Influence of UV Irradiation on the Properties of Goatskin Collagen Matrices[#]

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Abstract: Research on the effect of UV radiation on the properties of collagen is an area of potential interest owing to the fact that collagen is an important biomaterials finding immense use in various fields. In the present work, influence of UV irradiation on the mechanical properties, water vapor permeability, dimensional stability and surface morphology of goatskin collagen matrices was investigated. Air-dried goatskin matrices were submitted to treatment with UV irradiation (wavelength 312 nm) for different time intervals. The mechanical properties, water vapor permeability, dimensional stability and surface morphology of the matrices were investigated before and after UV irradiation. It was found that the tensile strength of the goatskin matrices increases slightly at the initial stages of irradiation (2-4 h), and decreases dramatically after irradiation for long time (8-64 h). Increasing the UV irradiation time leads to a decrease in elongation at break and tensile fracture energy. The water permeability decreases at the beginning of the UV irradiation (2-4 h) and increase after irradiation for longer time (8-64 h). UV irradiation results in a continuous decrease in samples dimension. Scanning electron microscopy observation shows that UV irradiation induces crack and loss of skin tone. It indicates that phototransformation has occurred during UV irradiation and changed the conformational state of collagen.

Key words: UV irradiation; collagen matrices; properties

1 Introduction

Studies on the effect of UV radiation on collagen have a wider application in medical, biomaterial, and tanning industry. It has been reported that the process of UV irradiation can induce crosslink into collagen fibrils. However, this is complicated by peptide bond scission events that may also occur through free radical mechanisms [1-4]. The photochemical reactions may be attributed to direct absorption by tyrosine/phenylalanine or to peptide bonds. The fine tuning of irradiation processes may therefore result in either the degradation processes or crosslinking processes of collagen fibers. The efficiency of these two types of reactions depends mainly on the sample preparation and irradiation dose. The cleavage of the peptide bond occurs to account for the observed reduction in viscosity of the rod-like triple helical molecule and concluded that the primary effect of UV was to generate reactive oxygen species in the water molecules around the collagen molecule, resulting in cleavage of the peptide bonds. This was supported by inhibition of degradation by free radical scavengers and anti-oxidants [5-7]. UVB irradiation has been shown to result in denaturation of the triple helix, followed by fragmentation of collagen chains. From this, it was supposed that sun-generated radiation containing UVB light can play a significant role in collagen photolysis and photo aging [8].

In this present investigation on the effect of UV irradiation on goatskin collagen matrices has been studied. This paper details the changes in mechanical parameters, water vapor permeability, dimensional

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stability, and surface morphology of goatskin collagen on UV irradiation.

2 Experimental

2.1 Materials

Pickled goatskins were supplied by Xinxiang Heitian Mingliang Leather Co., Ltd., Xinxiang, China. The other chemicals, NaCl, H_2SO_4, $NaHCO_3$, CH_3COONa are all analytical reagents.

2.2 UV irradiation

The samples were irradiated under air at room temperature using a UV-resistant Climate Testing Machine (Model ZN, Shanghai Linpin Experiment Equipment Co., Ltd.), which emits light mainly at a wavelength of 312 nm. Irradiation experiments were carried out on aluminum shutter at a distance of 5 cm from the light source. Various doses of UV irradiation were obtained by varying irradiation time (0-64h). After irradiation, all the samples were air-conditioned at the relative humidity of 65% (controlled with 36.7wt% H_2SO_4) for at least two weeks to a constant weight.

2.3 Characterization

2.31 Mechanical Properties

All the samples were air-conditioned in at the relative humidity of 65% (controlled with 36.7wt% H_2SO_4) for at least two weeks to a constant weight. Mechanical properties of samples before and after different intervals of UV irradiation were tested using a Microcomputer Control Electronic Universal Testing Machine (Model CMT6104, Shenzhen Sans Materials Testing Machine Co., Ltd.). The samples were stretched at a speed of 100 mm/min. The average of at least five tests was reported.

2.32 Water Vapor Permeability

All the samples were air-conditioned at the relative humidity of 65% (controlled with 36.7wt% H_2SO_4) for at least two weeks to a constant weight. Water vapor transmission rate (WVTR) was measured by permeation cup method. Fill the stainless steel cup (body diameter of 45.0 mm and top diameter of 30.0 mm) with 50mL distilled water. The sample was mounted to the top of the cup. An O-ring was used to hold the sample in place. After taking initial weights of the test cup, the cups were placed in a desiccator cabinet with 98wt% H_2SO_4. Weight loss measurements were taken by weighing the test cup with an electronic scale (Ohaus, Switzerland) after 24h of incubation. The water vapor transmission rate (WVTR) of the sample was calculated as follows:

$$WVTR=(w_1-w_2)/At\ (mg/(10cm^2\cdot 24h))$$

where w_1 is the weight of the cup before test (mg), w_2 is the weight of the cup after 24h of incubation (mg), A is the area of exposed film (7.07cm^2), and t is the time of experiment (h). There were at least three repetitions per experiment.

