Effects of heat treatment on the gel properties of the body wall of sea cucumber (*Apostichopus japonicus*)

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Abstract The sensory texture of sea cucumber (*Apostichopus japonicus*) was dramatically affected by heat treatment. In this study, sea cucumbers were heated under different thermal conditions (HSC), and divided into five groups (HSC-80, HSC-90, HSC-100, HSC-110, and HSC-120) according to the heating temperature (from 80 to 120 °C). The changes in texture, moisture, gel structure, and biochemical parameters of the HSC were investigated. With increasing heating time (from 10 to 80 min), the hardness and gel structure changed slightly, and the water activity decreased as the proportion of T_{21} increased by 133.33, 55.56, and 59.09% in the HSC-80, HSC-90, and HSC-100 groups, respectively. This indicated that moderate heating conditions (below 100 °C) caused gelation of sea cucumbers in HSC-80, HSC-90, and HSC-100 groups. However, as the water activity increased, the hardness declined rapidly by 2.56 and 2.7% in the HSC-110 and HSC-120 groups, with heating time increased from 10 to 80 min. Meanwhile, free hydroxyproline and ammonia nitrogen contents increased by 81.24 and 63.16% in the HSC-110 group; and by 63.09 and 54.99% in the HSC-120 group, as the gel structure of the sea cucumbers decomposed in these two groups. These results demonstrated that, severe heat treatment (above 100 °C) destroyed the chemical bonds, triggered the disintegration of collagen fibers and the gel structure of sea cucumbers, and transformed the migration and distribution of moisture, finally

causing the deterioration of the sensory texture of the sea cucumbers.

Keywords Sea cucumber · Heat treatment · Texture · Gel · Moisture

Introduction

Sea cucumber (*Apostichopus japonicus*) is one of the important economic marine species and is consumed extensively in China for its high nutrition and tonic value (Xu et al. 2015). *A. japonicus* is believed to contain many biologically and pharmacologically active compounds such as bioactive peptides, essential fatty acids, polysaccharides, and glycoproteins (Abuajah et al. 2015; Lee et al. 2012; Liu et al. 2012b; Wang et al. 2012; Yang et al. 2015). Especially in recent years, *A. japonicus* processing industries have developed increasingly fast and become a flourishing economic sector in China (Xu et al. 2015).

Apostichopus japonicus can be converted into edible products by different processing methods, including high temperature and high pressure processing (Peng et al. 2015), boiling processing, drying processing, and combined processing (Duan et al. 2007). All these processing techniques involve heating the sea cucumber, which could inactivate microorganisms and enzymes, change the gel properties, and affect the sensory texture of sea cucumbers (Gao et al. 2011). Earlier researchers reported that the gel properties of sea cucumbers were dramatically affected by the heating temperature and heating time (Liu et al. 2012a; Gao et al. 2011). However, the mechanisms of gelation and gel disintegration of the heated *A. japonicus* body wall remain unclear. This in turn restricts the development of the *A. japonicus* processing industry.

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It is necessary to study the changes in gel properties as well as gelation and gel disintegration mechanisms of the *A. japonicus* body wall when heated by different thermal treatments. In the present study, sea cucumbers were treated under different heating conditions (HSC). The textural differences were determined, and the variations in the moisture state and distribution were analyzed. Subsequently, gel structural changes were studied, and biochemical parameters were measured to investigate the gelation and gel deterioration mechanisms of HSC.

Materials and methods

Materials

The sea cucumbers *A. japonicus* were purchased from Nanshan aquatic products market of Qingdao (Shandong, China). All the chemicals and reagents used in this study were of analytical grade with high purity. The 2,4,6-trinitrobenzene sulfonic acid (TNBS) was obtained from Sigma-Aldrich (St. Louis, USA). Chloramine-T, isopropanol, ninhydrin, perchloric acid, fructose, 4-dimethylaminobenzaldehyde, and solvents were bought from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

Sample preparation

Fresh sea cucumbers *A. japonicus* (weight range from 100 to 110 g) were selected, gutted, and immediately washed with distilled water. Then, the sea cucumbers were heated at 80, 90, 100, 110, 120 °C for 10, 20, 40 and 80 min, separately. Five sea cucumbers were used in each heating condition. These sea cucumbers *A. japonicus* treated under different heating conditions were named as HSC. The sea cucumbers that were heated at 80, 90, 100, 110, and 120 °C were named the HSC-80, HSC-90, HSC-100, HSC-110, and HSC-120 groups, respectively. HSC samples were rapidly cooled to ambient temperature (25 ± 1 °C) in a cold-water bath. The free water on the surface of HSC samples was removed with a filter paper, and all experiments were performed at room temperature (25 ± 1 °C).

