International Journal of Fisheries and Aquaculture Vol. 4(10), pp. 209-216, November 2012
Available online at http://www.academicjournals.org/IJFA
DOI: 10.5897/IJFA12.027
ISSN 2006-9839 ©2012 Academic Journals

Full Length Research Paper

Rheological and structural properties of sea cucumber *Stichopus japonicus* during different heating temperature

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Accepted 21 August, 2012

Changes in tissue structure and rheological properties of sea cucumber meat during different heating temperature were studied. Tissue structure was observed using an optical microscope and scanning electron micrograph, and its characteristic values (Wm, Dm, Rm, C^*) were enumerated using image processing and analysis technique. Rheological properties (E_0, τ_1, η_1, rupture strength) and Texture profile analysis (TPA) parameters were obtained by stress-relaxation experiment and texture profile analysis experiment. The results indicated that the structure in heated samples had greatly changes comparing with the raw sample. Muscle fibers in heated samples shrank and subsequently reassembled, and the voids in muscular tissue tended to expand with heating temperature. The characteristic values (Dm and C^*) gradually increased with heating temperature, however the Wm and Rm showed irregular change. Rheological properties (E_0 and rupture strength) in heated samples were smaller than those in the raw samples and expressed the largest value at 70°C; however τ_1 and η_1 gradually increased with heating temperature. Rheological properties (E, τ, η and rupture strength) are mainly correlated with the characteristic values (Dm and C^*). These results confirmed that the changes in texture and rheological properties of sea cucumber meat were caused by thermal denaturation of proteins.

Key words: Sea cucumber, structure, rheological properties and thermal denaturation.

INTRODUCTION

Sea cucumber (*Stichopus japonicas)* has become important cultivated aquatic species in China and other Asian countries in recent years (Conand, 2004). In general, the body wall of raw sea cucumber is hard to masticate. However, it becomes tender after cooking. In food science, studies of sea cucumber mainly focused on the food feed, nutrition values, biological activity and structure of acid polysaccharide (Slater et al., 2009; Zamora and Jeffs, 2011; Stonik et al., 1998; Chen et al., 2011). However, few studies have considerations on the structural and rheological properties in sea cucumber meat. Previous studies on rheological and texture properties of catfish, abalone and salmon stated that factors contributing to the textural properties of meat are muscle fibers and connective tissue (Jiang et al., 2008; Gao et al., 2001; Kong et al., 2008), and there are quantitative correlations between rheological properties and structural characteristic values (Gao et al., 2002; 2003). In the case of heating treatment, tissue structure and textural properties from meat have significantly difference and the denaturation of proteins was assumed to contribute to these differences. Saito et al (2002) reported that there is a larger proportion of collagen against the total amount of protein in sea cucumber meat. There are few studies related on rheological properties of sea cucumbers after heating at different temperature. Therefore, the structural and rheological properties of heated sea cucumber meat were studied, and correlations between structural characteristic values and

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rheological properties were analyzed. In this paper, tissue structure was observed by an optical microscopy and scanning electron micrograph. Structural characteristic values were enumerated using image processing and analysis technique. Rheological properties, including stress-relaxation measurement, texture profile analysis and rupture strength experiment, were taken by using texture meter measurements.

MATERIAL AND METHODS

Raw sea cucumber (*S. japonicas)*, harvested in Qingdao, Shandong Province, China (average weight was 101.91 ± 18.42 g) were purchased at a retail store. Cleared away their viscera, the body meat were heated at 50, 70 and 100℃ for 15 min, respectively. The upper part of the meat was used as sample for these experiments after being cooled to room temperature.

Cooking loss

Cooking loss was calculated as the percent weight reduction of the heated samples comparing with the raw samples.

