

## DSC Analysis of Heat-induced Changes of Thermal Characteristics for Perimysium and Endomysium Collagen from Beef *semitendinosus* Muscle

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**Abstract:** The main objective of this study was to investigate the heat-induced changes of thermal characteristics for perimysium and endomysium collagen from beef *semitendinosus* muscle. Muscle samples were heated to an internal core temperature of 20, 40, 50, 60, 70, 80 °C and 90 °C in water bath and in microwave oven respectively. The changes of thermal shrinkage temperatures ( $T_o$ : onset temperature;  $T_p$ : peak temperature;  $T_e$ : end temperature) of perimysium and endomysium collagen for beef *semitendinosus* muscle during heating were analyzed by Differential Scanning Calorimeter (DSC). The results indicated that the thermal shrinkage temperatures ( $T_o$ ,  $T_p$  and  $T_e$ ) of perimysium and endomysium collagen both showed significant differences at different internal core temperatures during water-bath and microwave heating. And an internal core temperature of 60 °C was the critical heating temperature which affects thermal shrinkage temperatures of perimysium and endomysium collagen for both water-bath and microwave heated meat. The significant differences in thermal shrinkage temperatures between water bath and microwave heated beef muscle samples were attributed to the heat-induced changes in thermal characteristics of perimysium and endomysium collagen.

**Key words:** beef *semitendinosus* muscle; perimysium and endomysium collagen; thermal shrinkage temperatures; heat-induced changes; Differential Scanning Calorimetry(DSC)

## 来自牛半腱肌肌束膜和肌内膜胶原蛋白热力学特性分析

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**摘 要:** 探讨牛半腱肌肌束膜和肌内膜胶原蛋白热力学特性的热诱导变化。牛半腱肌分别采用水浴和微波加热到内部终点温度分别为 20、40、50、60、70、80 °C 和 90 °C, 用示差扫描量热法研究肌束膜和肌内膜胶原蛋白热力学特性(起始、最高和最终热收缩温度)在热处理过程中的变化。结果表明: 牛半腱肌肌束膜和肌内膜胶原蛋白的热收缩温度在两种热处理方式间都存在显著差异, 在两种热处理方式中, 内部终点温度 60 °C 是影响肌束膜和肌内膜胶原蛋白热收缩温度的关键加热温度。热诱导的肌束膜和肌内膜胶原蛋白热力学特性的变化是水浴和微波加热牛肉胶原蛋白热收缩温度存在差异的主要原因。

**关键词:** 牛半腱肌; 肌束膜和肌内膜胶原蛋白; 热收缩温度; 热诱导变化; 示差扫描量热

中图分类号: TS251.5

文献标识码: A

文章编号: 1002-6630(2012)07-0118-05

Changes of meat tenderness and texture during heating are partly resulted from the changes of collagen characteristics, including collagen contents and solubility

and thermal stability (thermal characteristics)<sup>[1]</sup>. Meat collagen characteristics, especially collagen contents and solubility, have been analyzed to obtain information on beef

收稿日期: 2011-04-12

基金项目: 国家自然科学基金项目(31101313); 重庆市教委科学技术研究项目(KJ110714);

重庆工商大学博士科研启动基金项目(2010-56-12); 重庆高校创新团队建设计划项目(KJTD201020)

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tenderness<sup>[2]</sup>. In addition, the thermal stability of connective tissue has been analyzed by measuring the onset ( $T_o$ ) and peak ( $T_p$ ) temperatures and enthalpy ( $\Delta H$ ) of thermal shrinkage of intramuscular connective tissue (IMCT) in order to figure out the role of connective tissue in meat tenderness<sup>[3]</sup>. The perimysial and endomysial collagen are the main components of IMCT, which play an important role in binding the myofibers into fasciculus, and finally skeletal muscle. Those two components prevent disorganization and propagating contraction power of the myofibers to the bone level during activity<sup>[4]</sup>.

Chang Haijun et al.<sup>[5]</sup> investigated the effects of thermal treatment with different modes (water bath and microwave) and internal core temperature (from 40 °C to 90 °C) on meat quality of beef *semitendinosus* muscle by examining the change of connective tissue collagen (mainly histological structure). However, few literatures have been reported on the comparative study of thermal characteristics (thermal stability) changes of perimysium and endomysium collagen for Chinese yellow bulls treated by water-bath and microwave heating. Therefore, the objective of the study was to compare the changes of thermal shrinkage temperatures of those two kinds of collagen in beef *semitendinosus* muscle by water-bath and microwave heat treatments.

