TEXTURE—STRUCTURE RELATIONSHIPS IN SCALLOP

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ABSTRACT

Warner-Bratzler shear, Instron compression and extension were screened for sensitivity to the textural changes caused by heating scallop (Placopecten magellanicus) *adductor muscle. Instron compression, expressed as hardness, was selected since it gave the greatest slope with respect to temperature. Previously frozen commercial scallop were heated to internal temperatures of 25 to 80°C. A linear increase in hardness of 0.033 N/g/°C occured during heating from 25 to 60°C; this increase was primarily a function of water loss. This was followed by a stepwise increase of 0.14 N/g/°C between 60 and 65°C which is considered to be a result of denaturation of myofibrillar proteins. Hardness continued to increase above 65°C at a rate of 0.055 N/g/°C. Quantitative scanning electron microscopic (SEM) measurement of the proportion of irregular muscle fibers, expressed as % damage, was performed on the same heated scallop used for texture analysis. Scallop heated from 25 to 50°C exhibited 30% damaged fibers; from 55 to 65°C, damage increased from 45% to 63%, paralleling the increase in hardness. Above 65°C, damage reached a maximum of 70%. The relationship between hardness and damage fit a linear model, with an* R^2 *of 0.86* ($P = 0.004$)*; thus, the microstructural measurement of damage to scallop muscle can be used to predict the textural property of hardness.*

INTRODUCTION

A major problem in meat texture research is natural variation in the composition of muscle (Khan 1977). Determination of the contribution of the individual structural components of muscle to overall meat texture

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should provide a basis for the interpretation of these natural textural variations. A two component model of meat texture composed of background toughness, contributed by connective tissue, and myofibrillar toughness, resulting from the postrigor state of the contractile proteins, was proposed by Marsh and Leet (1966). It would be desirable to relate the effect of changes in the microstructure of the myofibril to the myofibrillar component of toughness; however, it is particularly difficult to neutralize the effect of connective tissue in land animals since its quantity and quality vary within the muscle.

Placopecten magellanicus, a marine mollusc, possesses a specialized striated adductor muscle that is consumed as the seafood scallop. Historically, scallop have been prized for their tenderness (Kellogg 1910) but overcooking of the muscle results in toughness. Scallop adductor muscle could be useful as a model system to evaluate the effect of heating on myofibrillar toughness if the background toughness due to connective tissue is indeed minimal.

The striated adductor muscle of scallop is made up of sarcomeres composed of interdigitating thick and thin filaments but there are several fundamental differences compared to mammalian skeletal muscle. Scallop muscle fibers are ribbon shaped, about 1 by 10 microns in cross section and average 663 microns in length (Nunzi and Franzini-Armstrong 1981) which is much smaller and shorter than mammalian muscle. In cross-section, there are in the order of 100 times as many muscle fibers as there would be in a similar area of beef muscle. The same authors found A-band widths to vary by up to 40% leading to a large variation in sarcomere length. The paramyosin core of scallop thick filaments and the higher ratio of thin to thick filaments make the contractile apparatus of the sarcomere quite different from mammalian muscle.

The goal of this work was to objectively measure changes in cooked scallop muscle microstructure and relate them to corresponding changes in texture.

MATERIALS AND METHODS

Scallop Muscle and Sample Preparation

A 20 kg supply of frozen scallop, *Placopecten magellanicus,* of an average weight of 5.59 g (S.D. = 1.65 g) was obtained from the fall 1980 catch of a commercial fishery in Lunenberg, Nova Scotia. A pooled sample of 10 individual scallop was found to contain 86.0% moisture, 10.50% protein (N×6.25), 0.17% free lipid and 1.20% inorganic material. All

analyses were performed using Association of Official Analytic Chemists (AOAC) standard methods. Connective tissue was estimated as 0.57% of the scallop muscle by using the factor of 8.2 (Lawrie 1979) to convert hydroxyproline to collagen.

Frozen scallop were thawed at 4°C overnight then allowed to warm to 25°C in deionized water over a period of 2 h. A Haake Model E-53 thermostatically controlled circulating water bath (Saddle Brook, N.J.) was used to heat samples to a given internal temperature. Samples of each temperature treatment were removed from the water bath, blotted free of water and measured for weight loss, change in dimensions, texture and differences in microstructure. Dehydration was achieved without heat by holding the scallop under a partial vacuum of 350 mm Hg in a desiccator and monitoring weight loss.

