Influence of heat on protein degradation, ultrastructure and eating quality indicators of pork

Feng Huang, Ming Huang,* Xinglian Xu and Guanghong Zhou

Abstract

BACKGROUND: Heating temperature is an important factor affecting meat palatability. This study aimed to evaluate the influence of heating temperature on some eating quality indicators, protein degradation and ultrastructure of pork muscle fibres and their correlations.

RESULTS: Cooking loss (CL) increased gradually \((P < 0.05)\) with increasing temperature. Warner–Bratzler shear force (WBSF) increased in two separate phases from 25 to 50 °C and again from 60 to 100 °C \((P < 0.05)\), with a steady phase from 50 to 60 °C \((P > 0.05)\); conversely, a significant increase in pH \((P < 0.05)\) occurred between 50 and 60 °C. Strong correlations \((P < 0.01)\) among pH, CL, WBSF and colour parameters \(L^*\) and \(b^*\) were observed following the heating process. Increasing temperature induced gradual degradation of many muscle proteins, but myosin was not significantly degraded until 80 °C and actin showed no visible degradation throughout the whole heating process. Meanwhile, the structure of muscle fibres also changed significantly on heating, with sarcomeres contracting transversely and longitudinally and becoming condensed, but there was no occurrence of breakage within fibres.

CONCLUSION: Heating temperature has a great effect on eating quality indicators, protein degradation and ultrastructure of pork muscle fibres.

Keywords: Jinhua pork; temperature; eating quality; protein degradation; ultrastructure

INTRODUCTION

It is well known that cooking is an essential process to achieve palatable and safe meat products.¹ However, cooking of meat also leads to a decrease in its nutritional value, mainly resulting from vitamin and mineral losses.²,³ Consequently, maintaining a balance of multiple attributes is a requisite in the processing of cooked meat. Currently, factors affecting eating quality of meat in the cooking process mainly include cooking time, temperature and cooking method. Numerous new techniques (such as microwave, far-infrared radiation and ultrasound cooking) have been employed to cook meat, but cooking in water, a universal process in industry and households for its economy and simplicity, is also of significance and is believed to be a reliable method to optimise tenderness owing to rapid and more consistent increases in internal temperature.⁴

It is well documented that the final internal temperature has a great effect on ultimate palatability, as assessed by a combination of colour, tenderness, flavour, water-holding and juiciness.⁵⁻⁷ Further, the final temperature is considered to be important for structural changes such as transverse and longitudinal shrinkage of muscle fibres due to meat protein denaturation during the cooking process. Cooking has also been defined as the heating of meat to a sufficiently high temperature to denature proteins.⁸

Muscle proteins, accounting for approximately 200 g kg⁻¹ muscle, are of great significance for the maintenance of meat product structure. Basically they can be divided into three groups, namely myofibrillar (salt-soluble), sarcoplasmic (water-soluble) and connective tissue (insoluble) proteins. Myofibrillar proteins, the main component of muscle proteins (ca 550–600 g kg⁻¹ total proteins), can be further subdivided into three classes, i.e. structural proteins (myosin, actin, etc.), which build up the main structure of muscle, regulatory proteins (troponin, etc.) and cytoskeletal proteins (titin, nebulin, desmin, etc.), which support the whole myofibrillar structure. However, sarcoplasmic proteins mainly include enzymes involved in energy metabolism. Collagen is the main component of connective tissue proteins and is located extracellularly. Previous studies have revealed that sarcoplasmic proteins aggregate between 40 and 60 °C⁹ and that myosin and actin denature at temperatures lower than 50 °C and higher than 65 °C respectively.¹⁰

However, the majority of the reported researches were focused on a few changing traits of meat separately induced by heating, and little work has been done on relationships among characteristics of...
Materials and Methods

Meat samples and heating process

Eight Jinhua pigs were slaughtered humanely at Qingliang slaughterhouse (Zhejiang, China) according to standard procedures. Following chilling for 24 h, *Longissimus dorsi* muscles (from 12th thoracic vertebrae to 5th lumbar vertebrae) were removed from both sides of each carcass. Each *Longissimus* muscle was trimmed of visible fat and connective tissue and cut into five 70 mm × 45 mm × 25 mm (length × width × thickness) pieces. After weighing accurately with an electronic balance (JA 2003, Hangping, Shanghai, China) and measuring the length, width and thickness using vernier calipers, samples were placed in vacuum bags (unsealed) and heated to one of five designated internal temperatures (25, 50, 60, 80 or 100 °C) in a water bath. Upon reaching the designated temperature, samples were kept in the water bath for 5 min. These meat samples were used for subsequent analyses. Thermocouples (UA-920C, Uygao, Shanghai, China) were inserted into the geometric core of each steak to monitor the internal temperature.

