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Full Length Research Paper

Rheological changes of sea cucumber *Stichipus japonicus* during different heated times

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Changes in tissue structure, weight, volume and rheological properties of sea cucumber meat were studied. Sea cucumber *Stichopus japonicus* was heated at 70°C for 1, 2, 6, 10 and 24 h, respectively. Microscopic photograph revealed that the structure has significant changed. When heated from 1 to 24 h. The weight, volume, rupture strength, and elastic moduli of sea cucumber meat decreased greatly, but the relaxation time and viscosity showed positive correlation with heating time. These results confirmed that the change of texture and rheological properties of Sea cucumber meat was mainly due to thermal denaturation and gelatinization of collagen during heating.

Key words: Sea cucumber, tissue structure, rheological properties, collagen.

INTRODUCTION

Sea cucumber *Stichopus japonicus* is a highly priced mollusk and its meat is considered great delicacies all over the world. The edible worth of sea cucumber has already been strongly certified. And the physiological functions of sea cucumber have also been recorded (Su et al., 2003). In food science, studies of sea cucumber mainly focus on the nutrition values, biological activity, and pharmacological activity of the components, such as carotenoids, fatty acid, free sterol compositions, fucan sulfates, chondroitin sulfate, stiparin-inhibitor, tensilin and glycosides, these components have specially physiological functions (Postma et al., 2009; Chen et al., 2001; Kariya et al., 2004; Ismail et al., 2008; Ji et al., 2008; Duan et al., 2010; Marques et al., 2011).But these studies do not have a rheological consideration on sea cucumber meat.

Previous papers on rheological and texture properties of yellowtail salmon, jellyfish and abalone suggest that elements contributing to meat hardness (tenderness) are muscle fiber and connective tissue (Kimura et al., 1991; Gao et al., 2001, 2007). In the case of heating treatment, changes in texture are mainly attributed to heat denaturation of protein fibers. Sato et al. (2002) reported that sea cucumber meat contained a larger proportion of collagen against the total amount of protein. There have few studies relating to the rheological properties (elastic modulus E, relaxation time τ, viscosity η, and rupture strength RS) of heated sea cucumber meat. Therefore, we studied the structural and rheological properties of sea cucumber meat, and compared the changes with different heating time.

In this paper, structural changes were investigated by observing sea cucumber muscle and collagen fibril with Van Gieson staining, and by using texture meter measurements for the rheological properties (E, τ, η, and RS). Thermal measurement was conducted by differential scanning calorimetry.

MATERIALS AND METHODS

Raw sea cucumber *S. japonicus*, harvested in Qingdao, Shandong Province, China (average weight was 41.25 ± 6.94 g) were purchased at a retail store. The raw sea cucumbers were cleared away theirs visceras, and then heated at 70°C for 1, 2, 6, 10, and 24 h, respectively. After cooling for 30 min, the upper part meat was used as the sample for the experiments.

Preparation for light microscope

Sea cucumber samples were cut into 5 mm cube, embedded into tissue-tek (OCT compound) at-20°C,and formed into 10 mm cubes(Niitu and Hiramoto, 1982). These blocks were trimmed and sliced into sections of 10 μm thickness using a cryostat (model CM 1900; Leica Company Limited, U.S.A.).

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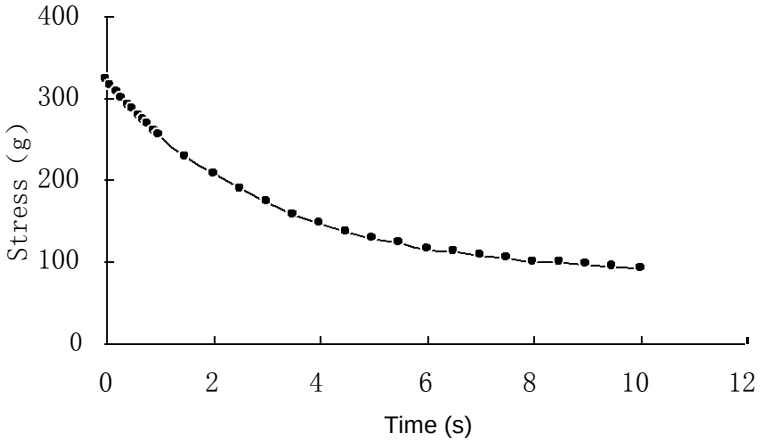

Figure 1. The stress-relaxation curve of sea cucumber meat.