2.33 Scanning Electron Microscopy

The samples were coated with a thin layer of gold by vacuum sputtering technique, and then observed with a scanning electron microscope (Quanta-200, FEI, The Netherlands).

3 Results and discussion

3.1 Mechanical properties

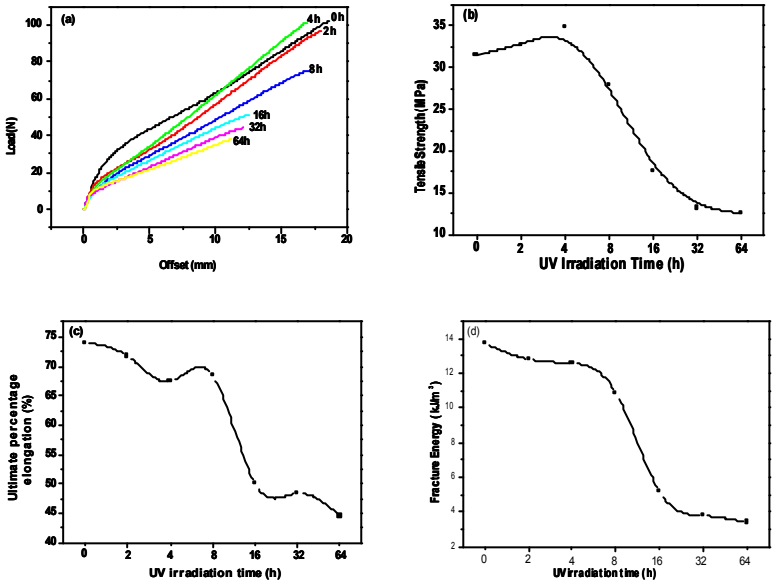


Fig. 1 Influence of UV irradiation on the mechanical properties of goatskin collagen matrices (a) load-strain curves, (b) tensile strength, (d) elongation at break, (d) tensile fracture energy

The influence of UV irradiation on the mechanical properties of samples was illustrated in Fig. 1. The characteristic load-strain curves of goatskin before and after UV irradiation was reported in Fig. 1(a). The general trend of the load-strain curves of goatskin after UV irradiation is very similar to that characteristic of control non-irradiated goatskin. After a very small initial stage, the material is stiff. Hereafter, the plots exhibit a more gradual slope, which remains approximately constant until the break point of the goatskin. The effect of UV irradiation is more apparent in this second linear part of the load-strain curve. Results of the dependence of tensile strength on the UV irradiation time are shown in Fig 1(b). It was found that the tensile strength of the goatskin matrices increases slightly in the initial stages of irradiation (0-4 h), and decreases dramatically after irradiation for long time (8-64 h). The increasing duration of UV irradiation leads to a decrease in ultimate percentage elongation (Fig 1(c)). It was concluded that UV irradiation had decreased the toughness of goatskin matrices. UV irradiation continuously decreases the tensile fracture energy as shown in Fig 1(d). The tensile fracture energy was decreased significantly from 13.7 to 3.4kJ m^{-3} after 64h of UV irradiation, a decrease of 303%. It was shown that less energy is required to break the sample due to the UV irradiation. The deterioration in mechanical performances is a consequence of the chain scission and cross-linking reaction within a fibril. Moreover, the loss of mechanical properties after long time of UV irradiation of collagen may be connected with breaking-up of inter- and intra-molecular hydrogen bonds and release of water which controls H-O-H-collagen bonds. The course of the chain scission and cross-linking reactions depends also on the water content in collagen samples.

