

## DSC Analysis of Heat-induced Changes in Thermal Characteristics of Connective Tissue 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 of connective tissue collagen from beef *Semitendinosus* muscle. Muscle samples were heated to an internal core temperature of 40, 50, 60, 70, 80 or 90 °C in a water bath and in a microwave oven respectively. The changes of filter residues content and thermal characteristics of connective tissue collagen were examined with internal core temperature. The results indicated that filter residues were increased during heating, and presented significant differences ( $P < 0.05$ ) at the internal core temperatures of 60, 70 °C and 80 °C between water bath and microwave heated meat samples. Internal core temperatures ranging from 40 to 60 °C were critical heating temperatures which affect thermal shrinkage temperatures of connective tissue collagen for both water bath and microwave heated meat samples. 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 connective tissue collagen.

**Key words:** beef *Semitendinosus* muscle; collagen; thermal shrinkage temperatures; heat-induced changes; differential scanning calorimetry (DSC)

## 牛半腱肌肉结缔组织胶原蛋白热力特性热诱导变化 DSC 分析

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**摘 要:** 探讨牛半腱肌肉结缔组织胶原蛋白热力特性的热诱导变化。牛半腱肌肉分别采用水浴和微波加热到内部终点温度分别为 40、50、60、70、80 °C 和 90 °C, 研究结缔组织滤渣和热力特性在热处理过程中的变化。结果表明: 结缔组织滤渣含量随着热处理温度的升高而增加, 当加热温度分别为 60、70 °C 和 80 °C 时, 结缔组织滤渣含量在两种热处理方式间存在显著差异( $P < 0.05$ )。在两种热处理方式中, 40 °C 至 60 °C 的内部终点温度是影响结缔组织胶原蛋白热收缩温度的关键加热温度。热诱导的结缔组织胶原蛋白热力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:** 牛半腱肌肉; 胶原蛋白; 热收缩温度; 热诱导变化; 差示扫描量热

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Heating (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>[1-2]</sup>. The texture

of meat is one of the most important quality properties, which has been studied for many years in different aspects. The effects of heating on the meat texture mainly resulting from the denaturation and dissociation of myofibrillar proteins, trans-

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versal and longitudinal shrinkage of meat fibers, aggregation and gel formation of sarcoplasmic proteins and solubilization of connective tissue collagen<sup>[3-5]</sup>. The texture of heated meat was related to the characteristics changes of connective tissue and collagen to a certain extent due to the thermal effects. On the other hand, it was also depended on the heating methods, heating temperature and time mostly.

Changes of meat tenderness and texture during heating are partly resulted from the changes of collagen characteristics, including collagen contents, solubility and thermal stability (thermal characteristics)<sup>[6]</sup>. Meat collagen characteristics have been analyzed to obtain information on meat tenderness, especially for collagen contents and solubility. In addition to, 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) when the role of connective tissue in meat tenderness has been studied<sup>[7]</sup>. Chang et al<sup>[2]</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 from the view point of connective tissue collagen (mainly histological structure). However, few literatures have been reported on the comparative study of heat-induced thermal characteristics changes (thermal shrinkage temperatures) of connective tissue collagen for Chinese yellow bulls during water bath and microwave heating.

In view of this, the present study was carried out to examine the changes of thermal shrinkage temperatures of connective tissue collagen in beef *Semitendinosus* muscle from Chinese yellow bulls during water bath and microwave heating.

## 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 (LvQi Meat Co. Ltd., Henan, China) by the Halal method within 48 h postmortem, during which time 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 Instruments and apparatus

Water bath (HH-42, Guohua, Changzhou, China); Microwave oven (600W, 2450MHz) (EM-2008MS1, Sanyang, Shanghai, China); Digital needle-tipped thermometer (HI145, HANNA Instruments, Italy); Waring-blendor (Ultra-Turrax T25 basic blender, IKA-WERKE, Germany); Freeze-dryer (Alpha 2-1.2, Christ, Germany); Multi cell Differential Scanning Calorimeter (MC-DSC)(TA instrument, USA); Electronic Balance (AUY120, Shimadzu, Japan).