The sections were then mounted on a glass slide and stained with Van Gieson stain (Kageyama and Watamabe, 1988). Light micrographs were taken with an Olympus BX51 optical light microscope.

Differential scanning calorimetry

Thermal measurements were carried out to determine the denaturation temperature of the samples with a differential scanning calorimeter (DSC) (200PC; NETZSCH Gerätebau GmbH, Germany) at heating rate of 2℃/min in the temperature range from 2 and 100°C (Gao et al., 2001). The wet weight of sample in an AL-sample capsule was 25.9 mg.

Rheological properties

Stress–relaxation measurement

Stress–relaxation experiments were carried out using a uni-axial compression and elongation type texture meter (model QTS-25I) at room temperature. The plunger was of a cylindrical type (0.3 cm i. d.) and was used to compress the samples (20 × 10 × 10 mm) at a constant strain of 20%. Before measuring, we confirmed that the samples were suitable for linearity when the volume was larger than 10 mm^3 by using the same plunger (that is 0.3 cm i. d.). The direction of applied stress was the same as the direction of observation by microscopy. All of the measurements were repeated six times for each sample.

Stress–relaxation curves were analyzed using the following progressive approximate method (Kimura et al., 1991; Gao et al., 2001, 2007 Sato et al., 2002; Niitu and Hiramoto, 1982; Kageyama and Watamabe, 1988; Iso et al., 1981). The typical stress-relaxation curve of sea cucumber meat was shown in Figure 1. The instantaneous modulus E_0 was calculated from the load when compression reached the maximum. The approximate equation of the stress–relaxation can be expressed as:

$$p(t) = e_0 \sum_{i=1}^{n} E_i \exp(-t/\tau_i) \quad (1)$$

in which p(t) is the stress at relaxation time; e_0 is the constant strain; t is the time; E_i is the elastic modulus of the i - th element; and τ_i is the stress–relaxation time of the i - th element. η_i is related to the viscosity of the i - th element, η_i, and E_i, as shown in equation 2:

$$\tau_i = \eta_i / E_i \quad (2)$$

E_0 is defined as follows:

$$E_0 = E_1 + E_2 + \ + E_n \quad (3)$$

Rupture strength measurement

Rupture strength was measured at room temperature by the same apparatus used for the stress–relaxation measurement. The cylindrical plunger (0.3 cm in diameter) compressed the samples at a rate of 20 mm/s. The experiment's results represented the average of six experimental values for each sample.

Statistical analysis

Statistical analysis on a completely randomized experimental design was performed using the SPSS statistics software. Significantly ($p < 0.05$) different property means were determined using Tukey's multiple range test.

RESULTS

Changes in weight and volume

The changes of weight and volume in sea cucumber meat during different heating time are shown in Table 1, which give a comparison of significant values that obtained from different heating time. When the sea cucumber meat was heated for 1h, its weight and volume decreased approximately 44 and 42%, respectively, and

Table 1. The changes of the weight and volume of heated sea cucumber meat.

Variable	Raw	1 h	2 h	6 h	10 h	24 h
Weight (g)	41.25±6.94^{a}	22.92±7.52^{b}	14.02±4.89^{c}	9.96±0.33^{d}	9.17±0.43^{d}	9.00±0.93^{d}
Volume (cm^3)	39.90±5.04^{a}	22.50±7.78^{b}	13.00±5.66^{c}	8.50±0.71^{d}	8.63±0.36^{d}	8.38±0.48^{d}

Values in the same row followed by different superscipt letters are significantly different ($P < 0.05$).

when it was heated for more than 1 h, the weight and volume decreased gradually. By the heating time for 6 to 24 h, weight and volume decreased approximately 78 and 79%, respectively, although the values are close.

