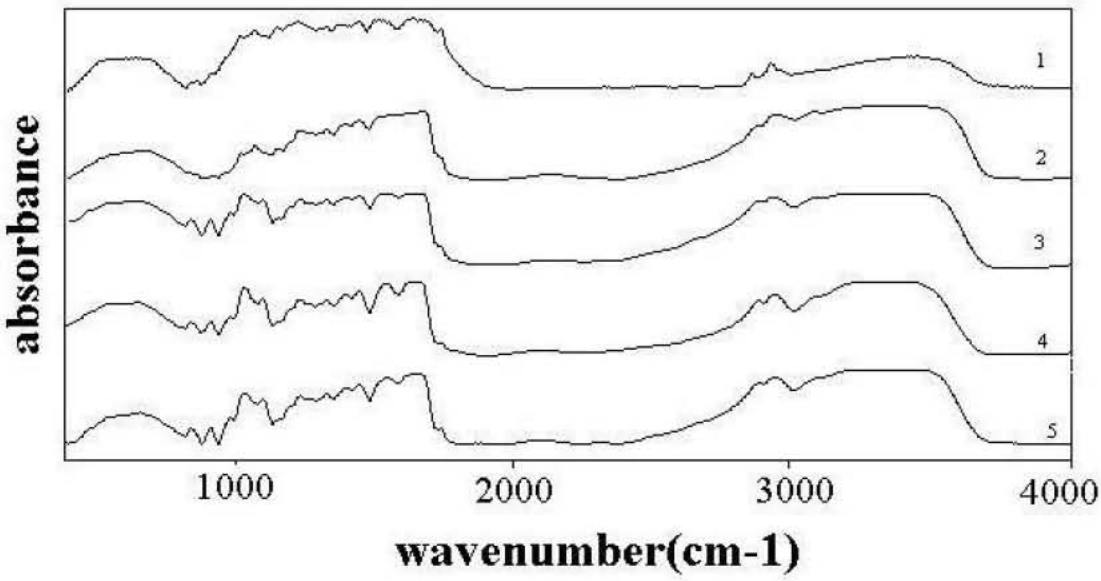

Fig. 4: FTIR spectra for: (1) bone gelatin powder, (2) bone gelatin film, (3) bone gelatin film contains glycerin, (4) bone gelatin film contains glycerin and 0.01%GTA and (5) bone gelatin film contains glycerin and 0.12% GTA

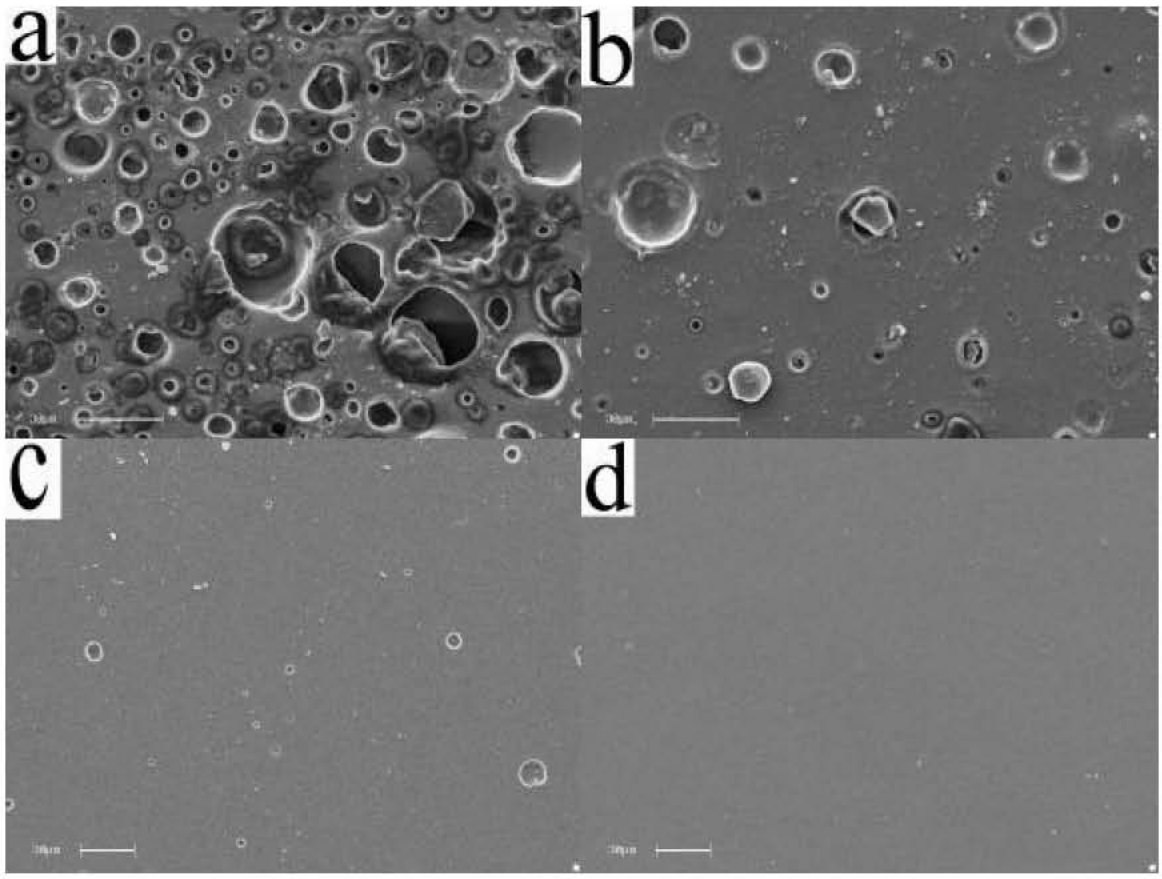

Fig. 5: SEM micrographs for gelatin films: (a) without GTA, (b) with 0.01% GTA, (c) with 0.1% GTA, (d) with 0.16% GTA (original magnification×1000)

As shown in Fig. 4 with increasing the GTA concentration the intensity of peaks increase at about 1110 cm^{-1}, which related to C—O vibration, show crosslinking has been occurred. Absorption in the region of 1000 to 1100 cm^{-1} is attributed to C—O vibration due to carbohydrates in collagen are associated with glycation of collagen and carbohydrates are required in the formation of pentosidine crosslinks [20].

With regards to SEM micrographs, the porosity of uncrosslinked gelatin film is reduced as GTA concentration increases and a smooth and void free surface induces (Fig. 5). The most of porosities are eliminated by increasing GTA concentration more than 0.01% in CBG gelatin films due to GTA crosslinking [21]. It confirms the increasing of film strength and decreasing the swelling percentage of the gelatin films on increasing GTA concentration.

CONCLUSION

Gelatin extraction from cow's bone using heat/pressure method is quicker as compared to conventional methods [4-8]. At the same time no chemicals are in this method. And the produced gelatin film is flexible. To improve the tensile strength of the gelatin film and its stability, glutaraldehyde as a crosslinking agent was used. Using 0.16%wt GTA in the gelatin film is sufficient to increase the stress at break by a factor of about 3.5 times with respect to uncrosslinked gelatin film and the strain at break is increased slightly. The swelling percentage is decreased on increasing GTA concentration and reaches almost to a minimum about 220% at 0.16%wt GTA. According to the SEM micrograph, decreasing the porosity in gelatin films as a result of increasing GTA concentration confirms the stabilization of the gelatin films. FTIR spectroscopy shows that conversion of gelatin powder to gelatin film and addition GTA to the films leads to increase crosslinking formation and molecular order.

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