Texture profile analysis of HSC

Texture profile analysis (TPA) was performed using a TMS-PRO texture profile analyzer (Food Technology Co., Virginia, USA). A two-cycle compression experiment was implemented using a 4 mm diameter cylindrical probe, at a speed of 50 mm/min, and the compression of the HSC sample was set to 70% (Hou et al. 2014). Each measurement was replicated three times. Parameters including hardness, adhesiveness, chewiness, springiness, and

cohesiveness were calculated according to the TPA curve method reported by Mitchell (2003).

Determination of moisture changes in HSC

Moisture content of HSC

Moisture content was analyzed following the AOAC Method 984.25 (AOAC 2000). The determination was conducted by drying pre-weighed samples in an oven at 105 °C until a constant weight was reached. Each sample was measured three times.

Water activity of HSC

A HD-3A water activity meter (Huake Electronic Instrument Co. Ltd., Wuxi, China) was used to assess the water activity of HSC. Prior to each measurement, the equipment was calibrated with saturated sodium chloride solution at 20 °C. To conduct the analysis, 1.0 g of the sample was placed in the cuvette of the meter. All measurements were performed in triplicate and under identical conditions.

Determination of transverse relaxation time (T_2)

Transverse relaxation times (T_2) were measured with a low-field nuclear magnetic resonance spectrometer (LF-NMR, NMI20, Niumag Electronic Technology Co. Ltd., Shanghai, China) using the Carr Purcell Meiboom Gill (CPMG) sequence (Zheng and Xia 2010). Samples were placed at the center of the LF-NMR coil situated at the center of the permanent magnetic field. T_2 measurement was performed in the LF-NMR before diffusion-weighted examinations with a τ -value (corresponding to the time between the 90° pulse and the 180° pulse) of 200 μ s. Typical CPMG test pulse parameters were employed, including a spectrometer frequency (SF) of 22 MHz, repetition time (TR) of 4000 ms, echo time (TE) of 200 μ s, and echo count (CE) of 3500.

T_1 -weighted and T_2 -weighted imaging of HSC

T_1 -weighted and T_2 -weighted imaging was performed using LF-NMR with a multi-spin echo sequence (MSE) employing the following parameters: 90° pulse width (P90) = 9.5 μ s, 180° pulse width (P180) = 19.0 μ s, repetition time (TR) = 4000 ms, echo time (TE) = 200 μ s, echo count (CE) = 3500. The proton density and the value of weighting were altered by adjusting TR and TE. To achieve T_1 -weighted imaging, the imaging parameters were as follows: D0 = 200, D4 = 1.0, D5 = 0.5, phase encoding gradient pulse amplitude (GA3) = 20. For T_2 -weighted imaging, the following parameters were

employed: D0 = 5000, D4 = 50, D5 = 50, GA3 = 25 (Hou et al. 2014).

van Gieson staining of HSC

The samples were cut into cube-shaped pieces (2 cm × 2 cm × 2 cm), fixed in 4% (v/v) neutral formaldehyde and dehydrated gradually, then embedded in paraffin. Subsequently, serial sections (7 µm thickness) of samples were cut. The van Gieson stain was used to visualize collagen fibers within the sections of HSC. The stained sections were then observed under an optical microscope (BX-41, Olympus, Tokyo, Japan) at 40× magnification.

Hydroxyproline content of HSC

The samples were homogenized (1:9 w/v) in distilled water, and the homogenates were centrifuged to collect the supernatants. Then 0.1 mL of supernatant was added into 1 mL 6 mol/L HCl and hydrolyzed at 130 °C for 4 h. The pH of the hydrolyzed solution was then adjusted to neutral, and diluted to 100 mL with distilled water. Subsequently, 2 mL of this diluted solution was combined with 1 mL of chloramine-T solution (0.05 mol/L), and incubated at room temperature for 20 min. To this mixture, 0.5 mL 35% (v/v) HClO₄ and 0.5 mL 10% (w/v) 4-dimethylaminobenzaldehyde were added, and the resultant solution was incubated at 60 °C for 15 min. The absorbance was measured at 560 nm using a spectrophotometer (UV-2802, Unico Co. Ltd., Shanghai, China). Each sample was measured three times. The hydroxyproline content was estimated using a calibration curve which was made with a hydroxyproline standard solution (10 µg/mL).