## 1 Materials and Methods

### 1.1 Materials

Beef *semitendinosus* samples were collected from 6 Chinese yellow bull (Simmental  $\times$  Nanyang crossbreed) (age: 24–30 months; live weight:  $(500 \pm 30)$  kg) carcasses slaughtered humanely in a commercial meat processing company (Luqi Meat Co. Ltd., Henan, China) by the Halal method within 48 h postmortem, during which the carcasses were hung by Achilles tendon in a 4 °C chiller. The visible subcutaneous fat and epimysial connective tissue were trimmed off and sliced into 2.54 cm thick cubes, perpendicular to the direction of the fiber. The samples were prepared in triplicate.

### 1.2 Apparatus

Water bath (HH-42, Guohua, Changzhou, China); Microwave oven (600 W, 2450 MHz) (EM-2008MS1, Shanghai, China); Digital needle-tipped thermometer (HI145, HANNA Instruments, Italy); Waring-basicblender (Ultra-Turrax T25, IKA-WERKE, Germany); Freeze-dryer (Alpha 2-1.2, Christ, Germany); DSC (Pyris 1, Perkin Elmer instruments, USA);

Pyris Manager Series (Pyris 1, Perkin Elmer, USA); Electronic Balance (AUY120, Shimadzu, Japan).

### 1.3 Heat treatment

Beef *semitendinosus* muscle steaks ( $2.5 \text{ cm} \times 5.0 \text{ cm} \times 5.0 \text{ cm}$ ) were packed in polypropylene bags, sealed and heated in a 95 °C water bath to the internal core temperatures of 40, 50, 60, 70, 80 °C and 90 °C, respectively. A laboratory microwave oven was operated at 2450 MHz and 250 W output power (the maximum output is 600 W). The  $30 \text{ cm} \times 20 \text{ cm} \times 25 \text{ cm}$  (width  $\times$  height  $\times$  depth) oven cavity houses a  $20 \text{ cm} \times 1.5 \text{ cm}$  (diameter  $\times$  height) turntable that rotates at 15 r/min. Meat steaks were heated in the above microwave oven to the same internal core temperatures as those resulted from water bath heating. Internal core temperature was measured using a digital needle-tipped thermometer equipped with a temperature probe inserted into the geometric center of meat steaks. Temperature changes were monitored constantly until the designed internal temperature was reached; after heat treatment, the steaks were chilled with cold running water to about room temperature (20 °C). Raw meat (unheated) kept at about 20 °C was used as the control samples (expressed as 20 °C in the following figures).

The experiment was in randomized block design, by which possible experimental errors due to non-uniform temperature transfer in heating could be averaged out over treatments<sup>[5]</sup>.

### 1.4 Perimysial and endomysial collagen preparation

The perimysial and endomysial portions of raw and heated meat samples were prepared and extracted according to the procedures of Light et al.<sup>[6]</sup> modified by Li Chunbao et al.<sup>[7]</sup>. Briefly, each meat sample (30 g wet weight) was cut into 1 cm cubes and homogenized in ice-cold 50 mmol/L  $\text{CaCl}_2$  for 30 s at full speed (about 5000 r/min) in a Waring Blendor. The homogenate was filtered through a layer of nylon net (1 mm<sup>2</sup> perforations) and the retentate on the filter was rehomogenized in 50 mmol/L  $\text{CaCl}_2$  and re-filtered. The process was repeated twice and then the filtrates, containing endomysium, was collected. The retentate on the filter was mainly the perimysium. The perimysium was washed three times in 1 g/100 mL sodium dodecyl sulphate (SDS) for 30 min at room temperature and SDS was removed by dialysis at 4 °C against distilled water (24 h), 40% methanol (24 h) and then distilled water (24 h).