3.2 Water Vapor Transmission Rate

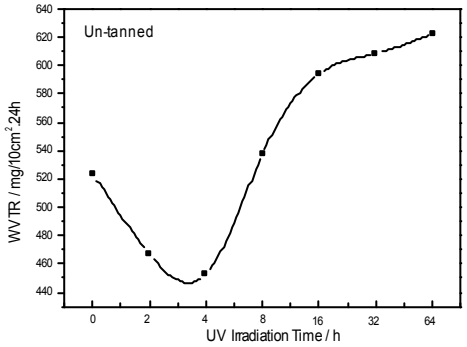


Fig. 2 Water vapor transmission rate of goatskin before and after UV irradiation

Water vapor transmission rate (WVTR) is defined as the amount of water vapor passing through a given area of a sheet or film in a given time, when the sheet or film is maintained at a constant temperature and when its faces are exposed to a certain relative humidity. Water vapor transmission rate for goatskin collagen before and after UV irradiation is shown in Fig. 2. WVTR decreases at the beginning of the UV irradiation (0-4 h) and increase after irradiation for long time (8-64 h). It is known that the diffusion of water vapor molecules through macromolecular material includes three steps: adsorption, diffusion and desorption. In a controlled environment (controlled water vapor pressure, relative humidity and temperature), the dominant parameters that may influence the WVTR include the crystallinity, the amount of hydrophilic groups and the pore characteristics of the material, e.g. pore volume, pore size, pore size distribution and pore microstructure. In the initial stages of UV irradiation, the amount of polar groups on collagen macromolecular chain may decrease due to the potential crosslinking reactions within fibrils during UV irradiation. This is obviously not in favor of the adsorption and diffusion of water vapor molecules throughout the leather matrices. With the irradiation time increase, however, chain scission reaction will result in the crack of the surface and the cleavage of collagen fibers, which decreases the porosity and increases the water vapor permeability.

3.3 Dimensional Stability

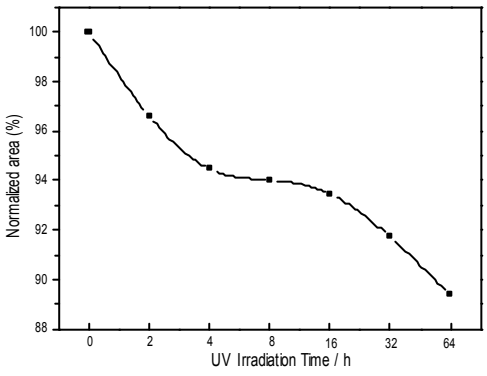
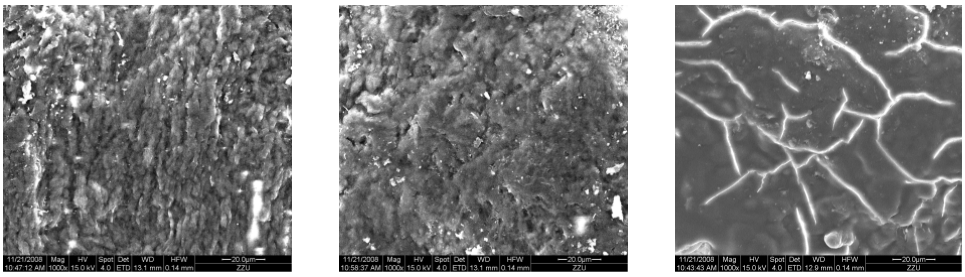


Fig. 3 Area changes of goatskin before and after different UV irradiation times

The area of each sample was measured before and after UV irradiation. The influence of UV irradiation time on the sample dimensional stability was studied and the results are illustrated in Fig. 3. It can be seen that a continuous decrease in the area of the goatskin matrices was found after UV irradiation, especially after being irradiated for a long time. The shrinkage of the weak part of collagen fiber during UV irradiation resulted in a decreased sample dimension.

3.4 Surface Morphology



(a) 0h **(b) 4h** **(c) 64h**

Fig. 4 Surface SEM images of goatskin matrix before and after UV irradiation

UV irradiation changes the physical and chemical properties of collagen by photochemical reaction. So it is expected that it changes the surface morphology of goatskin. The SEM images of goatskin matrices surface before and after UV irradiation are shown in Fig. 4. The photograph of non-irradiated goatskin matrix shows the multifasicular nature of the goatskin but the overall morphology is smooth. Short time (4h) irradiated sample exhibited some surface irregularity. However, as irradiation continued, sample exhibited some cracks and loss of skin tone (Fig. 4c). It indicates that phototransformation has occurred during UV irradiation and changed the conformational state of collagen.

4 Conclusions

The mechanical properties, water vapor permeability, dimensional stability and surface morphology of the goatskin matrices are sensitive to UV irradiation. Tensile strength of the samples increases slightly in the initial stages of irradiation (0-4 h), and decreases dramatically after long time of UV irradiation (8-64 h). Increasing UV irradiation time leads to a decrease in elongation at break and tensile fracture energy. The water vapor permeability decreases at the initial stage of UV irradiation (0-4 h) and then increases after UV irradiation for longer time (8-64 h). UV irradiation results in a continuous decrease in samples dimension. UV irradiation induces crack and loss of skin tone. The change of properties of goatskin collagen matrices after UV irradiation results from photochemical changes like cross-linking and chains scission.

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