Tissue structure

Preparation for optical microscopic

Raw and heated samples were cut into 5 × 5 × 5 mm^3 rectangular prism blocks, embedded in Tissue-Tek (O.C.T. compound) at -20℃, and then formed into 10 mm cubes (Niitu and Hiramoto, 1982). These blocks were trimmed and sliced into sections of 16 μm thickness using a cryostat (model CM1900; Leica Co. Ltd, USA). Then the sections were mounted on a glass slide and stained with Van Gieson staining (Kageyama and Watamabe, 1988). Light micrographs were taken with an Olympus BX51 optical microscopic.

Preparation for scanning electron microscopy (SEM)

Raw and heated samples were separated into 3 × 3 × 3 mm^3 rectangular prism blocks and fixed with glutaaldehyde phosphate buffer. The fixed samples were dehydrated with ethanol and substituted by isoamyl acetate, and then substituted samples were dried by critical point dry method and coated with gold palladium in a vacuum (Sukumar, Kohzoh, Maysuno, 2008). Observation was conducted by scanning electron microscopy (JSM-840; JEOL Co. Ltd, Japan).

Image processing and analysis technique

The morphometric analysis of muscle structure was carried out by a software package (Image Pro Plus 5.1, Media Cybernetics, Inc.) which could converse the optical micrographs of raw and heated samples to binary images. A binary image works only on two gray scales levels, black (0 to 127) and white (128 to 255). From the binary morphology, the following structural characteristic values were calculated:

Wm: width of myofibrils (μm),
Dm: distance between myofibrils (μm),
Rm: ratio of total myofibrils in full image domain (%),
Rvm: ratio of total voids in full image domain (%),
Cm: circularity of void area between myofibres.

The values of these morphometry parameters were calculated by the method using in chilled abalone meat (Gao et al., 2003), and defined $C^* = 4\pi/Cm$. The maximum value of real circle boundaries becomes one, 0.79 for square boundaries and 0.60 for equilateral triangular boundaries. The larger is the value of C^*, the more similar to a circle.

Rheological properties

Stress-relaxation measurement and analysis

Stress-relaxation experiment was carried out using a TMS-PRO Texture Analyzer (Food Technology Corporation, USA) at room temperature. Cylindrical probe, 4 mm in diameter, was used to compress the samples at a constant strain of 0.02. Figure 1 shows the typical stress-relaxation curve of sea cucumber meat.

Stress-relaxation curve was analyzed with the following progressive approximate method (Saito et al., 2002). Instantaneous modulus E_0 was calculated from the load when compression reached the maximum. The approximate equation of the stress-relaxation could be expressed as follows:

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

Where $\sigma(t)$ is the stress at relaxation time, e_0 is the constant strain, τ 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

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

Texture profile analysis (TPA)

Texture profile analysis of sea cucumber meat was done by the same apparatus used for stress-relaxation measurement. The double compression cycle test was performed according to the following testing procedure: cylindrical probe (4mm i. d.), final strain 0.50, time interval between first and second compression 5 s. The pre-speed, test-speed, and post-speed, were 0.5 mm/s, 1.0 mm/s, and 5.0 mm/s, respectively.

The following were quantified TPA parameters (Caine et al., 2003; Duan et al., 2010): Hardness (N) is peak force in the first compression cycle; springiness (*dimensionless*) is ratio of the length duration of force input during the second compression to that during the first compression; cohesiveness is the ratio of the positive force area during the second compression to that during the first compression; chewiness (N), is the product of hardness, springiness and cohesiveness.

Rupture strength experiment

Rupture strength was measured by the same texture analyzer at room temperature. The cylindrical probe (2 mm i. d.) compressed the samples at a rate of 2 mm/s. The peak of the force-time curve was regarded as the rupture strength value.

