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Thermal degradation behavior of collagen from sea cucumber (*Stichopus japonicus*) using TG-FTIR analysis

Leilei Si, Yan Fan, Yuekun Wang, Leilei Sun, Bafang Li, Changhu Xue, Hu Hou*

College of Food Science and Engineering, Ocean University of China, No. 5, Yu Shan Road, Qingdao, Shandong Province, 266003, PR China

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ABSTRACT

Collagen is the main protein of sea cucumber, and its structure and thermal properties were analyzed, which were related to the storage stability of sea cucumber. Collagen molecules (SCC) and collagen fiber from sea cucumber showed the similar amino acid composition. Fourier transform infrared (FTIR) spectra of SCC revealed amide A and I band shifted slightly when the temperature ranged from 20 °C to 100 °C, and X-ray diffraction indicated the distance between adjacent molecular chains decreased from 11.85 Å to 11.07 Å as the temperature increased from 20 °C to 100 °C. Thermal degradation behavior was analyzed by thermogravimetric analysis (TG) coupled with FTIR, and the degradation mechanism function of SCC could be described by $G(\alpha) = [-\ln(1-\alpha)]^{2/3}$, and the thermal degradation activation energy was in the range of 163–173 kJ/mol. As the temperature increased, the amino acids in the SCC began to degrade. CO_2, NH_3, H_2O, CH_4, NO_2 and HCN were released respectively along with various chemical reactions at different temperatures.

1. Introduction

Sea cucumber (*Stichopus japonicus*) has a great market value in East Asia because of its abundant nutrition. The major edible parts of sea cucumbers are the body walls, mainly consisting of collagen [1]. The physicochemical properties of sea cucumber collagen (SCC) greatly determine the distinctive textural properties of sea cucumber. Fresh sea cucumbers are easy to autolyze, and traditional processing is generally heated and dried for storage and sale.

Heat treatment is one of the most common approaches in sea cucumber processing, and the structure of collagen in the sea cucumber is changed. The thermal stability of collagen is a significant factor for the sea cucumber processing. In previous studies, most researches focused on the enzymatic degradation of collagen, and the determination of thermal denaturation temperature [2–5]. However, the thermal degradation behavior and mechanism of sea cucumber collagen was less reported.

Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction technology are effective methods to investigate the protein structure, and thermogravimetric analysis (TG) is the most important approach to speculate on the thermal degradation mechanism and activation energy [6]. The combination of a TG with a FTIR (TG-FTIR) is suitable for thermal degradation mechanism because of its high accuracy, sensitivity and real-time analysis. It has been used for analyzing degradation of the amino acid through TG-FTIR analysis [7,8]. By heating a sample on the TG, a sample released volatile materials or generated combustion components, and the components transferred to the FTIR cell and can be identified.

In this paper, amino acid compositions of SCC and collagen fiber were determined, and FTIR spectra and X-ray diffraction of SCC were analyzed when the temperature of SCC ranged from 20 °C to 100 °C. Thermal degradation behavior and mechanism were analyzed by TG analysis coupled with FTIR. It can provide a theoretical basis for processing of sea cucumber products.

2. Materials and methods

2.1. Materials and chemicals

Sea cucumbers (*Stichopus japonicas*), with the body weight of 110 ± 10 g, were bought from Nanshan market (Qingdao, China). All reagents were of analytical grade or the best grade available.

2.2. Preparation of sea cucumber collagen

Sea cucumber collagen (SCC) was prepared according to the method described by previous study with some modifications [9]. Sea cucumbers were dissected, eviscerated, and cut into pieces (0.5 cm × 0.5 cm × 0.5 cm). The pieces were washed with distilled water, and put into 4 mmol/L edetate disodium (EDTA-Na_2) in 0.1 mol/

* Corresponding author.
E-mail addresses: houhu@ouc.edu.cn, houhuouc@163.com (H. Hou).

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L pH 8.0 Tris-HCl (1:15, w/v) solution for 2 d with continuous stirring. They were filtered, washed, and stirred in distilled water (1:20, w/v) overnight. After removal of undecomposed pieces, the mixture was centrifuged at 6000 g for 10 min and the precipitate was collagen fiber (CF). Then CF was stirred in 0.1 mol/L NaOH solution (1:20, w/v) for 3 d. After centrifuged at 10,000*g* for 45 min, the precipitate was rinsed with distilled water to neutral and stirred in 0.5 mol/L acetic acid (1:20, w/v) containing 0.5% of pepsin for 2 d. After digestion, the solution was centrifuged at 10,000*g* for 20 min, and then SCC in the solution was salted out by NaCl. The precipitate was dissolved in 0.5 mol/L acetic acid, and dialyzed with 0.1 mol/L acetic acid for 1 d and distilled water for 2 d. All operations were performed at 4 °C. SCC was freeze-dried and stored at −20 °C in order to be used in the subsequent experiments.

2.3. Amino acid analysis

Lyophilized SCC, CF and the sea cucumber body wall were hydrolyzed in 6 mol/L HCl at 110 °C for 24 h, and the amino acid compositions of the samples were determined by an amino acid analyzer (Hitachi L-8900).