Measurement of ammonia nitrogen level

The samples were homogenized (1:9 w/v) in distilled water. Subsequently, the homogenates were centrifuged and the supernatants were collected to determine the ammonia nitrogen level of HSC using the ninhydrin colorimetric method (Rosen 1957). The absorbance at 570 nm was determined using a spectrophotometer (UV-2802, Unico Co. Ltd., Shanghai, China), and the ammonia nitrogen content was calculated according to the standard curve plotted using glycine solution.

Statistical analysis

Statistical analysis was performed using the SPSS statistical software package (version 18.0, SPSS, Inc., Chicago, IL, USA). All experiments were run in triplicate, and all the results were expressed as mean ± standard deviation (SD)

of three independent experiments. The experimental data were assessed by one-way analysis of variance (ANOVA), and significance of differences among treatment was obtained by the least significant difference (LSD). A *p* value <0.05 was considered statistically significant.

Results and discussion

Textural differences of HSC

As the best predictor of sensory texture of cooked meat (de Huidobro et al. 2005), TPA parameters were measured by texture profile analysis are shown in Table 1. Hardness reflects the force applied by the molar teeth to compress the food, as it is defined as the force (N) needed for deforming food particles to a certain extent (de Ávila et al. 2014). As shown in Table 1, the viscosity and restorability showed no regular changes, but the hardness decreased with increasing heating time (from 10 to 80 min) in all HSC groups (HSC-80, HSC-90, HSC-100, HSC-110, and HSC-120). The hardness in the HSC-110 and HSC-120 groups decreased sharply from 303.5 and 298 gf to 16.2 and 27.6 gf, respectively, while the hardness in the HSC-80, HSC-90, and HSC-100 groups declined smoothly (from 618 to 121 gf, 570.6 to 356.5 gf, and 266 to 96 gf, respectively). It can be observed that the average values of hardness in the HSC-80, HSC-90, and HSC-100 groups (at 537.68, 452.89, and 215.53 gf, respectively) were significantly higher than those in the HSC-110 and HSC-120 groups (at 168.47 and 128.18 gf, respectively). The relatively high level of hardness in these three groups might result from the gelation of sea cucumber body walls (Peng et al. 2015). Meanwhile, samples heated at 110 and 120 °C also showed the most rapid decline in chewiness. These data indicated that moderate heat treatment (lower than 100 °C) caused gelation in the HSC-80, HSC-90, and HSC-100 groups, but long heating times and high heating temperatures led to serious softening of the texture in the HSC-110 and HSC-120 groups. These results were in accord with a previous report (Gao et al. 2011).

When the heating time was increased from 10 to 80 min, the springiness increased from 0.78 to 0.95, 0.82 to 0.96, and 0.89 to 0.96 in the HSC-80, HSC-90, and HSC-100 groups, respectively. Meanwhile, it declined from 0.98 to 0.42 and 0.90 to 0.78 in the HSC-110 and HSC-120 groups, respectively. The cohesiveness of HSC also exhibited a similar tendency. Springiness can reflect how much the gel structure is broken down by the first compression during the TPA experiment. A previous study reported that low springiness due to the gel structure breaking into many small pieces during the initial TPA compression, but a high level of springiness occurs when the gel is broken into a few large

Table 1 Effects of different heat treatments on TPA parameters of heated sea cucumber *A. japonicus* body wall