Statistical analysis

Statistical calculations were performed using the Excel Statistics

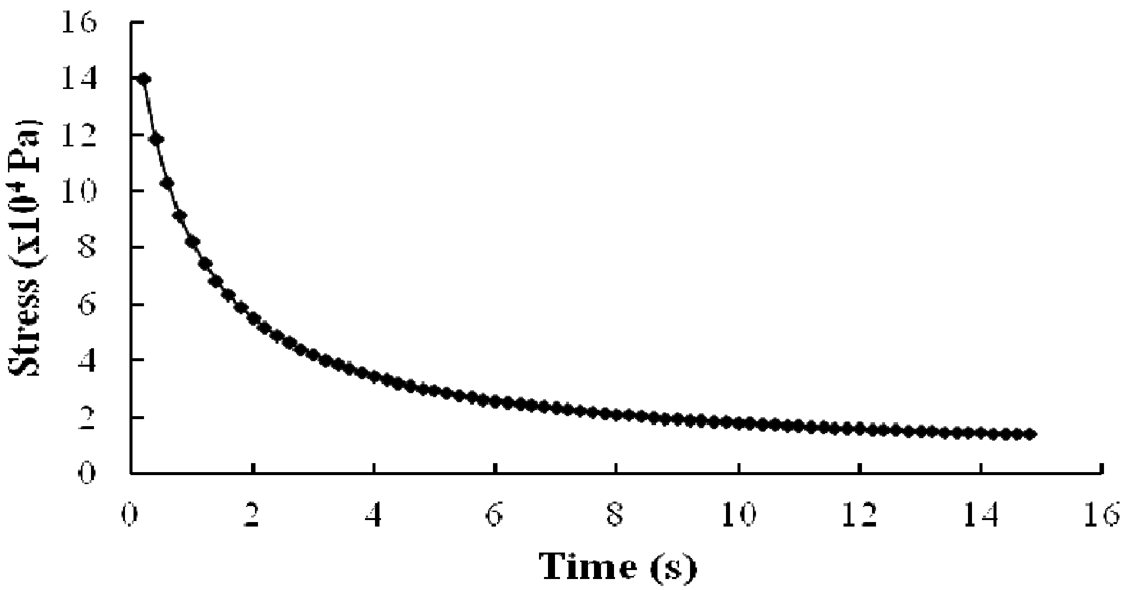


Figure 1. Typical stress-relaxation curve of sea cucumber meat.

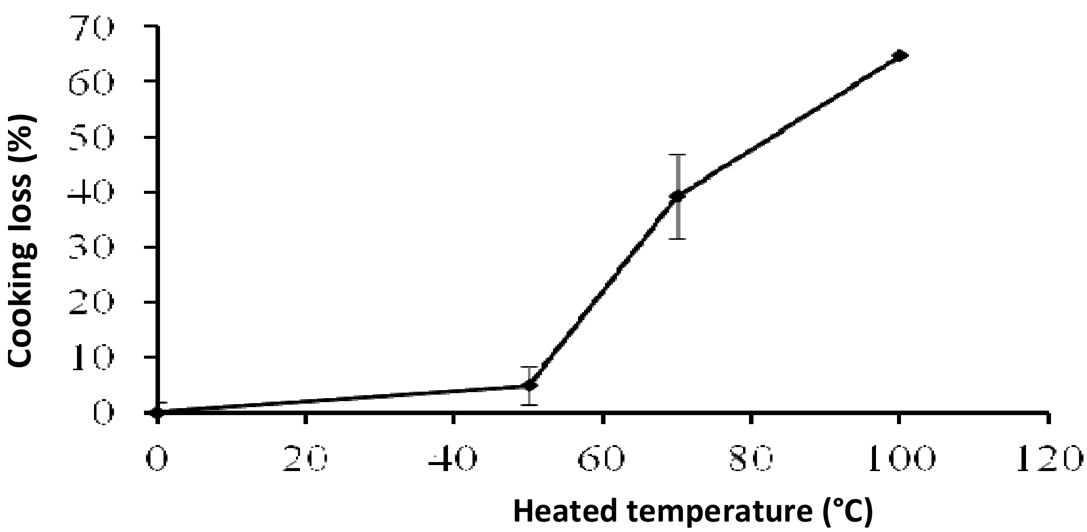


Figure 2. Cooking loss of sea cucumber meat at difference heating temperature.

software (Micro. Co.USA). Significantly ($P < 0.05$) difference property and between variable correlation were investigated with *SPSS* 17.0 statistical package. All data were expressed as means ± standard deviations.