Cooking loss

Samples were removed from the vacuum bags and cooled to room temperature. Superficial water was absorbed using blotting paper, then each sample was accurately weighed. Cooking loss (CL) was calculated as

\[
CL(\%) = \left(\frac{\text{raw weight} - \text{cooked weight}}{\text{raw weight}}\right) \times 100 \quad (1)
\]

Texture

After determination of cooking losses, heated samples were chilled at 4 °C for 24 h, then six 1.27 cm diameter cores were removed from each sample parallel to the muscle fibre orientation. Each core was sheared through once using a Warner–Bratzler shear machine (235G-R, Manufacturing Co., UK), and Warner–Bratzler shear force (WSBF) data were recorded.

Colour

The colour of heated meat samples was expressed in terms of *L*° (lightness), *a*° (redness) and *b*° (yellowness) values determined using a Minolta CR-400 Chroma Meter (Japan). For each sample, three measurements were made at internal locations and the values were averaged and recorded.

Protein extraction and sample preparation

For extraction of sarcoplasmic proteins, approximately 1.5 g of minced meat sample was homogenised with 150 mL of extraction buffer containing 10 mmol L⁻¹ ethylene diamine tetraacetic acid (EDTA) and 50 mmol L⁻¹ Tris (pH 8.3) in a polytron at a speed of 13 000 rpm for 10 s. This was repeated a further twice, with a 15 s cooling period between bursts. The homogenate was centrifuged at 15 000 × g for 30 min at 4 °C and the supernatant was collected as sarcoplasmic proteins.

Myofibrillar proteins were extracted according to the procedure of Etlinger et al., with some modifications. The pellet from sarcoplasmic protein extraction was homogenised with approximately 7.5 volumes of pyrophosphate relaxing buffer (PRB) buffer (100 mmol L⁻¹ KCl, 2 mmol L⁻¹ MgCl₂, 2 mmol L⁻¹ ethylene glycol tetraacetic acid, 1 mmol L⁻¹ dithiothreitol, 1 mmol L⁻¹ Na₃, 2 mmol L⁻¹ Na₄P₂O₇, 10 mmol L⁻¹ Tris-maleate, pH 6.8) in a polytron at a speed of 13 000 rpm for 10 s and the homogenate was centrifuged at 1000 × g for 10 min. The pellet was subsequently washed eight times, each wash with 10 volumes of low-salt buffer (same as PRB except for pyrophosphate being omitted). In the final step the myofibrils were suspended in Tris-EDTA buffer (10 mmol L⁻¹ Tris, 5 mmol L⁻¹ EDTA, pH 8).

The concentrations of sarcoplasmic and myofibrillar proteins were determined with a BCA Protein Assay Kit (Pierce, America). Samples were then mixed with treatment buffer (125 mmol L⁻¹ Tris, 40 g L⁻¹ sodium dodecyl sulfate (SDS), 250 g L⁻¹ glycerol), heated at 50 °C for 20 min and finally stored at −80 °C for subsequent SDS polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE

A 50 g L⁻¹ polyacrylamide continuous gel (acylamide/ bisacrylamide 100 : 1 w/w, 1 g L⁻¹ SDS, 0.5 g L⁻¹ tetramethylethylenediamine (TEMED), 1 g L⁻¹ ammonium persulfate (APS), 2 mmol L⁻¹ EDTA, 200 mmol L⁻¹ Tris-HCl, pH 8) was used for determination of myofibrillar proteins (mainly high-molecular-weight proteins). Two 125 g L⁻¹ polyacrylamide separating gels (acylamide/bisacrylamide 37.5 : 1 w/w, 1 g L⁻¹ SDS, 0.37 g L⁻¹ TEMED, 0.5 g L⁻¹ APS, 0.5 mol L⁻¹ Tris-HCl, pH 8.8) were used to detect changes in myofibril and sarcoplasmic proteins respectively. A 40 g L⁻¹ polyacrylamide gel was used as stacking gel. The running buffer contained 25 mmol L⁻¹ Tris, 192 mmol L⁻¹ glycine and 1 g L⁻¹ SDS (pH 8.3). The 50 and 125 g L⁻¹ gels were run on a Bio-Rad Mini-Protean II system (Bio-Rad Laboratories, Hercules, CA, USA) at a constant current setting of 5 mA per gel for 18 h and at a constant voltage of 120 V for approximately 2.5 h respectively. After electrophoresis, gels were stained for 1 h in an excess of 1 g L⁻¹ Coomassie brilliant blue R-250, 316 g L⁻¹ ethanol and 73 g L⁻¹ glacial acetic acid and then destained in an excess of the same solution without the Coomassie brilliant blue R-250. Gels were scanned (GT-800F, Epson, Japan) at a resolution of 600 dpi.

Muscle ultrastructure

Samples were cut into 2 mm × 3 mm × 10 mm pieces and fixed overnight at 4 °C by immersion in 100 g L⁻¹ pre-cooled glutaraldehyde in 0.1 mol L⁻¹ phosphate buffer solution (PBS, pH 7.4). Afterwards, samples were rinsed with PBS, post-fixed in 10 g L⁻¹ osmium tetroxide for 3 h, dehydrated in ethanol, exchanged in propylene oxide and embedded in Spurr’s resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by transmission electron microscopy (H-7650, Hitachi, Japan).

Eating quality and between eating quality and protein degradation and structure of muscle fibres. The objectives of this study were to evaluate the effect of heat treatment on pH, colour, cooking loss, Warner–Bratzler shear force, protein degradation and structure of muscle fibres and their correlations.
Above 60 °C, heating had a great impact on the colour of meat (Table 1). Internal temperatures from 25 to 50 °C significantly between 25 and 60 °C were not different. According to Duncan’s multiple comparison test, a significant difference (P < 0.05) was detected only between 50 and 60 °C, while increases in pH between 25 and 50 °C and between 60 and 100 °C were not significant (P > 0.05).

RESULTS
pH
Heating temperature had a significant effect on pH (P < 0.01) (Table 1). As internal temperatures increased, pH values increased consistently, which is in agreement with previously reported results.12 However, changes in pH at different temperature phases were not different. According to Duncan’s multiple comparison test, a significant difference (P < 0.05) was detected only between 50 and 60 °C, while increases in pH between 25 and 50 °C and between 60 and 100 °C were not significant (P > 0.05).

Cooking loss
The results for CL of meat samples are presented in Table 1. The final core temperature of the meat had a significant effect on CL (P < 0.01), which increased significantly with increasing core temperature and reached approximately 41% at 100 °C.

Texture
The final internal temperature of the meat also had a large impact on WBSF (Table 1). As the core temperature increased, WBSF of meat increased significantly (P < 0.05) in two separate phases from 25 to 50 °C and again from 60 to 100 °C, with little change (P > 0.05) between 50 and 60 °C.

Colour
Heating had a great impact on the colour of meat (Table 1). As the core temperature increased from 25 to 60 °C, values for L* increased (P < 0.05), reaching a peak (77.89) at 60 °C. Above 60 °C, L* values decreased slightly. Values for b* increased significantly between 25 and 60 °C (P < 0.05) and continued to increase above 60 °C, but not significantly (P > 0.05). With increasing temperature, a* values increased significantly up to 50 °C (P < 0.05) but then decreased markedly between 50 and 80 °C (P < 0.05), with no change between 80 and 100 °C (P > 0.05).