Group	Time (min)	Hardness (gf)	Viscosity (gf·s)	Springiness	Cohesiveness	Chewiness (gf ² ·s)	Restorability
HSC-80	10	618 ± 165.07 ^{ab}	40.03 ± 22.81 ^{af}	0.78 ± 0.05 ^a	0.65 ± 0.02 ^a	313.5 ± 75.35 ^{adeg}	0.43 ± 0.02 ^a
	20	948 ± 181.23 ^a	1.44 ± 0.27 ^b	0.84 ± 0.05 ^{abdj}	0.59 ± 0.05 ^a	410.87 ± 32.68 ^a	0.39 ± 0.07 ^{ah}
	40	463.7 ± 77.27 ^{bd}	13.71 ± 1.81 ^c	0.89 ± 0.03 ^{bdehk}	0.78 ± 0.05 ^{bf}	323.51 ± 62.45 ^{ade}	0.54 ± 0.04 ^{bc}
	80	121 ± 28.97 ^{ci}	2.29 ± 2.15 ^{be}	0.95 ± 0.01 ^c	0.84 ± 0.01 ^c	111.43 ± 19.80 ^b	0.65 ± 0.08 ^{bdef}
HSC-90	10	570.6 ± 70.12 ^b	2.24 ± 1.64 ^{be}	0.82 ± 0.05 ^{ab}	0.70 ± 0.02 ^d	351.25 ± 43.21 ^{ad}	0.50 ± 0.04 ^c
	20	488.67 ± 22.38 ^b	0.67 ± 0.98 ^b	0.97 ± 0.10 ^{cdeghk}	0.81 ± 0.05 ^{bc}	458.64 ± 5.16 ^c	0.58 ± 0.006 ^b
	40	395.8 ± 24.39 ^d	23.26 ± 36.34 ^{abcef}	0.93 ± 0.08 ^{bcddegh}	0.82 ± 0.03 ^{bc}	306.36 ± 38.78 ^{de}	0.60 ± 0.04 ^{bd}
	80	356.5 ± 73.58 ^{deh}	6.92 ± 4.20 ^e	0.96 ± 0.02 ^{cf}	0.84 ± 0.03 ^{bce}	257.75 ± 39.16 ^{eg}	0.63 ± 0.02 ^{de}
HSC-100	10	266 ± 22.34 ^{ef}	41.11 ± 6.69 ^{ag}	0.89 ± 0.01 ^{dek}	0.78 ± 0.04 ^b	177.13 ± 26.69 ^f	0.57 ± 0.03 ^{bg}
	20	258.5 ± 28.06 ^{ef}	14.34 ± 17.02 ^{bcef}	0.91 ± 0.02 ^{eh}	0.89 ± 0.03 ^{eg}	164.72 ± 18.98 ^f	0.69 ± 0.05 ^{ef}
	40	241.6 ± 21.78 ^f	22.15 ± 28.73 ^{abcef}	0.93 ± 0.08 ^{bcddegh}	0.87 ± 0.08 ^{bceeg}	167.94 ± 33.34 ^{fj}	0.71 ± 0.04 ^f
	80	96 ± 21.04 ^c	19.68 ± 24.53 ^{abcef}	0.96 ± 0.07 ^{cefh}	0.88 ± 0.06 ^{cefg}	81.60 ± 21.63 ^{hh}	0.67 ± 0.10 ^{bef}
HSC-110	10	303.5 ± 30.15 ^{eh}	2.60 ± 2.38 ^{be}	0.98 ± 0.04 ^c	0.87 ± 0.04 ^{ceg}	257.65 ± 29.68 ^{eg}	0.70 ± 0.03 ^f
	20	247.83 ± 15.35 ^f	4.70 ± 1.84 ^e	1.00 ± 0.04 ^{fg}	0.90 ± 0.01 ^g	236.35 ± 0.89 ^g	0.73 ± 0.02 ^f
	40	106.33 ± 16.60 ^c	24.24 ± 5.15 ^f	0.92 ± 0.02 ^h	0.82 ± 0.03 ^{bc}	69.61 ± 6.63 ^h	0.63 ± 0.04 ^{deg}
	80	16.2 ± 5.45 ^g	56.36 ± 38.99 ^{adf}	0.42 ± 0.19 ⁱ	0.25 ± 0.12 ^h	0.97 ± 0.66 ⁱ	0.44 ± 0.16 ^{abcegh}
HSC-120	10	298 ± 7.35 ^h	43.20 ± 4.72 ^a	0.90 ± 0.03 ^{chj}	0.69 ± 0.01 ^d	151.87 ± 6.28 ^f	0.40 ± 0.08 ^{ach}
	20	140.8 ± 14.75 ⁱ	21.09 ± 17.92 ^{acef}	0.94 ± 0.07 ^{cdeghk}	0.86 ± 0.04 ^{cefg}	112.74 ± 28.10 ^{bj}	0.68 ± 0.07 ^{df}
	40	46.33 ± 4.84 ^j	84.45 ± 15.29 ^d	0.64 ± 0.06 ^{ik}	0.23 ± 0.09 ^h	23.97 ± 0.17 ^k	0.33 ± 0.17 ^{ach}
	80	27.6 ± 8.10 ^g	31.87 ± 5.59 ^{fg}	0.78 ± 0.11 ^{abjk}	0.37 ± 0.06 ^h	3.43 ± 14.08 ⁱ	0.31 ± 0.02 ^h

Different superscript lowercase letters show differences between the rows ($p < 0.05$)

pieces (Huang et al. 2007). Thus, it can be concluded that the gel structure of *A. japonicus* body wall remained as large pieces and cross-linked at 80, 90, and 100 °C, but was destroyed significantly at 110 and 120 °C. It is reported that sensory texture of food is directly affected by moisture (Pearce et al. 2011); thus, the changes in TPA parameters might be due to the moisture changes in HSC.

