Development of Muscle Thermal Rigorometer and Characterization of Heat-induced Muscle Shortening of Tilapia
CHUNG-WEN SU AND MING-SHENG KONG

ABSTRACT: A muscle thermal rigorometer was constructed, allowing muscle shortening induced by dynamic heating or isothermal aging to be monitored. Operating isothermally like a traditional rigorometer at 10 °C, postmortem dorsal muscle shortening (SJgen) developing from 0% to 10% of its initial length in corresponding to RLgen developed fibredirection developing from 0% to 100% in 16 h was monitored for freshwater tilapia. Monitoring meat cooking in the dynamic heating mode, heat-induced shortening could be observed for all muscle samples possessing different degrees of rigor induced by 10 °C aging. The heat-induced shortening (Sdyn) plus its 10 °C aging shortening (SJgen) for each sample was the same, SJgen = Sdyn + SDLgen = 10%. Their heat-induced shortening peak temperatures (TTS) from 30 °C to 48 °C were inversely correlated with the sample RLgen from 0% to near 100%. These findings together with an additional calcium/adenosine triphosphate (ATP) model studies showed that the ATPase related myofibrillar contractile system was responsible for these low-temperature cooking shortenings, which along with TS values could thus be adopted as new rigor indices.

Keywords: tilapia, rigor, rigorometer, muscle shortening

Introduction
Heat-induced changes of muscle proteins greatly affect the functional and sensory properties of cooked meat products. Davey and Gilbert (1974) first reported that 2 stages of shrinkage occurred at low (40 to 50 °C) and high (65 to 75 °C) temperature ranges during cooking of beef and proposed that the stages were caused by thermal denaturation of myofibrillar and connective tissue proteins, respectively. For years, this two-stage shrinkage principle has been broadly accepted and adopted by meat industry to optimize the cooking process. Recent studies using differential scanning calorimeter (DSC) have confirmed that collagen denaturation leads to the shortening observed at the high temperature range (Beltran and others 1991). No endothermic peak indicating the occurrence of myofibrillar protein denaturation has been detected on DSC thermogram during heating of beef until it reached 55 °C (Quinn and others 1980; Parsons and Patterson 1986; Park and others 1993; Ma and Ledward 2004). This has left an unsolved question on the nature of low temperature muscle shortening. Meanwhile, Wright and others (1977) reported a 54 °C exothermic peak on the DSC muscle thermograms of freshly killed rabbit. This exothermic peak disappeared with the onset of rigor mortis. A similar finding was also reported in tilapia dorsal muscle by Park and Lanier (1988): The tilapia exothermic peak appearing at 50 °C was proposed to be related to the hydrolysis of adenosine triphosphate (ATP). These unexpected exothermic peaks in the low temperature region suggest that certain muscle changes might be caused by nonprotein denaturation. In Japan, a traditional hot water “arai” procedure is also an example of meat heating at a low temperature range, where prerigor fish meat being dipped in 50 °C water for 15 s to induce muscle shrinkage has been widely used to improve the biting quality of raw fish. Later, it was found that this procedure caused the release of large quantities of Ca++ from the sarcoplasmic reticulum (SR) and that this Ca++-induced myofibrillar ATPase activity and subsequent induction of a rapid rigor contraction responsible for the textural change (Okitani and others 1983; Watabe and others 1990, 1991; Ushio and Watabe 1994). It seems possible that the low temperature exothermic changes with DSC and the biochemical changes affected by the arai cooking operation are closely related to each other. However, it is currently unknown whether the shortening of muscle observed in this temperature range is related to heat-induced rigor mortis. From the results of previous studies, it was strongly suggested that cooking shrinkage and endothermic of meat occurring in higher temperature range of above 50 °C should most possibly be of protein denaturation in nature. However, no conclusive answer on the cause of the muscle shortenings and occurrence of DSC exothermic peaks had been drawn.

Traditionally, certain biochemical parameters (Iwamoto and others 1985; Iwamoto and Yamanaka 1986; Oka and others 1990) and external toughness or shortening indices (Schmidt and others 1968; Bito and others 1983; Iwamoto and others 1987; Korhonen and others 1990; Mochizuki and Sato 1994) have been used to monitor the development of the muscle rigor process under isothermal conditions. For this reason, a rigorometer (RM) was designed to record the strength and extent of shortening caused by the postmortem contraction of aged muscle at a fixed temperature (Buttkus 1963; Korhonen and others 1990; Devine and others 1999). In the present study, a modified version of an RM, named a meat/muscle thermal rigorometer (MTRM), was developed by adding a programmable heating unit in the muscle sample chamber to monitor muscle shortening either in isothermal mode or dynamic heating mode. The recorded heat-induced muscle shortening by dynamic heating was then compared with traditional rigor mortis parameters, that is, body rigor index (RIbody), pH, and ATP, to study the mechanism of
low-temperature shortening observed during cooking at low temperature, that is, 10 to 50 °C. Freshwater tilapia were used in this study as a model system.