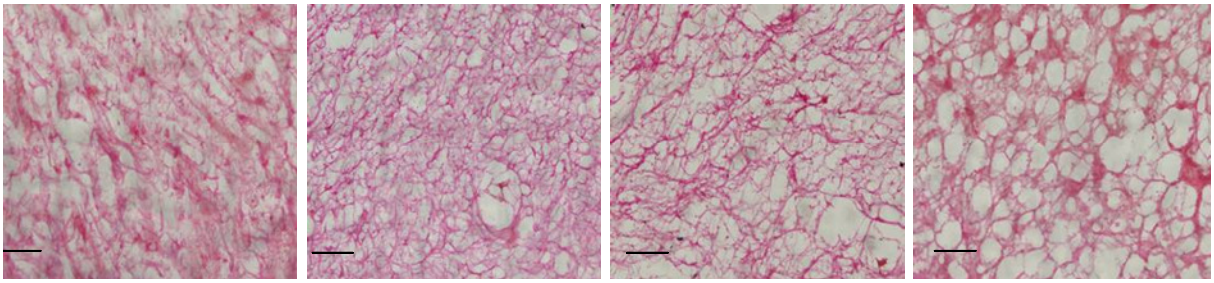
RESULTS

Cooking loss

Figure 2 shows the cooking loss of sea cucumber at different heating temperature. The cooking loss only had lower value (4.88%) when raw sea cucumber meat was heated at 50°C. The rapidly increasing of cooking loss occurred in the range 50 to 70°C. After heating at 100°C, the cooking loss had increased to approximately 64%, and moisture content decreased from 92.87 to 79.83%. Water-soluble components, like gelatin changed from collagen, also flowed out with water during thermal treatment.

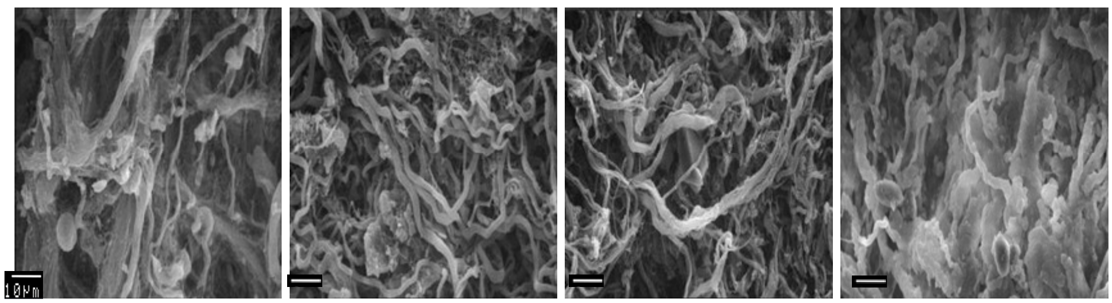
Muscle structure

Muscle structures of raw and heated samples observed



(a) (b) (c) (d)

Figure 3. Light microscopy of raw and heated sea cucumber stained with Van Gieson staining. (a) raw sea cucumber, (b) heating at 50℃ (c) heating at 70℃, (d) heating at 100℃. Bars: 30 μm.



(a) (b) (c) (d)

Figure 4. Views of raw and heated sea cucumber by a scanning electron micrographic. (a) Raw sample, (b) heating at 50℃, (c) heating at 70℃, (d) heating at 100℃.

by an optical microscopy are shown in Figure 3. The muscle fibers were stained with Van Gieson staining which dyed myofibrils yellow and collagen fibers red. Neither raw nor heated sample was dyed yellow, and raw sample was dyed red mostly. These results confirmed that collagen fibers were the mainly fibers in sea cucumber meat. For raw sample, muscle fibers in tissue were dense, and these fibers nearly parallel ordered with each other. In contrast, muscle fibers in heated samples shrank and subsequently reassembled, and the voids in muscular tissue tended to expand with heating temperature. After heating at 100℃, muscular tissue was porous and the voids in tissue were larger than those in the raw samples.