Moisture changes in HSC

Changes in water content and water activity of HSC

As shown in Fig. 1A, the water content declined significantly ($p < 0.05$) with increase of heating time in all HSC groups. The water content in the HSC-120 group declined most rapidly (from 82.67 to 76.64%), it indicating a significant loss of water holding capacity (WHC) of sea cucumbers heated at 120 °C. Meanwhile, the water content of HSC decreased as the heating temperature increased from 80 to 120 °C, when the samples were heated for 10, 20, 40, and 80 min. This might be due to the loss of WHC and the vaporization of free water from the samples. Similar phenomena have also been reported in the processing of pork (Ishiwatari et al. 2013) and beef (Isleroglu et al. 2015).

Earlier research suggested that water activity has a negative relationship with the binding degree of water to

food matter (Mathlouthi 2001). As shown in Fig. 1B, the water activity of HSC fluctuated between 0.92 and 0.97. It showed a declining trend with increased heating time in the HSC-80, HSC-90, and HSC-100 groups, indicating the increase of binding degree of water and the vaporization of free water at 80, 90, and 100 °C. In contrast, the water activity in the HSC-110 and HSC-120 groups increased from 0.939 to 0.963 and 0.926 to 0.951, respectively. The change of water activity in heated meat was elucidated as the muscle proteins denaturing during heating, leading to shrinkage of the protein network, which in turn exerted a mechanical force on the water between protein fibers (van der Sman 2007). This theory could explain the decrease of water activity in the HSC-80, HSC-90, and HSC-100 groups. However, sea cucumbers are mainly constituted of collagen fibers (Cui et al. 2007), which are dramatically different from muscle proteins and gradually disintegrate at high temperatures. The disintegration of collagen might contribute to the increase of water activity in the HSC-110 and HSC-120 groups.

The changes in water state in HSC

The information on the water state can be provided by LF-NMR spectroscopy on the basis of the relaxation time of the H nucleus (Chaland et al. 2000). The total relaxation

Fig. 1 Effects of different heating conditions on the water content (A) and water activity (B) of heated sea cucumber *A. japonicus* body wall. The bars represent SDs and values are means. Different lower case letters suggest significant differences ($p < 0.05$)

time can be divided into T_1 (longitudinal relaxation time) and T_2 (transverse relaxation time) components. T_2 is closely related to the degree of freedom of water, and can reveal the water state in the samples (Bertram et al. 2001). It was made up of three parts that represent different states of water: T_{21} (varying from 0 to 10 ms) represents the relaxation time of bound water, T_{22} (varying from 10 to 100 ms) is the relaxation time of immobilized water, and T_{23} (above 100 ms) reflects that of free water (Bertram et al. 2002).

According to Table 2, the proportion of T_{21} and T_{22} increased ($p < 0.05$) when the heating time was increased from 10 to 80 min, but the proportion of T_{23} decreased from 0.955 to 0.888, 0.968 to 0.899, and 0.937 to 0.859 in the HSC-80, HSC-90, and HSC-100 groups, respectively. It might result from the evaporation of free water (Bertram et al. 2001). In contrast, the proportion of T_{21} , T_{22} , and T_{23} show no regular tendency ($p > 0.05$) in HSC-110 and HSC-120 groups.

As shown in Table 2, the ranges of T_{21} , T_{22} , and T_{23} were 1.535–3.273, 16.450–60.844, and 112.348–313.008 ms,

respectively. The relaxation time T_{21} changed slightly ($p > 0.05$) during heating. There was an initial decrease in T_{23} at all heat periods from 80 °C (313.008 ms) until 90 °C, indicating a decrease in the degree of freedom of free water, and might result from gelation. Thereafter, there was an increase in the relaxation time T_{23} from 100 to 120 °C, which was much more marked in samples heated for 80 min (from 201.911 to 296.889 ms), followed by those heated for 40 min (from 249.461 to 265.498 ms), while the increases in those heated for 20 min (from 204.201 to 205.552 ms) and 10 min (from 178.940 to 214.275 ms) were not statistically significant. The relaxation time T_{22} also showed a similar tendency as that observed for T_{23} . T_{22} of HSC (heated for 10 min) declined from 60.844 to 23.637 ms when the temperature increased from 80 to 90 °C, but it increased significantly from 26.972 to 39.334 ms when the temperature increased from 100 to 120 °C. This pattern was also observed for the additional heating times. These data suggested that gelation caused by moderate heating conditions (lower than 100 °C), limited the movement of free water and immobilized water. Nevertheless, the degree of freedom of free water and immobilized