**Materials and Methods**

**Samples and sample preparation:**

Live freshwater tilapia (Oreochromis niloticus X O. aureus) with body length of 25 to 30 cm and weight of 600 to 800 g were live caught in aquaculture ponds and shipped on the same day to our laboratory. Upon arrival, they were kept in a well-aerated tank at room temperature for at least 24 h to allow recovery after transportation. Fish were sacrificed individually by rapidly capturing and wrapping them in a net to restrain their activity and then stunning them on the head with a large wooden hammer. After removing the head, bleeding, and eviscerating (all done in a 10 °C cold room), whole fish were used for determination of body rigor index (RIbody). The dorsal white muscle was used for pH and ATP measurements as well as MTRM analyses. Only fish considered to be healthy and unstressed as shown by sufficiently high initial pH immediately after slaughtering (generally between pH 7.0 and 7.2) were used. Each pH check experiment was repeated 5 times.

**RIbody**

Fish were placed along the bench edge at the midpoint of their body with tails hanging over the edge according to the modified method of Bito and others (1983). At fixed time intervals of 0.5 h, the D values were measured and the corresponding RIbody was calculated by the following equation

\[
\text{RI}_{\text{body}} = \left(\frac{D_0 - D}{D_0}\right) \times 100\%
\]

where \(D_0\) is initial height from the tail to the extended horizontal line of the bench, and \(D\) denotes height decrease, as rigor mortis develops. The data of RIbody changes were the averages of repeating experiment performed on 5 fish samples.

**Thermal rigorometer setup and analysis:**

**MTRM construction and setup**

The MTRM used in this study was constructed as shown in Figure 1. A flat rectangular stainless steel box consisting of a bottom part and a removable cover part of polyurethane foam to improve heat insulation was used as the sample chamber. A clip was welded at one end of the chamber to fix one end of the muscle samples. A fine-gauge nylon wire, attached to the other end of the horizontally laid sample by another small clip, extended out through a small hole of the chamber and turned vertically down to attach to a small lightweight metal position pointer. Actual muscle length was the distance between the 2 clips. The changes of muscle length were read as the distance that the position pointer traveled along the vertically attached stainless steel 80 mm ruler (0.5 mm resolution). An improved resolution of 0.02 mm could be resolved by using a magnifying lens (Peak Stand Micro 50x, West Yorkshire, England) with internal fine-scale marking placed in front of pointer and ruler. A T-type thermocouple (Digital Thermometer DE-305, A One Union Co., Taiwan, R.O.C.) was inserted inside the sample chamber to record the environment temperature of the sample. A small vial in the sample chamber was filled with water to keep a high-humid condition to prevent dehydration of the muscle sample. To control heating and cooling rates, the walls of the sample chamber were inter-layered with tunnels of circulating water, and the water temperature was controlled by a digital program controller (REX P-200, RKC Instrument Inc., Tokyo, Japan) through a water bath (TIT BL-20, Tungtec Instruments Co., Taiwan, R.O.C.).

**MTRM analysis**

Tilapia dorsal white muscle was cut into 50 × 10 × 2 to 3 mm (L × W × T) slices, longitudinally along the fiber orientation. The muscle slices were laid horizontally in the sample chamber, and one end was fixed by a clip inside the bottom of the chamber that had been coated with a thin layer of soybean oil to prevent muscle from sticking. A small vial was filled with water just before closing the chamber cover to keep the sample chamber at a near saturate relative humidity. For 10 °C isothermal analysis, the chamber was precooled to 10 °C before the sample was placed at time intervals of 0.5 h, a reading of S\(_{\text{pH}}\) was detected on the muscle slice being taken from the fish right after slaughter and pH value check. Five fish sample were used to perform the isothermal aging experiment and used to do the data average. For dynamic MTRM analysis, muscle samples were placed into the 10 °C chamber for different periods of time to afford different degree of muscle shortenings of rigor mortis, subsequently dynamic heating and heat-induced shortening were performed and measured. In this study, each S\(_{\text{dynamic}}\) curve was resulted from the average of 3 repeats with individual fish sample. A preliminary test was done at a fast heating rate of 5 to approximately 7 °C/min from 10 to approximately 50 °C to estimate the rough temperature range at which heat-induced shortening occurred. For precise tests, samples were heated at a heating rate of 3 °C/min from approximately 10 °C below the temperature determined in a preliminary test for the temperature at which shortening occurred. Fiber-direction length (FL) changes were read from the magnified ruler.