To illustrate the detail changes that occurred in the muscle fibers of heated samples, we studied with a SEM (Figure 4). Compared with Figure 3, the SEM photographs revealed the microstructural changes of fibers in sea cucumber meat after heating. Fibers bundles in raw sample were rich and loosely, and the gaps between fibers were large. However, the gaps in heated samples were smaller than those in raw sample. For heated samples, the SEM photographs were significantly different. Fibers bundles in tissue aggregated and the gaps between fibers become smaller after heating at 50℃, while coagulated blocks of muscular fibers were observed at 70℃. With continuous increase of the heating temperature, these fibers completely denatured and disintegrated, and then formed close structure at 100℃.

Enumerating muscle structure

For a marine mollusk, textural properties were thought to depend on tissue structure. Therefore, in this work, the images in Figure 3 are converted into binary morphology (Blatta et al., 2004) and are shown in Figure 5. Binary morphology works on two gray scale levels, white and black. In Figure 5, white pixels represented muscle fibers, while black pixels represented the voids in tissue. Using the image processing and analysis technique, the average values of Wm, Dm, Rm, Rvm and C^* were enumerated, and given them in Table 1. The Rm from heated samples was smaller than that from the raw samples. However the C^* from heated samples was bigger than that from the raw sample, which indicated that the extent of voids in heated meat approached to a real circle. The Dm and C^* gradually increased accompanied with increasing of heating temperature, however the Wm and Rm showed irregular changes. When fish were heated, cooking loss was significantly correlated with shrinkage ratio and the collagen solubility enhanced with heating (Kong et al., 2008). The correlations between cooking loss and structural characteristic

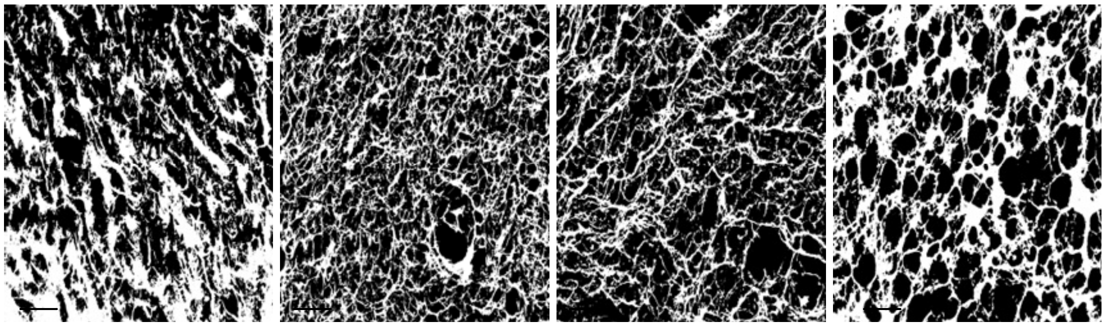


Figure 5. The binary morphology images of raw and heated sea cucumber samples. (a) Raw sample, (b) heating at 50°C, (c) heating at 70°C, (d) heating at 100°C. Bars: 30 μm.

Table 1. Structural characteristic values of sea cucumber heated at different temperature.