Table 2 NMR relaxation times T_{21} , T_{22} , and T_{23} and associated proportions $A-T_{21}$, $A-T_{22}$, and $A-T_{23}$ of heated sea cucumber *A. japonicus* body wall

Group	Time (min)	T_{21} (ms)	T_{22} (ms)	T_{23} (ms)	$A-T_{21}$	$A-T_{22}$	$A-T_{23}$
HSC-80	10	3.273 ± 0.707 ^{ac}	60.844 ± 4.225 ^a	313.008 ± 18.670 ^a	0.012 ± 0.003 ^{af}	0.083 ± 0.047 ^{adefghij}	0.955 ± 0.048 ^{acde}
	20	3.007 ± 1.473 ^{ab}	48.723 ± 6.451 ^{bi}	273.966 ± 36.664 ^{aef}	0.016 ± 0.007 ^{abf}	0.051 ± 0.006 ^{adef}	0.944 ± 0.011 ^a
	40	2.168 ± 0.592 ^{ab}	42.23 ± 6.451 ^{bchi}	177.673 ± 25.757 ^b	0.026 ± 0.006 ^{bef}	0.030 ± 0.033 ^{abcdef}	0.915 ± 0.035 ^{abe}
	80	2.041 ± 0.901 ^{ab}	32.248 ± 4.498 ^{cdifg}	200.923 ± 0.000 ^b	0.028 ± 0.006 ^{bcef}	0.084 ± 0.014 ^{agh}	0.888 ± 0.018 ^{bf}
HSC-90	10	2.900 ± 1.294 ^{ab}	23.637 ± 5.302 ^{defg}	233.266 ± 35.430 ^{abf}	0.027 ± 0.007 ^{bcef}	0.006 ± 0.009 ^b	0.968 ± 0.012 ^c
	20	1.732 ± 0.283 ^b	18.331 ± 0.996 ^e	138.925 ± 3.089 ^c	0.020 ± 0.003 ^{bf}	0.023 ± 0.003 ^c	0.957 ± 0.002 ^c
	40	1.535 ± 0.368 ^b	16.450 ± 2.346 ^e	112.348 ± 6.565 ^d	0.037 ± 0.013 ^{cdef}	0.029 ± 0.009 ^{ce}	0.934 ± 0.007 ^{ae}
	80	1.796 ± 0.228 ^b	21.755 ± 3.304 ^{ef}	116.252 ± 17.806 ^d	0.042 ± 0.004 ^{ce}	0.059 ± 0.008 ^{def}	0.899 ± 0.010 ^{bd}
HSC-100	10	1.598 ± 0.272 ^b	26.972 ± 5.152 ^{fg}	178.940 ± 27.919 ^b	0.022 ± 0.010 ^{bdf}	0.041 ± 0.011 ^{ef}	0.937 ± 0.007 ^{ae}
	20	1.892 ± 0.233 ^b	28.666 ± 3.569 ^g	204.201 ± 17.484 ^b	0.025 ± 0.007 ^{bef}	0.050 ± 0.004 ^f	0.926 ± 0.007 ^e
	40	1.817 ± 0.557 ^b	27.244 ± 1.916 ^g	249.461 ± 74.988 ^{abf}	0.027 ± 0.014 ^{abcdef}	0.050 ± 0.003 ^f	0.923 ± 0.016 ^{ade}
	80	1.865 ± 0.352 ^b	34.029 ± 10.142 ^{bfigh}	201.911 ± 33.159 ^{bg}	0.035 ± 0.011 ^{cdef}	0.106 ± 0.011 ^{ghj}	0.859 ± 0.020 ^{fh}
HSC-110	10	2.302 ± 0.665 ^{ab}	27.516 ± 2.136 ^g	191.899 ± 11.025 ^b	0.046 ± 0.011 ^{def}	0.068 ± 0.021 ^{afh}	0.886 ± 0.024 ^{bdfh}
	20	2.198 ± 0.922 ^{ab}	29.902 ± 2.202 ^g	223.173 ± 25.281 ^{befg}	0.034 ± 0.011 ^{bcddef}	0.086 ± 0.012 ^h	0.881 ± 0.020 ^{bth}
	40	2.507 ± 1.046 ^{ab}	45.364 ± 3.418 ^{bci}	266.765 ± 1.372 ^f	0.038 ± 0.007 ^{cdef}	0.139 ± 0.010 ^{ij}	0.824 ± 0.008 ^{gh}
	80	3.253 ± 0.686 ^a	37.689 ± 4.327 ^{eh}	249.595 ± 21.559 ^{fg}	0.043 ± 0.010 ^{ef}	0.079 ± 0.044 ^{aefhj}	0.885 ± 0.046 ^{beth}
HSC-120	10	2.839 ± 1.257 ^{ab}	39.334 ± 3.096 ^{bch}	214.275 ± 16.221 ^{bg}	0.040 ± 0.023 ^{bcddef}	0.074 ± 0.033 ^{afhj}	0.886 ± 0.051 ^{abefh}
	20	1.711 ± 0.659 ^b	25.012 ± 3.799 ^{fg}	205.552 ± 7.203 ^b	0.031 ± 0.011 ^{bcddef}	0.128 ± 0.024 ^j	0.841 ± 0.026 ^{gh}
	40	1.586 ± 0.708 ^b	39.698 ± 2.073 ^h	265.498 ± 21.787 ^{hi}	0.025 ± 0.011 ^f	0.159 ± 0.003 ^k	0.816 ± 0.010 ^g
	80	1.841 ± 0.729 ^{bc}	49.191 ± 1.741 ⁱ	296.889 ± 20.287 ^{ai}	0.034 ± 0.009 ^{cdef}	0.101 ± 0.025 ^{hj}	0.875 ± 0.026 ^{bh}