Percentage shortening (S) was calculated by comparing the lengths of muscle samples (FL\(_{\text{sample}}\)) during isothermal holding and progressive heating to its prerigor state (FL\(_{\text{prerigor}}\), using the following equation:

\[
S = \left(\frac{\text{FL}_{\text{prerigor}} - \text{FL}_{\text{sample}}}{\text{FL}_{\text{prerigor}}}\right) \times 100\%
\]

Peak shortening temperature (Ts) is defined as the temperature at which the maximum rate of muscle shortening occurred and was determined by using the MTRM in a dynamic heating mode. Accu...
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Rate Ts were estimated by taking the 1st-order derivative curves of MTRM thermograms (DMTRM thermograms) during development of muscle shortening. Muscle shortening resulting from the 10 °C isothermal holding and subsequent dynamic heating treatments were denoted as Si0°C and S dynamic respectively. At time intervals of 0.5 h, single reading of percentage shortening (S) was detected from the same muscle slice being taken right after slaughter and hanged on the MTRM.

Effects of ATP and Ca2+ additions and pH adjustment on muscle

Prerigor tilapia dorsal muscle slices were immersed in 4°C, 0.2 M lactate buffer, pH 6.9, containing either 0.5% or 1% (w/w) CaCl2 for 20 min to allow them to reach equilibrium. Some of the samples were further treated with ATP by transferring them into 4°C lactate buffer solutions containing 0.5% or 3% (w/w) ATP (Sigma Chem. Co., St. Louis, Mo., U.S.A.) for 20 min. The treated muscles were then placed in the MTRM sample chamber for dynamic heating analyses. Changes in sample length were recorded as described previously, and results were expressed as percent muscle shortening. To demonstrate the effect of pH on postmortem shortening, slices of prerigor tilapia muscle were dipped for 20 min in a 4°C lactate buffer, pH 6.3, which is the approximate ultimate pH resulting from postmortem aging. Muscle lengths before and after dipping were compared, and their MTRM thermograms were measured and compared. At least 3 repeats were performed to get average for each dipping treatment.

pH

Muscle pH was measured according to the method of Korhonen and others (1990). Five grams of dorsal muscle were removed from each fish, and fat and dark muscle was removed. The muscle was mixed with 45 mL distilled water and homogenized for 1 min with a Polytron PT-2000 homogenizer (Brinkman Instruments, Westbury, N.Y., U.S.A.) at 7000 rpm. pH of the mixture was read using an Orion SA-720 pH meter (Analytical Instrument Recycle Inc., Golden, Colo., U.S.A.). The changes of muscle pH, ATP, along with S10°C were adjusted to 6.5 with 1 N KOH, and the final volume was adjusted to 50 mL with 5% (w/w) perchloric acid (PCA). After homogenization, the supernatants from these washings were collected, and the pH was adjusted to 6.5 with 1 N KOH, and the final volume was adjusted to 50 mL with 5% (w/w) PCA. ATP was analyzed with high-performance liquid chromatography (HPLC) (Hewlett Packard, HP-1084, Columbus, Ohio, U.S.A.) with a reversed phase column (Zorbax-ODS column, Du Pont Instrument, Wilmington, Del., U.S.A.) by gradient elution (solution A: 1% triethyleneamine, pH 6.7; solution B: 20% methanol + solution A; gradient setting: 100/0 to 50/50 at 18 min) at a flow rate of 0.8 mL/min, 1.28 absorption unit full scale. Eluents were monitored at 254 nm. The ATP externally quantified using standard obtained from Sigma Chemical Co. ATP content was expressed as µmol/g meat (wet weight). Correlations of ATP samples for analysis were mentioned in pH measurements.