Parameter	Wm (μm)	Dm (μm)	Rm (%)	Rvm (%)	C*
Raw sample	6.71 ± 2.01^{a}	7.12 ± 2.65^{b}	41.99 ± 2.84^{a}	58.01 ± 2.84^{b}	0.39 ± 0.13^{c}
Heated sample					
50°C	2.38 ± 0.59^{b}	4.45 ± 1.42^{c}	33.22 ± 8.73^{ab}	66.78 ± 8.73^{ab}	0.53 ± 0.12^{b}
70°C	2.17 ± 0.63^{b}	6.64 ± 2.24^{bc}	30.70 ± 3.06^{b}	69.30 ± 3.06^{a}	0.52 ± 0.14^{b}
100°C	7.97 ± 4.12^{a}	15.9 ± 5.09^{a}	39.16 ± 2.64^{ab}	60.84 ± 2.64^{ab}	0.76 ± 0.07^{a}

The different superscripts of the same column values are significantly different ($p < 0.05$).

Table 2. Correlations between cooking loss and structural characteristic values.

	Cooking loss	Wm	Dm	Rm	C*
Cooking loss	1				
Wm	0.329	1			
Dm	0.829	0.789	1		
Rm	-0.097	0.907	0.459	1	
C*	0.875	0.356	-0.820	-0.033	1

values in sea cucumber meat are shown in Table 2. For sea cucumber meat, cooking loss was positively correlated with Dm (r = 0.829) and C^* (r = 0.875), but not significantly correlated to Wm or Rm. It is confirmed that positively correlations between characteristic values (Dm and C^*) and cooking loss existed in sea cucumber meat.

Rheological properties

Rheological properties, such as elasticity modulus $\boldsymbol{E}$, relaxation time $\boldsymbol{\tau}$, viscosity $\boldsymbol{\eta}$ and rupture strength, obtained from raw and heated samples are showed in Table 3. Rheological properties greatly changed during heating treatment. Elasticity modulus ($\boldsymbol{E_0}$, $\boldsymbol{E_1}$, $\boldsymbol{E_2}$) from raw sample were higher than those from heated samples. For heated samples, this elasticity expressed a highest value at 70°C. Relaxation times ($\boldsymbol{\tau_1}$) for heated samples tended to increase with heating temperature, while viscous modulus ($\boldsymbol{\eta_1}$) had the same reaction with heating temperature as relaxation time. Rupture strength for sea cucumber samples was the similar to elasticity modulus, and the highest value existed at 70°C.

On the other hand, the textural properties of meat also could be described by TPA parameters (Palka and Daun, 1999). TPA parameters obtained from raw and heated sea cucumber samples are given in Table 4. Table 4 also gives a comparison of significant values obtained from different heating temperature. Hardness values in heated samples were lower than that in raw sample, while springiness was much larger than raw sample. Hardness values in heated samples were significantly different, same significant difference in springiness. The hardness from heated samples expressed a highest value at 70°C, however springiness expressed the lowest value at the same temperature.

Table 3. Rheological properties of sea cucumber meat heated at different temperature.

Parameter	E_0 (×10^5Pa)	E_1 (×10^5Pa)	E_2 (×10^5Pa)	τ_1 (s)	τ_2 (s)	η_1 (×10^6Pa·s)	η_2 (×10^6Pa·s)	Rupture strength (N)
Raw sample	3.53 ±1.95[a]	1.85 ± 0.55[a]	2.31±1.41[a]	27.93 ± 12.52[b]	1.44 ± 0.07[b]	2.95 ± 0.29[ab]	0.33 ± 0.21[a]	31.95 ± 6.91[a]
Heated sample								
50℃	1.26 ± 0.34[a]	0.30 ± 0.08[b]	0.96 ± 0.28[a]	14.43 ± 5.26[b]	1.25 ± 0.08[b]	0.46 ± 0.29[b]	0.12 ± 0.04[a]	17.04 ± 4.79[b]
70℃	2.75 ±1.63[a]	1.64 ± 0.73[a]	1.12 ± 0.90[a]	37.83 ±15.78[b]	1.78 ± 0.34[ab]	6.01 ± 3.70[ab]	0.18 ± 0.14[a]	17.65 ± 4.40[b]
100℃	0.98 ± 0.51[a]	0.83 ± 0.43[b]	0.16 ±0.08[a]	97.22 ± 29.27[a]	2.11 ± 0.28[a]	7.42 ± 3.59 [a]	0.03 ± 0.02[a]	2.17 ± 0.07[c]

The different superscripts of the same column values are significantly different ($p < 0.05$).