Muscle structure

The structures of raw and heated sea cucumber meat observed by an optical microscope are shown in Figure 2. To discriminate collagen from muscle fibers, collagen and muscle fibers were stained with Van-Gieson staining method. Muscle fibers were dyed yellow and collagen fibrils red. Neither raw nor heated meat is stained yellow in Figure 2. Raw sea cucumber muscles were dyed red completely. However, the heated samples did not show the red staining. From 1 to 24 h, the structures changed significantly. For heated sea cucumber meat, the size of aperture among myofibrils increased gradually with heating time, and tissue structure seemed to have collapsed when the meat had been heated for 6 to 24 h.

Differential scanning calorimetry

In DSC thermogram (Figure 3), raw sea cucumber meat showed one peak at 57°C. The enthalpy change ΔH for raw sea cucumber meat was calculated from the area between the peak and the baseline, the result being 519 $mJmg^{-1}$. The thermograms of heated samples (1, 2, 10 and 24 h of heating) were overlap, and no peak occurs. This confirms that collagen in heated sea cucumber was completely gelatinized after heating.

Rheological properties

Rheological parameters obtained from heated sea cucumber meat are shown in Table 2. Table 2 also gives a comparison of significance values that obtained from different heating time. Elastic moduli (E_0, E_1) of heated sea cucumber meat were significantly different. When heated for 1 to 24 h, E_0 remarkably decreased at the first 2 h and then remained almost the same. The relaxation time (τ_1) and viscosity (η_1) of sea cucumber meats showed positive correlation with heating time. The rupture strength for sea cucumber meat was similar to elastic moduli, which decreased with heating time.

DISCUSSION

It is well known that sea cucumber meat contains a higher content of collagen than protein (Gao et al., 2001, 2007; Sato et al., 2002). The presence of collagen is assumed to contribute to tissue structure (Figure 2). In DSC thermogram (Figure 3), the collagen denaturation by DSC was studied. In previous studies (Brenner et al., 2009; Ando et al., 1992), the denaturation temperatures of among fish species, so the peak at 57°C was assumed to be due to the overlap of collagen. Therefore, it was assumed that the process by which collagen changed into gelation had been completed within 1 h, and the nutritional ingredient and water-soluble components of sea cucumber meat has been drawn out during longer heating time. Therefore, the heating time for 1h is suit for sea cucumber meat.

Rheological properties, such as elasticity E, relaxation time τ, viscosity η, and rupture strength (RS), changed greatly in heating treatment. It was reported in a previous study that, structural diversity of muscle connective tissue existed specifically and structural differences in connective tissue among fish species are clarified in relation with muscle firmness (Farahnaky et al., 2010; Taskaya et al., 2010). The resumed collagen network assumes to contribute to elastic moduli (Gao et al., 2001), and as discussed above, the weight and volume decreased all the heating time. So it could be considered that at the first 1 h the structure of sea cucumber muscle changed absolutely, consisting with the change in elastic moduli E_0, E_1 values; and from 6 to 24 h, the sea cucumber muscle structure kept relatively steady and E_0, E_1 values kept almost constant.

The heated sea cucumber meat, which was immersed in boiling water and experienced an outflow of denatured protein and moisture due to heating, recovered its water content from the surrounding boiling water. This water exchange maintained the muscle structure and affect to the relaxation time and viscosity (Binsi et al., 2009). Meanwhile, (Gao et al., 2004) have reported that viscosity was greater when the viscous force was smaller, whereas viscosity was smaller when viscous force was greater. Therefore, we considered that sea cucumber meat became less viscous when it was heated for longer time. The rupture strength is chiefly related to the firmness and energy as the meat is being ruptured. As discussed above, collagen in sea cucumber meat can be changed into gelation with 1 h. After heated for 2 to 24 h,