### 1.5 DSC analysis

DSC analysis of perimysium and endomysium were

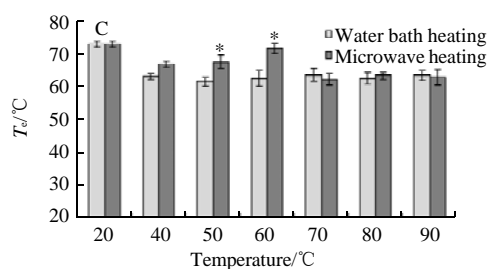
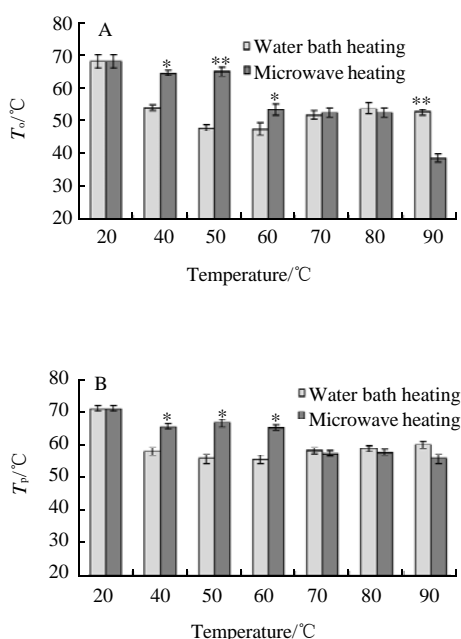
conducted as described by Chang Haijun et al.<sup>[8]</sup> with slight modifications. The purified perimysial and endomysial portions were concentrated by freeze-drying, and then the thermal shrinkage temperatures of perimysial and endomysial collagen were measured using DSC. The samples (10 mg) were accurately weighed in aluminum pans and hermetically sealed. The samples were heated from 20 °C to 100 °C at heating rate of 10 °C/min, and nitrogen was used as purge gas at flowing rate of 20 mL/min. An empty sample pan was used as the reference. Thermal shrinkage temperatures ( $T_o$ : onset temperature;  $T_p$ : peak temperature;  $T_e$ : end temperature) of perimysium and endomysium collagen were estimated from the thermogram using the software of Pyris Manager Series.

### 1.6 Statistical analysis

Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) 16.0 (SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) and Duncan's multiple-range test were carried out to determine significant differences in thermal shrinkage temperatures ( $T_o$ ,  $T_p$  and  $T_e$ ) of perimysial and endomysial collagen between water bath and microwave heated meat samples, and the effects were considered significant at  $P < 0.05$  (\*) and  $P < 0.01$  (\*\*). Error bars indicate  $\bar{x} \pm s$  of three replicates.

## 2 Results and Discussion

### 2.1 Thermal characteristics of perimysium collagen

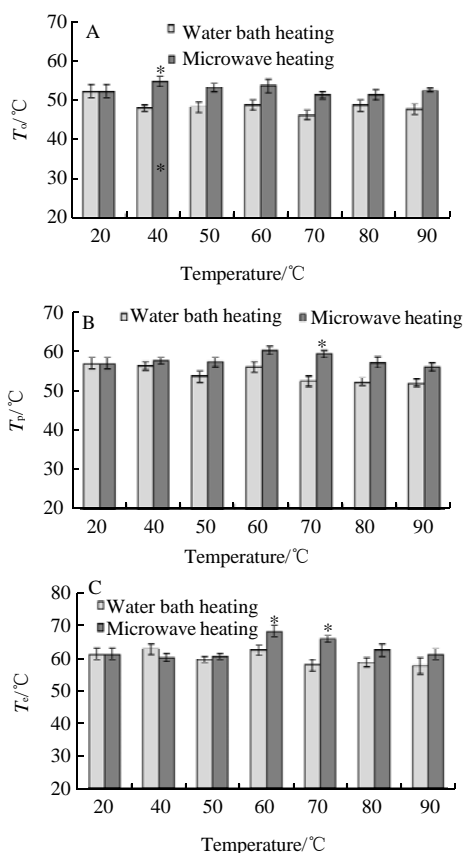


\*.Indicates the effects were considered significant at  $P < 0.05$ ; \*\*.Indicates the effects were considered significant at  $P < 0.01$ , between water bath and microwave heated meat samples. The same as follows.

**Fig.1 Change in thermal shrinkage temperatures of perimysium collagen of beef *semitendinosus* muscle during water-bath and microwave heating**

Thermal shrinkage temperatures, including onset ( $T_o$ ), peak ( $T_p$ ) and end ( $T_e$ ) temperature of perimysium collagen were shown in Fig. 1A, B and C, respectively. Significant differences of  $T_o$  between water bath and microwave heated meat were found at 40, 60 °C ( $P < 0.05$ ) and 50, 90 °C ( $P < 0.01$ ), respectively.  $T_p$  showed significant differences at 40, 50 °C and 60 °C ( $P < 0.05$ ). And significant differences of  $T_e$  were found at 50 °C and 60 °C ( $P < 0.05$ ).