Those results kept consistent with rheological properties (**E_0**, **E_1** and rupture strength). Cohesiveness tended to increase during heating; however chewiness expressed an irregular change.

DISCUSSION

In a previous paper, we published a large amount of experimental data demonstrating that thermal denaturation of muscle proteins is related to tissue structural changes(Gao et al., 2002; 2003). It is well known that raw sea cucumber meat contains a higher content of collagen than protein and the degree of aggregation for protein molecules increased with heat. Cui et al. (2007) has confirmed that the thermal stability temperature of pepsin-solubilized collagen which was extracted from sea cucumber meat was 57℃. In this study, the optical photographs revealed that muscular tissue in sea cucumber meat had greatly changed during different heating temperature (Figure 3). While SEM microphotographs also showed the denatured degrees for muscle fibers increased with heating temperature (Figure 4). After heating from 50 to 100℃, muscle fibers in sea cucumber meat respectively aggregated closely, formed blocks and denatured disintegrated. Therefore, it was confirmed that the presence of collagen and its denatured degree have greatly influence on muscular tissue for rich-collagen meat.

Structural characteristic values (Table 1) obtained from raw and heated sea cucumber meat has significantly changed with heating temperature. The gradually increasing of Dm and C^* suggested that the profile of voids in muscular tissue expanded after heating from 50 to 100℃, which was consistent with those voids tended to larger in optical photographs. As early mentioned, Dm and C^* were greatly influenced by cooking loss. It is well known that collagen is changed into gelatin which is a water-soluble fluid substance and muscle fibers is assembled and aggregated each other by heat denaturation. Therefore, it is supposed that the larger voids in heated sea cucumber meat are caused by the outflow of denatured protein for collagen-rich meat. In this paper, the texture properties were described in two fields: Rheological properties (Table 2) and TPA parameters (Table 3). Rheological properties (**E_0**, **τ_1**, **η_1**) and TPA parameter from sea cucumber meat were greatly changed after heating from 50 to 100℃. Matumoto and Yamano (1987) has reported that elastic modulus is greater when elastic force is smaller, whereas elastic modulus is smaller when elastic force is greater. As with the elastic, viscosity, **η**, is greater when viscous force is smaller. Therefore, we concluded that the heated sea cucumber meat was more elastic than the raw meat and, similarly, more viscous than the raw meat. TPA parameter springiness also confirmed that the heated meat was more elastic than the raw meat.

Previous studies have reported that structural diversity of muscle connective tissue existed specifically and structural differences in connective tissue among fish species were clarified in relation with muscle firmness (Farahnaky et al., 2010; Johnston et al., 2000). In this present study, rheological properties, such as rupture strength, and TPA parameter hardness from heated sea cucumber meat has a largest value after heating at 70℃. Shrinkage of muscle fibers were observed at the same temperature by optical and SEM microphotographs. It is well known that rupture strength and hardness is related chiefly to firmness and energy as the meat was being ruptured. In previous studies, the extent of muscle fiber deformation during cooking depends on the compression stress applied by collagen fibers and the resistance of the muscle fibers to compression, while the compression force applied by collagen network on muscle fibers bundles depends upon the amount of collagen present and its thermal solubility (Lepetit et al., 2000). It was confirmed that the largest value of rupture strength or hardness in heated samples was caused by denaturation and aggregation of muscle proteins and the subsequent shrinkage and dehydration (Kong et al., 2007; 2008; Chen et al., 2011). Meanwhile, solubilization and gelatin of collagen contribute to the decrease of rupture

Table 4. TPA parameters of sea cucumber meat heated at different temperature.