### 1.3 Heating treatment

Beef *Semitendinosus* muscle steaks (2.5 cm  $\times$  5.0 cm, 5.0 cm) were packed in polypropylene bags, sealed and heated in 95 °C water bath to the internal core temperatures of 40, 50, 60, 70, 80 °C and 90 °C respectively. A laboratory microwave oven operating at 2450 MHz and 250 W output power (the maximum output is 600 W) was used. The 30 cm  $\times$  20 cm  $\times$  25 cm (width  $\times$  height  $\times$  depth) oven cavity houses a 20 cm  $\times$  1.5 cm (diameter  $\times$  height) turntable that rotates at 15 r/min. Meat steaks were heated in an above microwave oven to the same internal core temperatures as those resulting 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 the completion of heating treatment, the steaks were then 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.

The experimental design was a randomized block type with possible differences in results among the differences for non-uniform temperature transfer in heating was averaged out over treatments<sup>[2]</sup>.

### 1.4 Connective tissue collagen (connective tissue filtering residues) preparation

Filtering residues were prepared and determined as described by Li et al<sup>[8]</sup> and Chang et al<sup>[9]</sup> with some modifications. 50 g wet weight of raw and heated meat samples was cut into 0.5 cm<sup>3</sup> cubes and was homogenized in 50 mL of ice cold CaCl<sub>2</sub> (50 mmol/L) for 30 s at 3000 r/min a Waring-Blendor. The homogenate was filtered through a layer of nylon net (1 mm<sup>2</sup> perforations) and the material retained on the filter was re-homogenized in 50 mL of CaCl<sub>2</sub> and re-filtered. The process was repeated for three times. The material retained on the filter, designated as filtering residues, was freeze-dried till a constant weight was reached. The contents

of filtering residues were calculated as a percentage of the initial wet sample weight. Filtering residues were mainly the composition of perimysia and endomysia connective tissue portions, and a spot of myofibrillar portions<sup>[8]</sup>.

### 1.5 DSC analysis

DSC analysis of connective tissue collagen was conducted as described by Chang et al<sup>[10]</sup> with slight modifications. Thermal shrinkage temperatures of connective tissue collagen were measured using DSC. The samples (10 mg) were accurately weighed into aluminum pans and hermetically sealed. The samples were heated from 10 °C to 100 °C at heating rate of 2 °C/min. An empty sample pan was used as the reference. The thermal shrinkage temperatures ( $T_o$ : onset temperature;  $T_p$ : peak temperature;  $T_e$ : end temperature) of connective tissue collagen were estimated from the thermogram using the software of DSC Manager Series (TA instrument, USA).

### 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 filtering residues contents and thermal shrinkage temperatures ( $T_o$ ,  $T_p$  and  $T_e$ ) 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 mean  $\pm$  SD of three replicates.

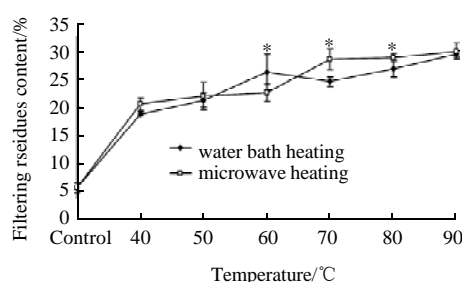
## 2 Results and Analysis

### 2.1 Filtering residues contents of connective tissue

Filtering residues contents of water bath or microwave heated beef *Semitendinosus* muscle samples versus different internal core temperatures are shown in Fig.1. Heating treatment had significant effects ( $P < 0.01$ ) on filtering residues. Generally, the filtering residues contents of water bath and microwave heated samples had a tendency to increase with increasing internal core temperature. The results were consistent with those reported by Li et al<sup>[11]</sup>. Compared with the control samples, filtering residues were increased during heating, and presented the significant differences ( $P < 0.05$ ) at internal core temperature of 60, 70 °C and 80 °C between water bath and microwave heated meat samples.