Texture Analysis

Compression measurements were made using an Instron UTM, Model 1122 (Burlington, Ont.) equipped with a 2000 g load cell. Ten samples of each treatment were compressed to 50% of their original height between a 10 cm^2 flat disc and the platform of the load cell. Both the crosshead and chart speeds were fixed at 1.67×10^{-3} m/s. The method used was similar to the Instrumental Texture Profile Analysis with only the initial peak, referred to as hardness, being used (Breene 1975). The maximum force was recorded and adjusted for sample weight to N/g.

Tensile properties were measured in the same Instron using a 2000 g load cell. Crosshead speed was fixed at 1.67×10^{-3} m/s and chart speed was set at 3.33×10^{-3} m/s. Samples of muscle bundles were excised *in situ* from scallop adductor muscles immediately postmortem and were equilibrated to temperatures of 25, 55 and 80°C. They were then attached to fiber clamps on the load cell and crosshead by a combination of soft thread strengthened with methacrylate glue and emery cloth to prevent slippage. Extension was carried to the breaking point and the response recorded on a chart recorder calibrated to 0.985 N full scale. Ten samples of each treatment were tested and the weight and length of each were recorded. Measurements were taken of the extension at rupture, peak breaking force and work to rupture.

Shear measurements were made using a modified Warner-Bratzler apparatus with a single blade at a crosshead speed of 0.043 m/s. The instrument was calibrated for 20 N full scale response. Fifteen determinations of each treatment were made. Shear force was measured across the fiber axis and adjusted for sample weight to N/g. Force-deformation

curves were examined using Moller's method for the differentiation of myofibrillar and connective tissue components of toughness (Moller 1981).

Scanning Electron Microscopy

Samples were prepared for scanning electron microscopy by excising a 5 mm cube of muscle from the centre of each specimen. The samples were fixed in glutaraldehyde and post-fixed in osmium tetroxide using the method of Moir (1977). They were subsequently dehydrated using an ethanol series followed by critical point drying with liquid carbon dioxide. Sections were mounted on aluminum stubs using a conductive carbon cement. A Technics sputter coater (Alexandria, VA) was used to provide a thin conductive layer of gold-palladium. The samples were examined using an ETEC (Hayward, CA) scanning electron microscope, with an accelerating voltage of 10 kv.

Muscle microstructure was quantitatively evaluated in order to objectively assess changes in structure resulting from heat treatment. For each treatment a block of fixed dehydrated tissue, prepared for SEM as previously described, was cut along the fiber axis into six sections. The surface was intentionally roughened by breaking the block rather than slicing it. Each section was mounted with a fiber surface parallel to the surface of the stub. A standard sputter coat of gold-palladium was applied. The stub was then examined under low magnification (100×) in the SEM. To assure that the results of the evaluation were unbiased all specimens examined were coded and randomized by a third party who retained the code until the results were analyzed. All statistical analyses were performed using the Statistical Analysis System (Barr and Goodnight 1972). Measurements were taken directly from the image on the cathode ray tube of the SEM using a clear plastic template bearing a grid calibrated to read 2 μm per division at a magnification of 2600×.

For each field, a count of the number and size of fibers was made. To obtain a reasonable number of fibers within a field and still allow complete rotation of the template over the CRT, a Y axis of 24 μm was selected. To permit easy estimation of the percentage of irregular surface a 20μm X axis (10 units on the template) was chosen. The area measured was 480 μm^2. Four structural features were measured:

1. apparent packing was determined as the percentage of the area of the field occupied by fibers;
2. fiber damage was estimated as the percentage of total fiber surface

judged to be irregular compared with the smooth appearance of unaltered muscle fibers;

3. broken fibers were recorded as the percentage of fibers in a field showing transverse gaps that appear to break the fiber;
4. the regular waves on the surface of the fiber were thought to be due to a repeating structure of the sarcomere which would permit an estimation of sarcomere length. The measurement of the average distance between waves over 10 μm of one fiber in each field was expressed as apparent sarcomere length. No count was taken when the surface was perfectly smooth.