Parameter	TPA parameters			
	Hardness (N)	Springiness (-)	Cohesiveness (-)	Chewiness (N)
Raw sample	32.85 ± 3.87[a]	0.27 ± 0.05[c]	0.18 ± 0.05[b]	1.67 ± 0.61[b]
Heated sample				
50℃	8.86 ± 1.86[b]	0.54 ± 0.14[b]	0.07 ± 0.01[c]	0.38 ± 0.24[b]
70℃	26.34 ± 7.87[a]	0.40 ± 0.09[bc]	0.36 ± 0.07[a]	3.97 ± 1.69[a]
100℃	3.79 ± 0.74[b]	1.03 ± 0.03[a]	0.43 ± 0.06[a]	1.73 ± 0.45[b]

The different superscripts of the same column values are significantly different ($p < 0.05$).

Table 5. Correlations between structural characteristic values and rheological properties.

	Wm	Dm	Rm	C^*	E_0	τ_1	η_1	Rupture strength
Wm	1							
Dm	0.789	1						
Rm	0.907	0.459	1					
C^*	0.356	0.820	-0.033	1				
E_0	-0.046	-0.439	0.205	-0.833	1			
τ_1	0.694	0.989*	0.327	0.862	-0.464	1		
η_1	0.304	0.765	-0.098	0.726	-0.258	0.847		
Rupture strength	-0.187	-0.715	0.205	-0.985*	0.866	-0.777	-0.707	1

*.Correlation is significant at the 0.05 level.

strength and hardness after heating at 100℃.

The heated sea cucumber meat, which was immersed in hot water and experienced an outflow of denatured proteins and moisture due to heating, formed unique muscular structure. Simultaneously, rheological properties reflected by $\boldsymbol{E_0}$, $\boldsymbol{\tau_1}$, $\boldsymbol{\eta_1}$ and rupture strength also greatly changed. Gao et al. (2002) reported that rheological properties were negatively correlated with characteristic values of myofibrils in abalone meat. Correlations analyses were conducted to evaluate the relationships between rheological properties and structural characteristic values. In Table 5, Dm was highly positively correlated with $\boldsymbol{\tau_1}$ (r = 0.989) and $\boldsymbol{\eta_1}$ (r = 0.989), was negatively correlations with rupture strength (r = -0.715). C^* was negatively correlated with instantaneous modulus $\boldsymbol{E_0}$ (r = -0.833), rupture strength (r = -0.985). However, Wm and Rm were not significantly correlations with rheological properties. In other words, $\boldsymbol{E}$ and rupture strength were negatively correlated with characteristic values (Dm, C^*), which are consistent with Gao report. However, $\boldsymbol{\tau}$ and $\boldsymbol{\eta}$ were positively correlated with characteristic values (Dm). Therefore, rheological properties reflect by $\boldsymbol{E}$, $\boldsymbol{\tau}$, $\boldsymbol{\eta}$ and rupture strength are mainly correlated with the characteristic values (Dm and C^*) in heated sea cucumber meat.

Conclusion

Based on these results, we confirmed that the muscular structure in heated sea cucumber meat due to cooking loss and denaturation of collagen. Rheological properties ($\boldsymbol{E}$, $\boldsymbol{\tau}$, $\boldsymbol{\eta}$ and rupture strength) and TPA parameters were greatly changed after heating. Rheological properties ($\boldsymbol{E_0}$ and rupture strength) in heated meat expressed the largest value at 70℃. The shrinkage and dehydration of muscle fibers were assumed to result in the largest value. Cooking loss positively correlated to structural characteristic values (Dm, C^*). Meanwhile, rheological properties reflect by $\boldsymbol{E}$, $\boldsymbol{\tau}$, $\boldsymbol{\eta}$ and rupture strength are mainly correlated with the characteristic values (Dm and C^*). These results suggested that the change in texture and rheological properties of sea cucumber during heating was caused by denaturation of proteins. Compared with raw sea cucumber meat, heated meat had higher springiness, viscosity and lower hardness.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 31071631) and Qingdao Technology Development Project (No. 2011-5-028-QT).

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