The Correlation between Rheological Properties and Characteristic Values of Structure for Steamed Abalone Meat

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The quantitative correlation between rheological properties and characteristic values of structure for steamed abalone meat was studied. Abalone *Haliotis discus* was steamed in a food steamer for 1 h, 2 h, or 3 h, then cut up and separated it into cross- and vertical sections. Changes and characteristic values were enumerated by image processing and analysis techniques. Rheological properties were determined by stress-relaxation experiments; in raw abalone meat, those properties were mainly correlated with the characteristic values of collagen fibrils. For steamed meat, there was clearly a negative correlation between two of the characteristic structural values: the distance between myofibrils (D_m) and the void area between myofibrils (A_m), and rheological properties. This negative correlation between structural and rheological characteristic values (elastic moduli, relaxation time, and viscosity) has been expressed by logarithmic expressions. The results suggested that rheological properties are quantitatively influenced by the characteristic values of structure.

Keywords: abalone, steam, structure, image processing, rheology

Abalone is a highly prized mollusk worldwide, and the taste of the meat is a great delicacy. In food science, studies of abalone have shown a seasonal correlation of extract constituents, food texture and taste after extended cooking, rupture strength, and collagen content (Hatae *et al.*, 1995, 1996; Olachea *et al.*, 1993; Watanabe *et al.*, 1992). We also studied rheological properties and structural changes of both raw and heated abalone meat (Gao *et al.*, 2001, 2002). In raw abalone meat, the rheological properties of elastic modulus, relaxation time, and viscosity were influenced by the changes of collagen in muscle tissue; in heated abalone meat, collagen in connective tissues was converted to gelatin and the texture of the meat was tender (Gao *et al.*, 2001). The changes in rheological properties resulted from a difference in heating methods, heating time and myofibrillar structures (Gao *et al.*, 2002). These studies revealed a qualitative correlation between rheological properties and myofibrillar structure, but were not evaluated from a quantitative viewpoint. For the study of abalone, the quantitative correlation between rheological properties and characteristic values of the structure are understood, so that it is possible to create a food with special texture. The purpose of this study was to identify some of the characteristic structural values and to make a quantitative correlation. Image processing and analysis were employed to be enumerated the structure of raw and steamed abalone meat.

Image processing has recently become a promising method of food texture analysis. This technology can not only expedite and automate the assessment process for some important food qualities but also enhance objectivity of the measurement results. Image processing has found application in manufacturing, agriculture, food processing, medicine and other fields. Several

researchers have reported that textural properties which heavily depend on cellular structure and structural characteristics such as cell size, density and uniformity strongly influence the sensory properties of a product (Alina, 1962; Barret & Peleg, 1992a, b; Gao & Tan, 1996a; Smolarz *et al.*, 1989). For a marine mollusk, however, textural properties were thought to depend on not only fibrillar diameter but also to be influenced by other structural parameters: distance between myofibrils, ratio of total myofibrils in full image domain, ratio of total voids in full image domain, the void area between myofibrils, and circularity of that area. Therefore, in this work, characteristic values of raw and steamed abalone structure were enumerated by image processing and analysis technique. Meanwhile, structural changes were investigated by observing abalone myofibrils and collagen fibrils with an optical microscope and a scanning electron microscope, and by measuring rheological measurements in terms of stress-relaxation and rupture strength experiments. The quantitative correlation between rheological properties and characteristic values of structure are given by logarithmic expressions.

Materials and Methods

Materials Kuro-awabi, *Haliotis discus*, harvested in Chiba prefecture and with a total weight of 320–370 g including the shell, were purchased at a retail store. Abalones were shelled and cooked in a bamboo food steamer for 1 h, 2 h, or 3 h, respectively. After cooling for 30 min at room temperature, the middle part of the meat was cut out and then the muscle was cut vertically and cross-wise as a sample. An explanation of vertical and cross-wise was reported previously (Gao *et al.*, 2001).

Samples of an optical microscopic examination Steamed abalone meat was cut into 5 mm cubes, embedded in Tissue-Tek (OCT compound) at -20°C , and formed into 10 mm cubes (Nii-tu & Hiramoto, 1982). These cubes were trimmed and sliced into

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sections 10 μm thick using a cryostat, then mounted on a glass slide and stained with Van Gieson stain (Kageyama & Watanabe, 1988). Optical micrographs were taken with an Olympus MF optical microscope.