RESULTS AND DISCUSSION

Instrumental Texture

Preliminary studies were conducted to determine the rheological method that responded with the greatest sensitivity to textural change caused by controlled heating of scallop muscle. Timed immersion in a boiling water bath was found to produce a thermal gradient within the scallop, but thermal equilibration in a constant temperature waterbath was found to minimize this gradient effect. Softness of the muscle made clamping of samples for extension testing difficult but the problem was overcome by using methacrylate glue. However, under SEM the fibers were observed to disengage rather than break due to their short fiber length (ca. 0.6 mm). Since this does not reflect the strength of the myofibril, this method was rejected. Shear values, determined using the Warner-Bratzler method, increased as treatment temperature increased. The shear force-deformation curve, interpreted using the method developed by Moller (1981), showed a negligible connective tissue component. This agrees with the assayed content of connective tissue, 0.57% of whole muscle, which is below the lower range of connective tissue of beef and pork muscles (Lawrie 1979). Hardness increased in a similar manner to the shear values but gave a greater slope as temperature increased. Consequently, hardness was chosen as the texture measurement to correlate with microstructural data.

The relationship between temperature and weight loss is shown in Fig. 1. There is a large weight loss between 40 and 50°C and another sharp increase between 55 and 60°C which levels out at 45% above 70°C. A linear equation was found to fit the data over the temperature range examined (Table 1). Weight loss was affected slightly by holding time (range—7 to 10 min) since higher internal temperatures required longer equilibration time. Analysis of the cooking water showed that less than

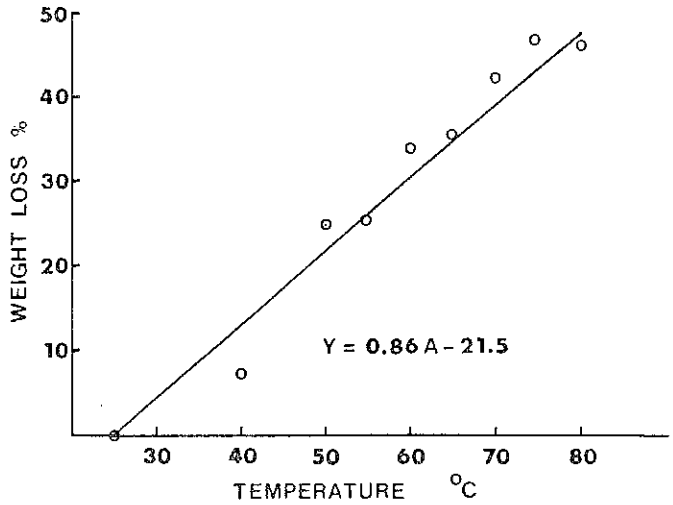

FIG. 1. THE EFFECT OF INTERNAL TEMPERATURE ON THE WEIGHT LOSS OF PREVIOUSLY FROZEN COMMERCIAL SCALLOP
(Y = % weight loss, A = temperature °C)

1% of the total scallop protein was lost. Since other components are minor by comparison it is concluded that the weight loss was mainly water.

A linear model composed of both weight loss and temperature gave a significant correlation with hardness (Table 1). The predicted values deviated from the actual values mainly in the region of 60°C. Subsequent treatment of the data as two sets, 25 to 60°C and 65 to 80°C, improved the fit for both portions of the curve (Fig. 2). Regression data (Table 1) showed that the increase in hardness with respect to temperature is 0.033 N/g/°C below 60°C and 0.057 N/g/°C above 65°C while in between those temperatures hardness increased at 0.14 N/g/°C. The linear increase in hardness to 60°C paralleled weight loss. The effect on the hardness of scallop that had been dehydrated without heating indicated an increase in hardness of 0.038 N/g/% weight loss (Table 1). When the data for scallop heated to 60°C were pooled with the data for dehydration without heating the fit of the regression was almost unchanged.