Different superscript lowercase letters show differences between the rows ($p < 0.05$)

Fig. 2 Low field nuclear magnetic resonance images of sea cucumber *A. japonicus* body wall treated by different heating conditions: **A** the T_1 -weighted images and **B** T_2 -weighted images

water increased at high temperatures (above 100 °C), in agreement with the changes in the moisture and water activity of HSC.

Collagen can restrict the mobility of water molecules because of its highly organized structure (Shoulders and Raines 2009). Thus, the structural integrity of collagen fibers can be indirectly revealed by the amount of different states of water, which is reflected by the transverse relaxation time. According to this principle, it is reasonable to conclude that moderate heat conditions (lower than 100 °C) led to the gel formation in HSC, but high temperature (above 100 °C) caused dramatic disintegration of the gel in HSC.

Changes in the distribution of bound water in HSC

The LF-NMR technique can be implemented to determine the distribution of water by monitoring the proton signal without damaging the samples (Pearce et al. 2011). However, because of the high moisture content in sea

cucumbers, the proton signal of HSC is so strong that proton density imaging is not appropriate to distinguish the different water states. Thus, T_1 -weighted imaging, which can reveal short relaxation signals, can be adapted to the analysis of sea cucumber (Zhang et al. 2012).

Figure 2A showed the water distribution in the sea cucumber body wall under different heating conditions. When samples were heated at 90 °C for increasing time periods, the T_1 -weighed imaging became brighter; this indicated that the proportion of bound water increased. Meanwhile, the bright outer layer of the sea cucumber body wall faded as the heating time prolonged, it demonstrated that the proportion of outer bound water decreased. The brightness of HSC decreased and became uniform when sea cucumbers were heated at 100, 110, and 120 °C, compared with those of samples heated at 80 and 90 °C, suggesting that the proportion of bound water declined gradually and that the bound water was distributed more uniformly when the temperature increased. This corresponds to the results of the changes in the water state in HSC.

Fig. 3 Effects of different heat treatments on the collagen structure of heated sea cucumber *A. japonicus* body wall (van Gieson stain, magnification $\times 40$)

Changes in the distribution of free water in HSC

In T_2 -weighted imaging, the signal area is observed to be bright as the free water content is high (Lai and Hwang 2004). As shown in Fig. 2B, the brightness of samples heated for 40 min decreased with increasing heating temperature. It indicated the loss of free water in HSC. The brightness of HSC decreased when the samples were heated at 90 °C from 10 to 80 min, whereas the brightness of the outer part of HSC increased. This phenomenon indicated that the total free water content decreased and the superficial free water content increased with prolonged heating. This is consistent with the results of the T_1 -weighted images, in which the outer bound water decreased. These observations indicated that when the samples were heated at 90 °C, part of the superficial bound water transformed into free water as the heating time

increased. So that the outer free water increased and the outer bound water decreased. Therefore, the results demonstrated that heat treatment can considerably influence the distribution of water and it might be related to the thermal changes in the gel structure of *A. japonicus*.