Results and Discussions

Isothermal MTRM and RIfiber

Changes in postmortem muscle shortening and fiber rigor index development during isothermal aging at 10 °C (Figure 2a) indicated that freshwater tilapia muscle started to shorten from the Spreigor value of 0% to the maximum Sfull-rigor value of approximately 10%. Taking the full rigor shortening as 100%, the progressive rigor development was quantitatively calculated and defined as RIfiber, according to Eq. 1:

\[ R_{ifiber} = \left( \frac{S_{sample}}{S_{full-rigor}} \right) \times 100\% = \left( \frac{S_{sample}}{100\%} \right) \times 100\% \] (1)

Changes RI fiber of tilapia muscle while holding at 10 °C can be divided into 3 different stages: (1) prerigor stage (0 to 1 h) where no significant shortening occurred and RI fiber remained approximately 0%, (2) in-rigor stage (1 to 16 h) where muscle shortening went from RI fiber of 0% to 100%, and (3) full-rigor stage (beyond 16 h) where RI fiber remained at 100%, and essentially no further muscle shortening nor relaxation occurred for an extended period of time. No attempt was made to observe the occurrence of post-rigor or resolution of rigor.

Other physicochemical changes associated with isothermal rigor development

Rbody is one of the simple and widely accepted methods to observe rigor mortis development in terms of apparent body stiffness, especially for small and medium size fish. Figure 2b shows that the isothermal development of Rbody measured at 10 °C increased from 0% to 100%, that is, inversely proportional to RIfiber, so that same 3 stages of rigor mortis development could be observed. The result indicated the feasibility of using RIfiber as an additional rigor mortis index.

Figure 2c and 2d show the changes in ATP content and pH during 10 °C isothermal postmortem rigor development of tilapia muscle. These are the 2 parameters that have been commonly used to monitor development of rigor induced by 2 different postmortem rigor-inducing physicochemical pathways. Muscle ATP decreased from its prerigor concentration of approximately 4.0 µmol/g meat to a minimum of 0.1 to approximately 0.2 µmol/g meat after 16 h postmortem. No further depletion of ATP occurred during aging to 24 h postmortem. Immediately after slaughter, tilapia muscle pH ranged between 7.0 and 7.2 but dropped to the ultimate pH, about 6.4, 14 h postmortem.

By comparing the changes of apparent rigor indices, RIfiber and Rbody to those of the physicochemical rigor indices, pH, and ATP contents, it was evident that the development of RIfiber and Rbody were more closely related to the changes in ATP concentration during the 1st 16 h of postmortem aging. However, the postmortem pH-fall was not severe and not in good correlation to the changes of other rigor indices, declining from about 7.0 to its ultimate value of 6.4 in 14 h. It was suggested by studying a vast amount of meat that only if the muscle pH-fall dropped to below 6.0, approaching an approximate muscle protein pl value, would isoelectric coagulation or shortening be effectively induced (Hultin 1984; Oka and others 1990). This further excluded the pH-fall to be an important role in effecting the postmortem shortening of tilapia. Thus, it can be concluded that muscle shortening caused by actomyosin complex formation due to enzymatic depletion of ATP is the major physiochemical pathway directly leading to rigor mortis of fish muscle.

Heat-Induced muscle shortening monitored by dynamic heating MTRM

When muscle samples that had been held at 10 °C for different
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periods of time to develop different postmortem shortening values of $S_{10^\circ C}$ and $R_{I_{fiber}}$ were subjected to dynamic heating from 10 to 50 °C (a to h in Figure 3a), the overall muscle shortenings, $S_{overall}$, obtained by the two-stage treatments in MTRM were the same, that is: $S_{overall} = S_{10^\circ C} + S_{dynamic} = 10\%$ regardless of the differences in muscle shortening at 10 °C. $S_{overall}$ with heating is the same as the full-rigor shortening at 10 °C. Thus, it could be concluded from the MTRM results that heat-induced rapid muscle shortenings were due to rigor mortis development.

Figure 3b is the derivative thermal rigorometry (DMTRM) diagram obtained by taking the 1st-order derivative of each curve in the MTRM diagrams shown in Figure 3a. The activation temperatures of shortening, $T_s$, can thus be accurately determined. The $T_s$ values decreased from 47.8 °C to 30.4 °C, as rigor mortis developed from prerigor to the very near full-rigor prior to dynamic heating. In other words, the longer the muscle was aged at 10 °C, the less thermally stable the muscle became. Among them, no muscle shortening of dynamic heating could be detected for the true full rigor sample.