Quantitative Microstructure

Samples of all the temperature treatments of scallop were prepared for scanning electron microscopy. At 25°C (Fig. 3A) samples showed the

Table 1. Linear regression of scallop rheological and structural data

Dependant Variable	Equation	df	R^2	Probability
Frozen commercial scallop				
Weight Loss (%)	y = 0.86a - 21.5	11	0.960	0.001
Hardness (N/g)	y = 0.084a - 0.027b - 2.10	11	0.900	0.001
Hardness < 60°C	y = 0.033a - 0.004b - 0.34	8	0.998	0.002
Hardness > 65°C	y = 0.057a + 0.028b - 2.27	6	0.999	0.009
Damage (%)	y = 0.87a + 1.16	8	0.84	0.0005
Damage (%)	y = 0.92b + 24.9	8	0.82	0.0008
Ambient Dehydration				
Hardness (N/g)	y = 0.038b + 0.398	25	0.85	0.0001
Pooled Dehydration				
Hardness (N/g)	y = 0.034b + 0.482	31	0.84	0.0001
Texture as a Function of Structure				
Hardness (N/g)	y = 0.061c - 1.16	8	0.85	0.0004

a = temperature (°C) b = weight loss (%) c = damage (%)

smooth surface typical of unheated muscle fibers. As heating progressed, the surface lost its regular appearance and fibers developed waves or kinks (Fig. 3B). At 80°C (Fig. 3C) the fiber surfaces became extremely irregular with waves extending the visible length of most fibers.

Apparent fiber packing did not correlate with sample treatment. In order to expose the surface topography the muscle samples were broken rather than sliced, thus creating an artifact. This was also true of the number of breaks recorded for each sample. In addition, the attempt to measure sarcomere length from the frequency of surface waves did not give reliable results. The measurement that proved to relate to heating was the percentage of irregular fiber surface or damage. This figure compensated for variation in fiber packing by using a percentage of the exposed surface. The relationship between % damage and temperature reasonably fit a linear regression (Table 1), but the plot (Fig. 4) is more sigmoidal. Duncan's Test indicated that the process of microstructural change takes place in three stages. The samples treated at 25, 40 and 50°C were unaffected by heating and were not significantly different

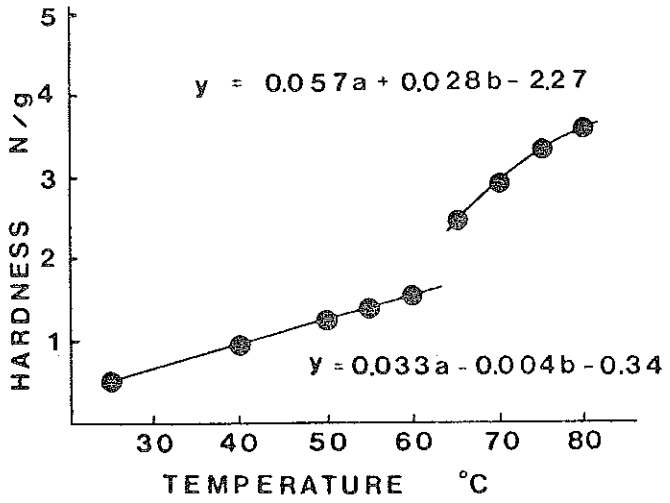

FIG. 2. THE EFFECT OF INTERNAL TEMPERATURE ON THE HARDNESS OF PREVIOUSLY FROZEN COMMERCIAL SCALLOP
y^1 = hardness (N/g) for temperature $\leq 60°C$
y^2 = hardness (N/g) for temperature $\geq 65°C$
a = temperature (C), b = weight loss (%)

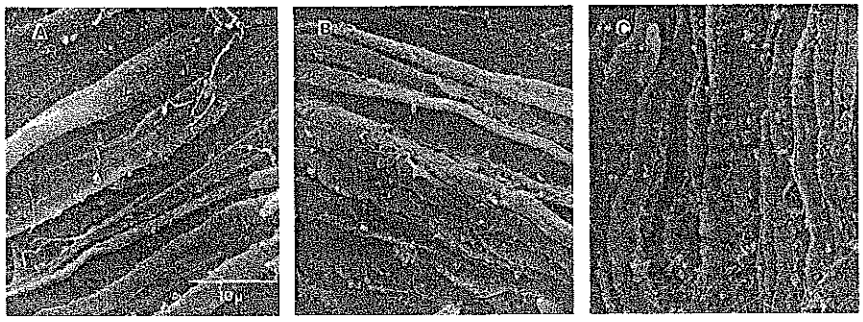

FIG. 3. SCANNING ELECTRON MICROGRAPHS OF PREVIOUSLY FROZEN SCALLOP HEATED TO AN INTERNAL TEMPERATURE OF: A. 25°C, B. 55°C, AND C. 80°C.