### 2.2 Thermal characteristics of endomysium collagen



**Fig.2 Change in thermal shrinkage temperatures of endomysium collagen of beef *semitendinosus* muscle during water-bath and microwave heating**

As shown in Fig. 2,  $T_o$ ,  $T_p$  and  $T_e$  of endomysium collagen exhibited significant differences between water-bath and microwave heating at 40 °C (Fig. 2A), 70 °C (Fig. 2B), 60 °C and 70 °C (Fig. 2C) ( $P < 0.05$ ), respectively. According to previous studies<sup>[1,5]</sup>, the internal core temperatures from 40 °C to 60 °C were critical heating temperatures which affect the thermal shrinkage temperatures and mechanical strength of connective tissue collagen for both water bath and microwave heated meat samples. And the perimysial and endomysial collagen were separated purposefully from the connective tissue for thermal characteristic research in this study. Because the water-bath heating is a non-uniform heat transfer treatment and the microwave treatment is operated in short time, the extent of the denaturation of myofibrillar proteins and collagen by these two treatments is different. Therefore, the thermal characteristics of perimysial and endomysial collagen by water bath and microwave heating exhibited differences<sup>[5]</sup>. Based on those viewpoints, in this study, it is concluded that internal endpoint temperature of 60 °C was critical heating temperature which affects thermal shrinkage temperatures of perimysial and endomysial collagen in both water-bath and microwave heated meat.

Thermal treatment is an important step for the conversion of inedible (raw) meats to edible meats, and has an obvious effect on the eating and sensory quality of meats, especially on the meat tenderness and texture<sup>[2]</sup>. The effects of thermal treatment on the proteins lead to drastic change of meat texture, including denaturation and dissociation of myofibrillar proteins, transversal and longitudinal shrinkage of meat fibers, aggregation and gel formation of sarcoplasmic proteins and solubilization of connective tissue collagen<sup>[9-11]</sup>. Owing to the thermal effects on the thermal characteristics (thermal stability) of connective tissue collagen (perimysial and endomysial collagen), the texture of heated meat was partly related to the characteristics changes of connective tissue and collagen. Because of the differences in heating modes in the study, and the changes of perimysial and endomysial components (manifested as denaturation, solubilization, gelation, aggregation, and the heterogeneous ultrastructure appearance), thermal characteristics of these collagen at different internal core temperature during water bath and microwave heating were various.

According to Bailey et al.<sup>[12]</sup> study,  $T_p$  of collagen from mammals is around 65 °C, but it is various for different muscles and animal species. They reported that  $T_o$  is considered to describe the least stable collagen and the  $T_p$  is a measure of

the average stability of collagen during heating, and the temperature of thermal shrinkage ( $T_s$ ) and the temperature of thermal melting ( $T_m$ ) are corresponding terms for  $T_p$ . Based on this standpoint, the  $T_p$  values of perimysial collagen decreased during heating compared with the raw meat; this was probably resulted from the weakening of the average stability of collagen because of the heat induced gelation and denaturation.

Kijowski et al.<sup>[13]</sup> reported a  $T_p$  of 65.3 °C (heating rate 10 °C/min) in isolated IMCT of chickens and Aktas et al.<sup>[14]</sup> found a  $T_p$  of 69.2 °C (heating rate 5 °C/min) in that of old cows. Voutila et al.<sup>[15]</sup> reported that  $T_o$  and  $T_p$  of connective tissue from porcine *M. semimembranosus* were around 60 °C and 65 °C, respectively (heating rate 5 °C/min). Although the DSC samples were not pure collagen in this study, the thermal shrinkage temperatures of collagen were similar to that reported on whole meat. Nevertheless, minor difference also existed because of the different muscles, animal species and collagen kinds.

### 3 Conclusion

According to these results, the thermal stabilities (expressed as thermal shrinkage temperatures) of connective tissues collagen in beef *semitendinosus* muscle changed during water-bath and microwave heating. Significant differences of  $T_o$ ,  $T_p$  and  $T_e$  for perimysium and endomysium collagen were observed at different internal core temperature during heating. Due to the thermal exposure, perimysium and endomysium collagenous tissues denatured and melted, and the internal core temperature of 60 °C was critical heating temperature which affects thermal shrinkage temperatures of perimysium and endomysium collagen for both water bath and microwave heated meat. Based on those viewpoints, it is concluded that the significant differences in thermal shrinkage temperatures between water bath and microwave heated beef muscle samples were attributed to the heat-induced changes in thermal characteristics of perimysium and endomysium collagen.

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