Figure 4 demonstrates an excellent linear correlation of $T_s$ observed by MTRM in dynamic heating mode with $R_{I_{fiber}}$ values of muscle samples measured before heating. The results reflected that these values could be used to establish standard curves to evaluate tilapia dorsal muscle using MTRM dynamic heating thermograms. However, caution should be observed if muscle samples were subjected to heating near 50 °C for an extended period of time in MTRM, because shortening due to heat denaturation may occur.
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This temperature is too close to about 53 °C, at which myofibrillar protein denaturation of tilapia muscle occurs (Poulter and others 1985; Park and Lanier 1988).

Effect of ATP and Ca²⁺ on heat-induced muscle shortening

To further understand the nature of heat-induced rigor shortening observed during dynamic heating in the MTRM, the effects of pH adjustment of prerigor muscle and of ATP and/or Ca²⁺ addition to prerigor muscle were studied. Prerigor muscle samples that had been previously immersed in various concentrations of CaCl₂ solutions were subjected to dynamic MTRM heating, and the resultant changes in sample length are reported as percent muscle shortening (Figure 5a). The results indicated that Ca²⁺ enhanced the development of muscle rigor mortis. Among the treatments, dipping in 0.5% CaCl₂ only induced partial rigor of muscle, whereas 1% CaCl₂ resulted in full rigor. Figure 5b shows the MTRM dynamic heating thermograms of prerigor muscle slices treated with various levels of CaCl₂ followed with treatment in 0.5% or 3% ATP. Significant partial recovery of RIfiber was observed by the treatment with 3% ATP for both partial and full-rigor muscles whose rigor was induced by the addition of Ca²⁺. However, no significant muscle shortening was observed by dipping prerigor tilapia dorsal muscle in a 4 °C lactate buffer, pH 6.3, for 20 min to facilitate the muscle reaching its minimum postmortem pH value while ATP still remained at a higher level. These results reflected that muscle shortening that occurs in the low temperature range of 30 to 50 °C during early stage of cooking is caused by heat-induced rapid rigor mortis through the muscle-ATPase-related enzyme system rather than an isoelectric coagulation caused by the pH decrease in the muscle.

Conclusions

The MTRM has been shown to be an effective instrument for monitoring muscle shortening in freshwater tilapia between 10 and 50 °C either in an isothermal or dynamic heating mode. In the isothermal mode, different stages of rigor mortis development and corresponding muscle shortenings could be directly measured by MTRM. In the dynamic mode, the temperature of the maximum rate of heat-induced rapid muscle shortening, Ts, could be calculated. Because the linear relationship is established between Ts and RIfiber as shown in Figure 4, it is evident that the Ts value measured by the dynamic MTRM can be used as a new indices along with the traditional indices (S isothermal, RIfiber, RIbody) measured by the isothermal mode to identify the extent of rigor mortis in equal effectiveness. The results of this study reflected that the muscle shortening and dehydration of low temperature cooking, up to about 50 °C, of tilapia muscle was heat-induced rapid rigor mortis in nature, instead of protein denaturation. They further give the illustration the nature of the conventional Japan 49 °C hot-water “aria” treatment of fresh sashimi to improve the texture quality.

It was found that undesirable preslaughter conditions, such as starvation, stress, and so on, should be prevented to assure no complication on the experiment results. Furthermore, it is also critical in muscle-related studies to have well-defined sample preparation and aging procedures to ensure reproducible results. Currently, automation of MTRM using a computerized system as well as application of MTRM to monitor muscle changes with high temperature cooking are in progress.

Acknowledgments

The authors wish to express their sincere appreciation to Northern Taiwan Inst. of Science and Technology (NTIST) and Natl. Taiwan Ocean Univ. (NTOU), Taiwan, for their equipment and support of this study. Special thanks to Professor Haejung An, Seafood Lab., Oregon State Univ., for her kind encouragement and help on the preparation of this paper.

Figure 4—Comparison of Ts measured by MTRM in dynamic mode with the RIfiber of tilapia dorsal muscle sample determined prior to MTRM heating

Figure 5—MTRM thermograms of prerigor tilapia dorsal muscle with dynamic heating mode at the heating rate of 3 °C/min. The muscle samples have been treated by (a) dipping in 0.2 M lactate buffer, pH 6.9, containing 0% to 1% (w/w) CaCl₂ or (b) dipping in the above-mentioned lactate buffer containing CaCl₂ followed by subsequent dipping in 0.2 M lactate buffer, pH 6.9, containing 0.5% to approximately 3% (w/w) ATP.
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References