($P \leq 0.05$). The samples heated to 65°C and higher reached a maximum of visible damage and were also not significantly different ($P \leq 0.05$). A transition zone between 50 and 65°C is similar to the increase in hardness over the same temperature range.

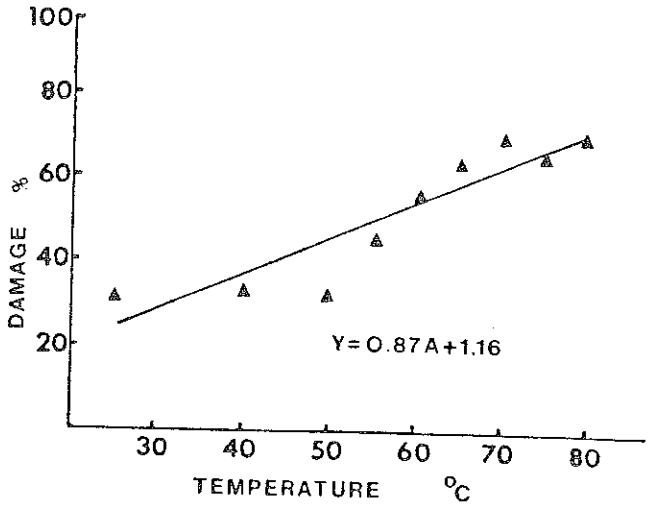

FIG. 4. THE EFFECT OF INTERNAL TEMPERATURE ON MICROSTRUCTURAL DAMAGE (%) TO PREVIOUSLY FROZEN COMMERCIAL SCALLOP MUSCLE

Texture-Structure Relationships

The structural response of previously frozen scallop muscle was measured on the basis of surface changes to the individual fibers. Linear regression analysis (Table 1) of the damage measurement gave a strong correlation with treatment temperature ($R^2 = 0.84$) and with weight loss ($R^2 = 0.82$). Hardness also related well to changes in temperature and weight loss. To examine the relationship between texture and structure, damage was used as the independant variable in the linear regression of hardness (Table 1). Hardness fits a linear function of damage quite well ($R^2 = 0.85$). Thus the microstructural measurement of damage to scallop muscle can be used to predict the textural property of hardness (Fig. 5).

SUMMARY AND CONCLUSIONS

Quantitative microstructural data on muscle may be obtained using SEM. This measurement relies on a surface change in the muscle fibers. Dehydration was found to be responsible for the change in appearance of scallop muscle fibers below 60°C. The rapid transition noted in both hardness and damage between 60 and 65°C is attributed to the denaturation of myofibrillar proteins.

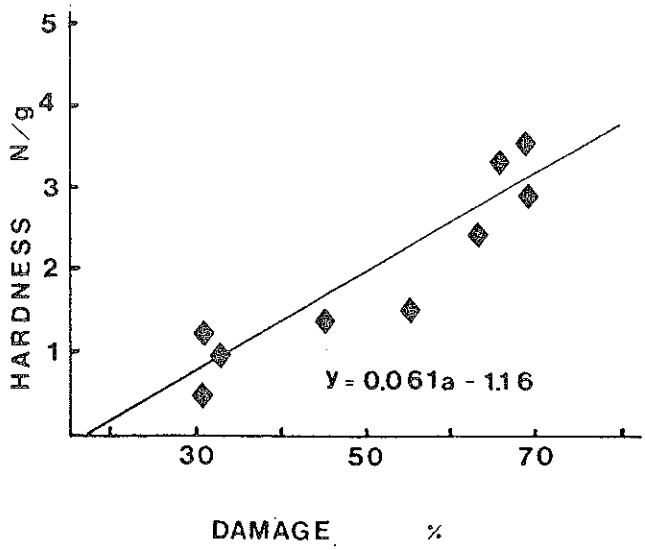

FIG. 5. THE RELATIONSHIP BETWEEN HARDNESS OF PREVIOUSLY FROZEN COMMERCIAL SCALLOP AND % MICRO-STRUCTURAL DAMAGE, MEASURED BY SEM

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