Image processing and analysis technique The image processing system used was a software package (Optimas, Bioscan Inc., West Dayton, WA). The optical micrographs of raw and steamed abalone meat were transmitted by image scanner (GT-6500WIN 2, Seiko Epson Corp.) and used as original images. To threshold this original image from 256 bites, a binary morphology which works on two gray scale levels, black (0 to 127) and white (128 to 255), was obtained. From the binary morphology, the following characteristic values of structure were calculated:

- W_m : width of myofibril (μm)
- D_m : distance between myofibrils (μm)
- R_m : ratio of total myofibrils in full image domain (%)
- R_{vm} : ratio of total voids in full image domain (%)
- A_m : void area between myofibrils (μm^2)
- C_m : circularity of void area between myofibrils.

The value of W_m is calculated by line morphometry. The value of D_m is averaged by the length of the line which crosses the longest axis of voids among the myofibrils. The value of C_m is given by the ratio of the perimeter length squared divided by the void area between myofibrils, $(\text{perimeter})^2/\text{area}$. This is a dimensionless number with a minimum value, $4\pi=12.57$, achieved only for circular boundaries. The value is 16 for square boundaries and 20.78 for equilateral triangular boundaries. For this definition, the value of C_m is smaller, the circular boundaries are more similar to a circle. In this paper, we changed the formula of this definition and expressed it by C_m^* , that is, $C_m^*=4\pi/C_m$. Therefore, the maximum value of real circle boundaries becomes one, and is 0.79 for square boundaries, and 0.60 for equilateral triangular boundaries. The larger is the value of C_m^* , the more similar to a circle are the circular boundaries.

Moreover, for collagen fibrils, the above characteristic values of structure were expressed as W_c , D_c , R_c , R_{vc} , A_c , C_c^* , respectively.

Stress-relaxation measurement and analysis Stress-relaxation experiments were carried out using a uni-axial compression and elongation type rheometer (Tensipressor, Model TTP-50BX, Taketomo Electric Co., Ltd.) at room temperature. A cylindrical

type plunger (3.6 mm in diameter) was used to compress the vertical and cross sections of the samples. The constant strain was 0.05.

Stress-relaxation curves were analyzed using a progressive approximate method (Iso *et al.*, 1981; Kimura *et al.*, 1991). The typical stress-relaxation curve of raw abalone meat is shown in Fig. 1, and its approximate equation can be expressed as follows:

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

where $p(t)$ is stress, e_0 is the constant strain, t is the time, E_1 is the elastic modulus of i -th element ($E_0 = E_1 + E_2 + \dots + E_n$, the instantaneous modulus), τ_i is the stress-relaxation time of i -th element ($\tau_i = \eta_i/E_i$, and η_i is the viscosity of i -th element). When $\ln p(t)$ is plotted against t , a straight line is obtained at large values of τ . The first term of relaxation time, τ_1 , which indicates the largest relaxation time, and the 1-st term of elastic modulus, E_1 , can be calculated from the slope of the straight line. Then, the value of η_1 is calculated from the values of E_1 and τ_1 . Once the values of E_1 and τ_1 are determined, $\ln(p(t) - e_0 E_1 e^{-t/\tau_1})$ is plotted against t . Again a straight line is obtained at large values of τ . Then the 2-nd term of relaxation time, τ_2 , and the 2-nd term of elastic modulus E_2 can be calculated from the slope of the straight line. Thus the viscosity η_2 can be calculated as before. In this study, two trials was made and all the measurements were repeated 8 times for each sample.

Rupture strength measurement Rupture strength was measured using the same apparatus used in the stress-relaxation measurement at room temperature. A cylindrical plunger (2.7 mm in diameter) compressed the vertical and cross sections of samples at a rate of 2 mm/s. The results shown in this paper represent the average of 8 experimental values for each sample.

Statistical analysis The data were compiled to determine standard descriptive statistics including mean and standard deviation. Intra-sample comparison between groups was analyzed via analysis of values, ANOVA. For this analysis a p -value of 0.05 or less was chosen to indicate a significant difference between groups.