Changes in collagen fibers in HSC

The thermal changes in the gel structure in HSC were determined by observing collagen fibers through the van Gieson stain technique, which is widely used as a counterstaining method for reflecting the histological properties of muscle and connective tissue. The van Gieson stain images of HSC are shown in Fig. 3. Collagen fibers maintained the organized and ordered cross-linking structure in samples heated at 80, 90, and 100 °C for 10 min, resulting in the gelation of HSC. However,

Fig. 4 Effects of different heating conditions on the contents of free-hydroxyproline (A) and ammoniacal nitrogen (B) of heated sea cucumber *A. japonicus* body wall. The bars represent SDs and values are means. Different lower case letters suggest significant differences ($p < 0.05$)

collagen fibers shortened, became loose and disordered as the samples were heated at 110 and 120 °C for 10 min, indicating the thermal disintegration of the gel structure upon high temperature treatment. Meanwhile, it can be observed that the collagen fibers disintegrated as the heating time prolonged (from 10 to 80 min) at 100, 110, and 120 °C, similar to previous research (Gao et al. 2011). Especially when *A. japonicus* samples were heated at 120 °C, their collagen fibers collapsed rapidly after just 10 min heating treatment.

A previous study reported that the fabric or network structures of meat proteins and collagen play a critical role in maintaining the texture and moisture of foodstuffs (van der Sman 2007). According to this theory, thermal disintegration of the gel structure is likely to be the main reason for moisture migration and changes in the moisture distribution, which then lead to the deterioration of sensory texture. Earlier research suggested that chemical bonds such as hydrogen bonds and peptide bonds contributed most to the structural stability of collagen (Shoulders and

Raines 2009). Thus, the degradation of collagen fibers of HSC might be due to the rupturing of chemical bonds.

Changes in free hydroxyproline content of HSC

As the unique amino acid in collagen, hydroxyproline has traditionally been used in the quantitative analysis of collagen (da Silva et al. 2015). Free hydroxyproline is observed when the chemical bonds of collagen are broken. Thus, free hydroxyproline content can be used to determine the degree of deterioration of collagen in heated sea cucumbers.

The changes in the free hydroxyproline of HSC are shown in Fig. 4A. The content of free hydroxyproline increased only slightly ($p > 0.05$) and stayed below 10 mg/10 g protein in the HSC-80, HSC-90, and HSC-100 groups. However, it increased from 8.42 to 15.26 and 8.48 to 13.83 mg/10 g protein with the heating time increased from 10 to 80 min, when the temperature reached 110 and 120 °C. This indicated that the collagen of HSC degraded

dramatically when heat temperature exceeded 100 °C. This could be the main reason for the gel deterioration of HSC.

Changes in ammonia nitrogen levels of HSC

The amino group of collagen becomes exposed when the hydrogen bonds and peptide bonds rupture, therefore, the degradation level of collagen chemical bonds can be inferred from the level of ammonia nitrogen (Peng et al. 2015).

As shown in Fig. 4B, the ammonia nitrogen level of HSC fluctuated between 1.94 and 3.30 $\mu\text{mol/g}$ in the HSC-80, HSC-90, and HSC-100 groups. However, it increased from 3.42 to 5.58 $\mu\text{mol/g}$ in the HSC-110 group and from 3.91 to 6.06 $\mu\text{mol/g}$ over time in the HSC-120 group. It was especially observed when the samples were heated at 120 °C for 80 min, where the ammonia nitrogen level reached 6.06 $\mu\text{mol/g}$. This indicated that the hydrogen bonds and peptide bonds of the collagen fibers were relatively intact when the sea cucumbers were heated below 100 °C, but ruptured quickly at high temperature (above 100 °C), causing the decomposition of collagen fibers and the destruction of the gel structure.

Conclusion

Sea cucumbers *A. japonicas* heated under different thermal conditions (HSC) showed significant differences in sensory texture. Heat treatment at 80, 90 and 100 °C caused the gelation of sea cucumbers by rearranging the collagen fibers. This then increased the water activity and the proportion of bound water. However, at 110 and 120 °C, the sensory texture of HSC deteriorated as the moisture content, water state, and water distribution of HSC changed dramatically, and these resulted from the thermal destruction of the gel structure of sea cucumbers. Meanwhile, the collagen fibers decomposed and became disordered as the contents of free hydroxyproline and ammonia nitrogen increased rapidly with prolonging of heating time at 110 and 120 °C. On these grounds, it is confirmed that moderate heat treatment (lower than 100 °C) caused gelation by rearranging collagen fibers, but severe heat treatment (above 100 °C) led to thermal degradation of the gel structure by breaking chemical bonds in collagen fibers. This caused changes in the water state and water distribution, resulting in the textural changes in the sea cucumbers. This study gives further support to the investigation of the mechanism by which changes occur in sea cucumbers during heating and industrial processing.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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