2.4. FTIR spectroscopy

SCC samples were treated under different temperatures (20, 40, 60, 80 and 100 °C) for 30 min. FTIR spectra were obtained from discs containing SCC samples and dry potassium bromide (KBr) extruded together. The spectra were measured using infrared spectrophotometer (Nicolet iS10) from 4000 to 400 cm^{-1} at a data acquisition rate of 4 cm^{-1} per point.

2.5. X-ray diffraction

The X-ray diffraction of SCC was performed on an X-ray diffractometer (Bruker D8 ADVANCE) with the range from 5° to 40° at the scanning rate of 4°/min.

2.6. Thermogravimetric analysis

The thermal degradation characteristics of SCC samples were monitored using a thermogravimetric analyzer (Netzsch TG 209 F3). For each experimental run, 10 mg of SCC samples were heated from 35 °C to 600 °C at the heating rate of 5, 10, 15, 20 and 30 °C/min under the N_2 atmosphere (the flow rate of 50 mL/min). The TG curves and derivative thermogravimetry (DTG) curves were studied by curve-fitting analysis using Netzsch Proteus software.

2.7. TG-FTIR experiment

TG-FTIR instrument (Netzsch TG 209 F3 linked with Bruker Tensor 27) was used to assess the weight loss ratio of samples and generation of evolved gas. In the TG experiment, 10 mg of SCC sample was heated from 35 °C to 600 °C at the heating rate of 10 °C/min under the N_2 atmosphere (the flow rate of 50 mL/min). Meanwhile, FTIR spectrometer was applied to detect the evolved gases. The FTIR spectra were obtained in the range of 4000–600 cm^{-1} at a resolution of 4 cm^{-1}.

2.8. Thermal degradation kinetics

The conversion degree (α) can be calculated by the Eq. (1):

$$\alpha = \frac{m_i - m_t}{m_i - m_\infty} \quad (1)$$

where m_i is the initial mass of the sample, m_t is the mass at time t, and m_∞ is the mass of the sample at the end of the experiment.

Basing on the degradation reaction theory [10,11], the kinetic Eq. (2) for SCC could be described as follows:

$$\frac{d\alpha}{dt} = k \cdot F(\alpha) \quad (2)$$

where t is time (in min), F(α) is the reaction mechanism function, and k is the reaction rate constant, which can be obtained by Arrhenius Eq. (3):

$$k = A \cdot e^{\left(-\frac{E}{R \cdot T}\right)} \quad (3)$$

where A is the pre-exponential factor (1/min), E is the activation energy (kJ/mol), R is the gas constant (J/(mol K)) and T is the absolute temperature (K).

The heating rate (°C/min) is assumed as β = dT/dt, and the combination of Eqs. (2) and (3) gives:

$$\frac{d\alpha}{dT} = \frac{A}{\beta} \cdot e^{\left(-\frac{E}{R \cdot T}\right)} \cdot F(\alpha) \quad (4)$$

There are many methods deduced by this equation to study the degradation kinetics of samples. In this study, the thermal kinetics of SCC was studied preliminarily by Flynn-Wall-Ozawa (FWO) method [12,13] and Satava-Sestak method [14]. The activation energy (*E*) can be obtained without the kinetic mechanism function. After that, the thermal degradation mechanism function can be inferred by combining the Satava-Sestak method.

3. Results and discussion

3.1. Amino acid composition analysis

The physicochemical properties of collagen, especially thermal stability, are closely related to the amino acid composition of collagen [15]. As shown in Table 1, the amino acid compositions of SCC, CF and sea cucumber body wall were quite similar, which implied collagen was the main protein in sea cucumber. Glycine levels in SCC, CF and sea cucumber body wall were 345, 341 and 324 residues/1000 residues, respectively, which accounted for approximately one third of the whole residues in collagen peptide chains, and played an important role in the helical structure of collagen [16]. The imino acid (proline and hydroxyproline) was 173 residues/1000 residues in SCC, lower than that in seabass scale collagen (193 residues/1000 residues) [17] and tilapia collagen (207 residues/1000 residues) [18], but higher than that in bighead carp scale collagen (156 residues/1000 residues) [19]. Generally speaking, the collagen of the terrestrial mammals has more imino acid than that of the marine animals [20,21]. The thermal stability of collagen was related to imino acid level, because the stability of a polypeptide and the triple helix of collagen could be enhanced by the

Table 1
Amino acid compositions of collagen (SCC), collagen fiber (CF) and the body wall of sea cucumber (residues/1000 amino acid residues).

Amino acid	SCC	CF	Sea cucumber body wall
Asp	52	51	61
Thr	26	28	23
Ser	38	38	41
Glu	89	86	103
Gly	345	341	324
Ala	131	136	132
Cys	2	2	2
Val	19	18	17
Met	1	2	2
Ile	12	15	13
Leu	23	20	20
Tyr	2	3	3
Phe	10	11	9
His	7	8	6
Lys	25	24	23
Arg	45	48	50
Pro	102	86	90
Hyp	71	83	81