Results and Discussion

Enumerating muscle structure The structure of the cross- and vertical sections of raw and steamed abalone meats observed by an optical microscope is shown in Fig. 2 (the morphology of raw abalone meat was quoted from our previous study) (Gao *et al.*, 2001). The muscle fibers were stained with Van Gieson staining which dyed myofibrils yellow and connective tissue red. Raw abalone meat connective tissue was dyed red and dispersed in muscle fibers. But the steamed abalone meat did not show red staining. These results were in agreement with those we reported previously (Gao *et al.*, 2001). The morphology shown in Fig. 2 was transmitted to image processing and converted to binary morphology, Fig. 3. Binary morphology works on two gray scale levels, white and black. In Fig. 3, white pixels represent the voids between muscles and black pixels represent by muscle fibers. Using the image processing and analysis techniques, the distributions and average value of D_m and A_m of raw and steamed abalone meat are shown in Fig. 4, and W_m , R_m , R_v , and C_m^* , are represented in Table 1. For the raw samples, the values of W_m , D_m , and A_m were smaller than those of steamed ones, in contrast, the value of R_m in raw abalone meat was higher than that in steamed

Fig. 1. The stress-relaxation curve of raw abalone meat.

Fig. 2. Optical micrographs of raw and steamed abalone meat stained with Van Gieson stain. (A) Vertical section of raw sample. (B) Cross section of raw sample. (C), (D), (E) Vertical section of steamed sample. (C) steamed 1 h, (D) steamed 2 h, (E) steamed 3 h. (F), (G), (H) Cross section of steamed sample. (F) steamed 1 h, (G) steamed 2 h, (H) steamed 3 h. Scale bars represent 30 μm .

Fig. 3. The binary morphology of raw and steamed abalone meat with image processing. (A) Vertical section of raw sample. (B) Cross section of raw sample. (C), (D), (E) Vertical section of steamed sample. (C) steamed 1 h, (D) steamed 2 h, (E) steamed 3 h. (F), (G), (H) Cross section of steamed sample. (F) steamed 1 h, (G) steamed 2 h, (H) steamed 3 h. Scale bars represent 30 μm .

meat. These results were agreement with the observation by optical microscope, and it was demonstrated that the formation of the larger voids is caused by a flowing out of gelatin from the extending space surrounded by denatured muscle fibers. For steamed abalone meat, the values of W_m and R_m of both cross and vertical sections are similar after steaming between 1 h and 3 h. The

D_m and A_m of the vertical section increased continuously with steaming time, but in the cross section these areas were rapidly decreased with steaming for 2 h. As steamed abalone meat was heated by water vapor, the meat juice, *i.e.* the moisture of samples with some denatured proteins such as gelatin, flowed out only under gravity, that is, the cascading direction was parallel to

Fig. 4. The distributions and average values of D_m and A_m of raw and steamed abalone meat.

Table 1. Structural characteristic values of raw and steamed abalone meat.

	$W_m (\mu m)$	$R_m (\%)$	$R_v (\%)$	C_m^*
Raw abalone				
Vertical section	2.7 ± 0.8^a	61.0 ± 1.4^a	39.0 ± 0.5^a	0.5 ± 0.2^a
Cross section	1.8 ± 0.4^b	58.8 ± 2.6^b	41.2 ± 0.9^b	0.4 ± 0.2^{ab}
Steamed abalone				
Vertical section				
1 h	3.2 ± 2.5^c	47.1 ± 2.4^{cd}	52.9 ± 0.9^{ce}	0.4 ± 0.2^b
2 h	3.3 ± 1.3^c	49.3 ± 0.4^c	50.7 ± 0.7^d	0.5 ± 0.1^a
3 h	3.5 ± 1.3^c	46.6 ± 2.2^{cd}	53.4 ± 1.9^{ef}	0.4 ± 0.1^{ab}
Cross section				
1 h	3.3 ± 1.3^c	46.4 ± 2.6^{cd}	53.6 ± 0.7^{cf}	0.4 ± 0.1^{ab}
2 h	3.3 ± 1.6^c	48.3 ± 1.0^c	51.7 ± 1.3^c	0.4 ± 0.1^{ab}
3 h	3.4 ± 0.7^c	45.9 ± 1.2^c	54.1 ± 0.2^f	0.4 ± 0.1^b

Values in the same column followed by different superscript letters are significantly different ($p < 0.05$). $n=65$

the vertical section. Thus, we can assume that the different directions of the cross and vertical sections resulted in different structure of muscle fibers. Meanwhile, it was also determined that there was a difference in characteristic values of the structure of the two sections.

Rheological properties Rheological properties of elasticity, viscosity, and food texture changed greatly with heat treatment, and those of both vertical and cross sections of raw and

steamed abalone meat are shown in Table 2. Elastic moduli E_0 , E_1 , and E_2 of raw abalone meat were higher than those of steamed abalone meat because of the low density muscle structure as shown in Fig. 2. Elastic moduli of the vertical and cross sections of raw abalone meat were significantly different, while no significant differences were observed in elastic moduli of the two sections of the steamed meat. The elastic moduli obtained from vertical sections tended to decrease during steaming, and for the cross section, these moduli were highest when steamed for 2 h. It was assumed that the movement of water and the dripping direction influenced the elastic moduli of the sections with steaming.