Filtering residues were mainly the mixed composition of perimysium and myofibrillar portions and not purely the connective tissue portions. Attempts have been made to

separate the perimysium from both raw and heated meat samples, but it was impossible to separate perimysium completely from muscle fiber. This was because that the mixed myofibrillar and connective tissue proteins make it more difficult to homogenate and separate perimysia protein (connective tissue collagen) from the mixed components perfectly. Therefore, this part was targeted to explore the relationship between filtering residues contents and other thermal characteristics of connective tissue collagen during heating treatment (Table 1).



\*.Effects were considered significant at  $P < 0.05$ , between water bath and microwave heated meat samples. The follows are same.%, percentage of the initial sample wet weight.

Fig.1 Change in filtering residues contents of water bath and microwave heated beef *Semitendinosus* muscle samples with internal core temperature

### 2.2 Thermal characteristics of connective tissue collagen

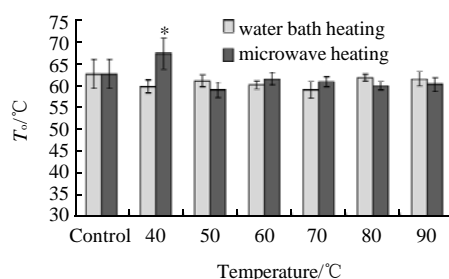
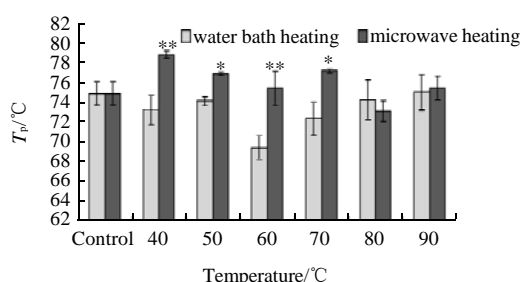


Fig.2 Change in onset temperature ( $T_o$ ) of connective tissue collagen from water bath and microwave heated beef *Semitendinosus* muscle samples with internal core temperature

The thermal characteristics of connective tissue collagen analyzed by DSC during heating treatment are depicted in Fig.2, 3 and 4. As can be seen from the figures, onset thermal shrinkage temperature ( $T_o$ ) of connective tissue collagen (filtering residues) were manifested significant differences at an internal core temperature of 40 °C between water bath and microwave heated meat samples (Fig.2), and there were no large differences for other heating temperature during those heating methods. On the whole, there were fewer

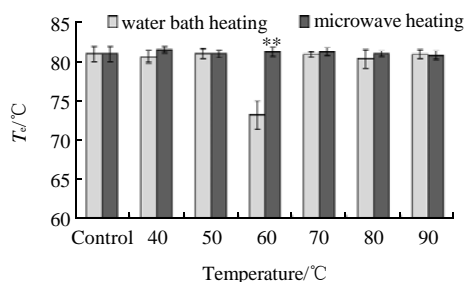
changes in the  $T_o$  of microwave heated beef meat samples compared with water bath heated meat samples to an identical internal core temperature.

For the peak thermal shrinkage temperature ( $T_p$ ) changes of connective tissue collagen,  $T_p$  of microwave heated meat samples were higher than water bath heated samples at internal core temperatures of 40 °C ( $P < 0.01$ ), 50 °C ( $P < 0.05$ ), 60 °C ( $P < 0.01$ ) and 70 °C ( $P < 0.05$ ) (Fig. 3). Meanwhile, the end thermal shrinkage temperature ( $T_e$ ) of microwave heated meat samples was significant higher than water bath heated samples at 60 °C ( $P < 0.01$ ) (Fig. 4).