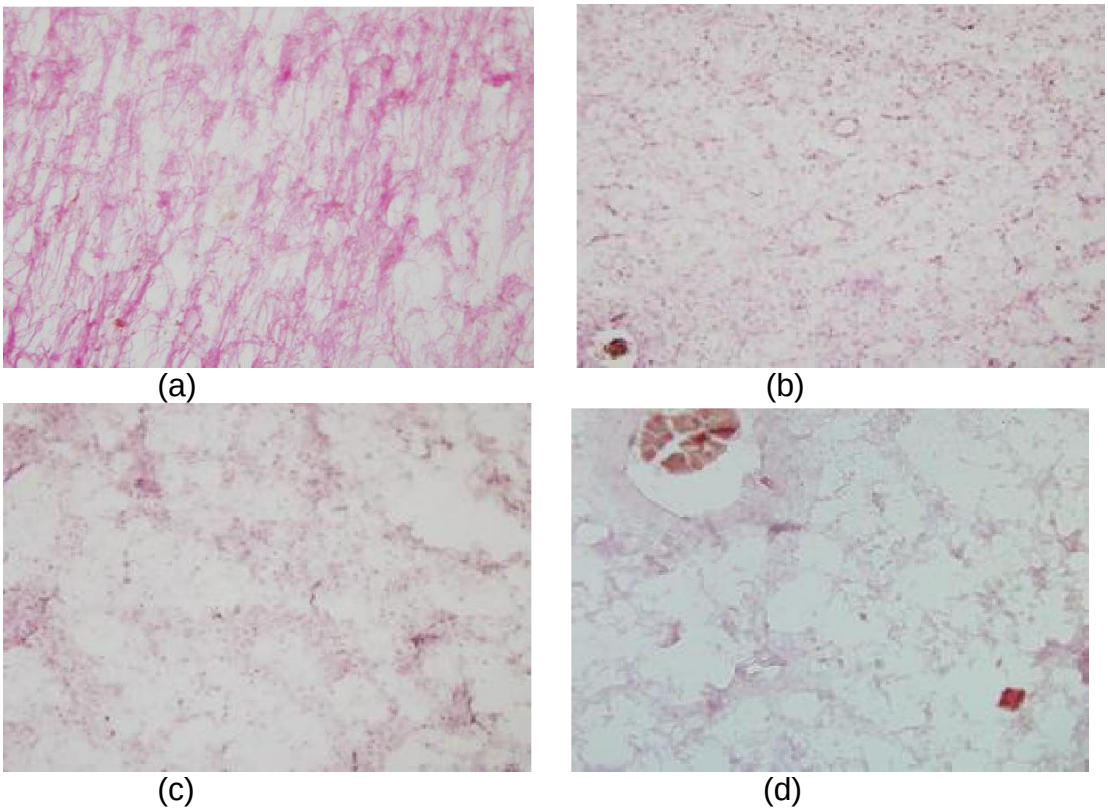

Figure 2. Light microscopy of heated sea cucumber stained with Van Gieson staining; (a) raw sea cucumber; (b) 1 h of heating; (c) 10 h of heating; (d) 24 h of heating.