Relaxation times for steamed abalone meat were longer than those for raw meat (Table 2). When the steamed meat is masticated, coagulated proteins interfere with muscle fibers sliding against each other. It was concluded that the contraction of muscle fibers accompanied increasing viscous components and reduced elastic components. Table 2 also shows that the relaxation time of both vertical and cross sections of the steamed meat tended to decrease for two hours after steaming between 1 h to 3 h. Viscosities obtained from both sections of the meats had the same reaction with steaming time as elastic moduli. Viscosities of the vertical sections tended to decrease after steaming between 1 h to 3 h, whereas those of cross sections steamed for 2 h were

higher than other steaming times. As above, when abalone meat was steamed, because of the different cascading direction of denatured proteins, the movement of moisture and denatured proteins in vertical and cross sections were different. This difference resulted in changes in viscosity for both sections.

The rupture strengths for the vertical and cross sections of steamed meat were less than those for raw meat. For steamed abalone meat, the rupture strengths were similar at each steaming time (Table 2). Rupture strength of meat steamed for 1 h was greatest among that steam for 1 h to 3 h. It is known that the rup-

ture strength is mainly related to firmness of the meat. Our previous paper (Gao *et al.*, 2001) reported that thermal denaturation of meat steamed for 1 h was complete because no peak occurred in the DSC curve. It also described that myofibril proteins and collagen showed a difference in denaturation temperature, with the temperature of the middle part of the meat reaching the denaturation temperature of collagen in less than 12 min in steaming. Collagen in the abalone meat was heated enough to change into gelatin within 1 h. After steaming for 2 h or 3 h, the denatured protein and moisture flowed out under gravity. Since there were

Table 2. Rheological characteristic values of raw and steamed sample.

	E_0	E_1	E_2	τ_1	τ_2	η_1	η_2	Rupture strength
	(MPa)			(s)		(MPa·s)		(MPa)
Raw abalone								
Vertical section	3.00±0.83	1.67±0.50	1.33±0.48	12.40±1.67	1.51±0.25	20.80±4.81	1.01±0.78	1.64±0.20
Cross section	0.97±0.40	0.54±0.23	0.42±0.17	16.34±4.19	1.53±0.19	8.87±4.32	0.65±0.21	1.34±0.08
Steamed abalone								
Vertical section								
1 h	0.72±0.05 ^a	0.66±0.06 ^{ab}	0.17±0.03 ^b	82.05±8.47 ^a	1.83±0.63 ^{ab}	53.90±5.28 ^a	0.30±0.03 ^a	1.11±0.08 ^b
2 h	0.67±0.03 ^b	0.55±0.09 ^d	0.13±0.05 ^a	73.07±9.55 ^b	2.68±0.88 ^a	40.10±6.50 ^d	0.33±0.04 ^a	0.82±0.09 ^a
3 h	0.65±0.05 ^b	0.47±0.06 ^c	0.18±0.03 ^b	64.00±11.59 ^c	1.52±0.53 ^b	30.00±4.43 ^b	0.27±0.07 ^a	0.83±0.09 ^a
Cross section								
1 h	0.83±0.08 ^c	0.65±0.05 ^a	0.19±0.05 ^b	78.28±8.43 ^{ab}	1.78±0.54 ^{ab}	49.80±9.92 ^c	0.34±0.07 ^a	1.00±0.11 ^b
2 h	0.84±0.03 ^c	0.71±0.04 ^b	0.13±0.02 ^a	80.33±11.45 ^{ab}	1.93±0.68 ^{ab}	57.00±8.81 ^{ac}	0.25±0.16 ^a	0.71±0.05 ^c
3 h	0.75±0.06 ^d	0.63±0.02 ^a	0.13±0.05 ^a	63.38±11.25 ^c	2.43±0.50 ^a	39.60±7.07 ^{ad}	0.31±0.06 ^a	0.75±0.08 ^{ac}

Values in the same column followed by different superscript letters are significantly different ($p < 0.05$). $n=8$

Fig. 5. Changes in rheological properties and characteristic values of structure in relation to the steaming time of abalone meat. (A) and (B) vertical section (C) and (D) cross section. (□) D_m , (△) A_m , (▲) E_1 , (■) τ_1 , (●) η_1 .

no substances such as exchange water to maintain the structure of myofibrils, the myofibrils were released and continuously squeezed. Therefore, the rupture strength of meat steamed for 1 h was less than raw abalone meat, but higher than that steamed for 2 h and 3 h.