\*\*.Effects were considered significant at  $P < 0.01$ , between water bath and microwave heated meat samples. The follows are same.

**Fig.3** Change in peak temperature ( $T_p$ ) of connective tissue collagen from water bath and microwave heated beef *Semitendinosus* muscle samples with internal core temperature



**Fig.4** Change in end temperature ( $T_e$ ) of connective tissue collagen from water bath and microwave heated beef *Semitendinosus* muscle samples with internal core temperature

From the thermal shrinkage temperature changes of connective tissue collagen, we can get conclusions that the internal core temperatures from 40 °C to 60 °C were critical heating temperatures which affect thermal shrinkage temperatures of connective tissue collagen for both water bath and microwave heated meat samples.

According to Bailey et al reports [12],  $T_p$  of collagen from mammals is around 65 °C but it is different 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. Kijowski et al [13] reported  $T_p$  of 65.3 °C (heating rate 10 °C/min) in isolated connective tissue collagen of chickens, Aktas et al [14] found  $T_p$  of 69.2 °C (heating rate 5 °C/min) in that of old cows. Voutila et al [15] 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 our study, the thermal shrinkage temperatures of collagen were similar to that reported on whole meat, nevertheless, minor difference were also existed because of the different muscles, animal species and collagen kinds for testing.

Due to the thermal exposure, collagenous tissues denatured and melted. Chang et al [10] reported that an internal core temperature of 60 °C was a critical heating temperature which affected thermal shrinkage temperatures of perimysium and endomysium in water bath or microwave heated beef meat. Another report indicated that internal core temperatures of 60 °C and 65 °C both had a critical effect on meat textural properties owing to the thermal denaturation of collagen in water bath or microwave heated beef meat [16]. On the other hand, because there were great differences in heat transmission and heating time both for water bath and microwave heating treatment, the distinctive characteristics for microwave thermal treatment were the non-uniform heating and short heating time, therefore, there were some differences for thermal characteristics of connective tissue collagen between heated meats by those two methods in this study.

Based on those viewpoints, the authors 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 connective collagen.

### 2.3 Correlation analysis

**Table 1** Correlation analysis between thermal characteristics changes of connective tissue collagen during water bath and microwave heating ( $n=21$ )

Item	water bath heating				Item	microwave heating			
	FR	$T_o$	$T_p$	$T_e$		FR	$T_o$	$T_p$	$T_e$
FR	1	0.395	0.708*	0.684	FR	1	0.608	0.798*	0.506
$T_o$		1	0.757*	0.384	$T_o$		1	0.892**	0.650
$T_p$			1	0.799*	$T_p$			1	0.901**
$T_e$				1	$T_e$				1

Note: FR, Filtering residues contents;  $T_o$ , Onset thermal shrinkage temperature of connective tissue collagen (filtering residues);  $T_p$ , Peak thermal shrinkage temperature;  $T_e$ , End thermal shrinkage temperature; \*, Correlation is significant at  $P < 0.05$ ; \*\*, Correlation is significant at  $P < 0.01$ .

Correlation coefficients among the traits of filtering residues contents and thermal characteristics ( $T_o$ ,  $T_p$  and  $T_e$ ) were listed in Table 1. All coefficients were correlated positively each other, filtering residues contents were correlated positively ( $P < 0.05$ ) with  $T_p$  for water bath and microwave heated meat samples;  $T_o$  value correlated with  $T_p$ , and  $T_p$  value correlated with  $T_e$ .

### 3 Conclusions

As stated above, heating treatment had significant effects on filtering residues contents. Generally, the filtering residues contents of water bath and microwave heated samples had a tendency to increase with increasing internal core temperature. Due to the thermal exposure, collagenous tissues denatured and melted, the internal core temperatures from 40 °C to 60 °C were critical heating temperatures which affect thermal shrinkage temperatures of connective tissue collagen for both water bath and microwave heated meat samples. Based on those viewpoints, we can get the conclusion 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 connective tissue collagen.

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