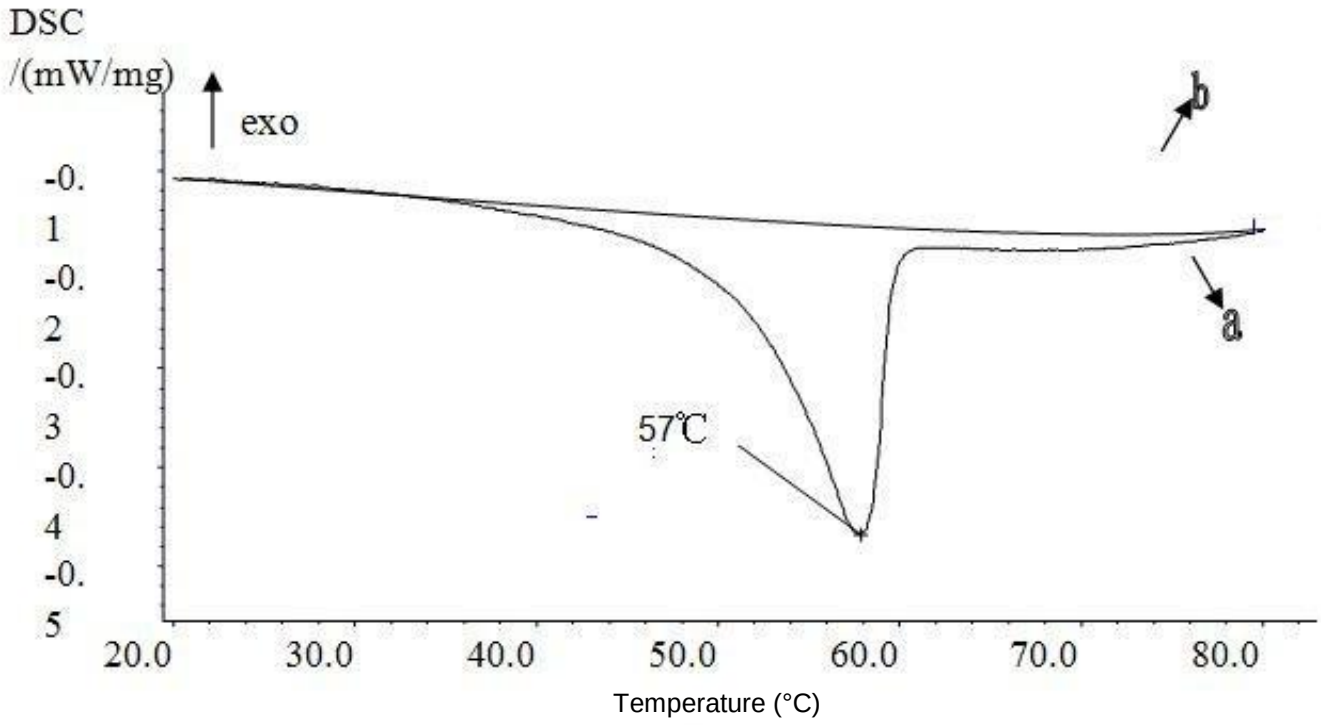

Figure 3. Differential scanning calorimetry patterns of sea cucumber sample; (a) raw sea cucumber sample, (b) heated sea cucumber samples (1, 2, 10 and 24 h).

Table 2. The rheological properities of sea cucumber meat during different heating time.

h	E_0 ($\times10^6$dyn/cm^2)	E_1 ($\times10^6$dyn/cm^2)	τ_1 (s)	η_1 ($\times10^6$dyns/cm^2)	RS (N)
Raw	62.20±12.23^{d}	32.51±6.63^{d}	5.14±0.88^{e}	125.01±11.90^{d}	130.40±13.07^{f}
1	2.13±0.24^{a}	1.89±0.25^{a}	35.78±13.22^{a}	65.63±18.14^{a}	19.22±3.94^{a}
2	1.32±0.02^{b}	1.20±0.05^{b}	45.87±8.26^{a}	55.83±6.90^{a}	16.53±2.96^{ab}
6	1.08±0.08^{c}	1.05±0.09^{bc}	103.66±79.70^{b}	109.80±28.25^{b}	13.43±2.66^{bc}
10	0.91±0.12^{c}	0.86±0.09^{c}	114.29±22.71^{c}	98.96±23.25^{b}	7.54±1.17^{d}
24	0.96±0.19^{c}	0.90±0.17^{c}	161.04±20.27^{a}	144.68±77.95^{c}	4.21±0.53^{e}

Values in the same column followed by different superscript letters are significantly different ($p < 0.05$), n=6.

the denatured protein and moisture flow out under gravity. Therefore, the rupture strength of meat heated 1h was smaller than raw meat, but higher than meat heated for other times.

Conclusion

Based on these results, we confirmed that the difference of rheological parameters (elastic modulus *E*, relaxation time τ, viscosity η, and rupture strength *RS*) of heated sea cucumber meat resulted from a difference in muscle structures. In firmness, sea cucumber meat was suitable for a short heating time within 1 h.

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