The quantitative correlation between rheological properties and characteristic values of structure To illustrate their correlation in abalone meat, the structural and rheological characteristic values of vertical and cross sections were projected into Fig. 5, which shows that D_m and A_m of vertical sections were increased accompanied by decreasing elastic modulus (E_1), relaxation time (τ_1), and viscosity (η_1) of the 1st element (Fig. 5-A and -B). Compared with the samples steamed for 1 h, the amount of increase in D_m and A_m was 36% and 60%, respectively, whereas the reduction of E_1 , τ_1 , η_1 was 10%, 25%, and 22%, respectively. For the cross section (Fig. 5-C and -D), no correlation was observed between rheological properties and characteristic val-

ues of structure. In general, when D_m and A_m are increased, the E_1 is decreased while τ_1 and η_1 are increased. It was therefore clearly a negative correlation between myofibrillar characteristic values (D_m , A_m) and rheological properties (E_1 , τ_1 , η_1) in vertical sections of steamed abalone meat. Results of the logarithmic expressions between structural and rheological characteristic values (Fig. 6) suggested that the rheological properties were quantitatively influenced by the characteristic structural values.

In raw abalone meat, elastic modulus of the 1st element (E_1) in vertical section meat was about three times as large as that of cross section meat, while the characteristic values, D_m , R_m , R_{mv} , A_m , and C_m^* of the two sections were similar. Only W_m of the vertical section was different to that of the cross section. As expressed above, W_m was influenced by E_1 , but it can't be assumed that the larger difference of E_1 in both sections only resulted in W_m . Watabe and Yoneda (1997) reported that abalone meat which contained 30–50% collagen of the total amount of

Fig. 6. The logarithmic expressions between rheological properties and characteristic values of structure in vertical sections of steamed abalone meat. ($\blacktriangle$) elastic modulus (E_1), ($\blacksquare$) relaxation time (τ_1), ($\bullet$) viscosity (η_1).

Fig. 7. The binary morphology of collagen structure with image processing. (A) Vertical section, (B) Cross section. Scale bars represent 1 μm .

Table 3. Characteristic values of collagen fibrils of raw abalone meat.

	W_c (μm)	D_c (μm)	R_c (%)	R_{vc} (%)	A_c (μm^2)	C_c^*
Vertical section	0.11 ± 0.06	0.32 ± 0.14	44.07 ± 0.80	55.93 ± 1.61	0.29 ± 0.13	0.57 ± 0.22
Cross section	0.13 ± 0.08	0.55 ± 0.23	46.91 ± 2.14	53.09 ± 0.72	0.50 ± 0.19	0.28 ± 0.08

$n=73$

protein and collagen greatly affected the firmness of the meat. Our previous paper (Gao *et al.*, 2001) also studied the collagen structure of vertical and cross sections of abalone meat and confirmed that this structure was very different in the two sections. As true of steamed abalone, the collagen structure of both sections of raw abalone meat obtained by cell-maceration/SEM method were transmitted to image processing and converted to binary morphology (Fig. 7). In Fig. 7, we enumerated the collagen structure by image processing and analysis technique and the results are shown in Table 3. W_c , R_c , and R_{vc} for both sections were similar, but D_c , A_c , and C_c^* of the cross section of collagen were almost double those ones of the vertical section. The collagen fibrils of raw abalone meat is believed to have a linear and fibrous structure which is difficult to deform in cross section; In contrast, it has a network structure which is easily deformed in vertical section. According to these large differences in the two sections of raw abalone meat, it is assumed that the rheological properties are mainly correlated to the characteristic values of collagen fibrils. But the ratios of two components, the width of myofibrils (W_m) and collagen structural factors (D_c , A_c , C_c^*), which influence the elastic modulus of the 1st element (E_1) in raw abalone meat are not yet known. Our next task will be to continue this research.

Based on these results, it can be confirmed that rheological properties are mainly correlated with the characteristic values of collagen fibrils in raw abalone meat. Steamed meat was influenced by the characteristic values (D_m , A_m) of myofibrils. A negative correlation was confirmed between D_m or A_m and E_1 , τ_1 , or η_1 in steamed abalone vertical section meat. These results suggest that rheological properties are correlated quantitatively with the characteristic structural values of abalone meat.

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