Table 1. Values of pH, cooking loss (CL), Warner–Bratzler shear force (WBSF) and colour (L*, a*, and b*) of cooked pork longissimus at different internal temperatures

<table>
<thead>
<tr>
<th>Parameter</th>
<th>25</th>
<th>50</th>
<th>60</th>
<th>80</th>
<th>100</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.63 ± 0.11a</td>
<td>5.76 ± 0.12a</td>
<td>5.95 ± 0.06b</td>
<td>5.96 ± 0.08b</td>
<td>6.05 ± 0.12b</td>
<td>1.82524 × 10⁻⁴</td>
</tr>
<tr>
<td>CL (%)</td>
<td>0.93 ± 0.31a</td>
<td>7.38 ± 1.55b</td>
<td>16.90 ± 1.93c</td>
<td>35.70 ± 1.34d</td>
<td>41.37 ± 0.31e</td>
<td>3.99139 × 10⁻¹⁷</td>
</tr>
<tr>
<td>WBSF (kg)</td>
<td>2.31 ± 0.38a</td>
<td>4.06 ± 0.29b</td>
<td>4.09 ± 0.07b</td>
<td>7.67 ± 1.13c</td>
<td>8.68 ± 0.15c</td>
<td>1.46689 × 10⁻⁸</td>
</tr>
<tr>
<td>Colour L*</td>
<td>48.46 ± 1.13a</td>
<td>72.18 ± 2.81b</td>
<td>77.89 ± 0.64d</td>
<td>77.19 ± 0.42cd</td>
<td>75.10 ± 0.42e</td>
<td>3.49700 × 10⁻¹⁴</td>
</tr>
<tr>
<td></td>
<td>5.99 ± 0.89a</td>
<td>9.26 ± 0.59c</td>
<td>8.03 ± 0.56b</td>
<td>5.38 ± 0.35a</td>
<td>5.32 ± 0.44a</td>
<td>1.79535 × 10⁻⁷</td>
</tr>
<tr>
<td></td>
<td>2.30 ± 0.26a</td>
<td>8.21 ± 0.71b</td>
<td>9.67 ± 0.79c</td>
<td>10.33 ± 0.34cd</td>
<td>10.66 ± 0.17c</td>
<td>1.99534 × 10⁻¹²</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation. Means with the same letter in a row are not different at 95% level.

Table 2. Correlation coefficients among pH, cooking loss (CL), Warner–Bratzler shear force (WBSF) and colour (L*, a*, and b*) of cooked pork longissimus during cooking process

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>CL</th>
<th>WBSF</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1.000</td>
<td></td>
<td></td>
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<tr>
<td>CL</td>
<td>0.772**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>WBSF</td>
<td>0.752**</td>
<td>0.943**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L*</td>
<td>0.706**</td>
<td>0.666**</td>
<td>0.622**</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a*</td>
<td>-0.211</td>
<td>-0.559*</td>
<td>-0.491*</td>
<td>0.156</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>b*</td>
<td>0.784**</td>
<td>0.789**</td>
<td>0.759**</td>
<td>0.963**</td>
<td>0.028</td>
<td>1.000</td>
</tr>
</tbody>
</table>

* P < 0.05; ** P < 0.01.

Correlation analysis for heating pH, CL, WBSF and colour
The results of correlation analysis for pH, CL, WBSF and colour are presented in Table 2. Strong correlations (P < 0.01) among pH, CL, L* and b* were observed during the cooking process. Values for a* only showed significant correlations with CL and WBSF (P < 0.05).

Degradation of muscle proteins
As the temperature of the core increased, sarcoplasmic proteins were gradually degraded (Fig. 1(a)). At 50 °C, protein bands with a molecular weight of ca 100 kDa disappeared, the content of proteins with a molecular weight of ca 43–44 kDa distinctly decreased but the content of other proteins increased. At 60 °C the content of proteins (possibly myoglobin) with a molecular weight of ca 34 kDa increased continuously but other protein bands became weak and even disappeared. At 80 °C, all sarcoplasmic protein bands became weak or disappeared. No visible change in any of the protein bands was observed between 80 and 100 °C. Overall, the degradation of many sarcoplasmic proteins occurred between 50 and 60 °C.

Myofibrillar proteins had greater thermal stability than sarcoplasmic proteins (Figs 1(b) and 1(c)). As the core temperature increased, the density of myosin heavy chain (MHC) remained almost unchanged at temperatures up to 80 °C but decreased significantly at 100 °C (Fig. 1(b)). Little visible change occurred in the density of actin (Fig. 1(b)) in the temperature range 25–100 °C. Protein bands with molecular weights of ca 15 and 47–60 kDa appeared at 60 °C and their densities increased with increasing internal temperature. Using 50 g L⁻¹ continuous SDS-PAGE gels, we found that nebulin was not altered up to 60 °C but was slightly degraded at 80 °C and completely disappeared at 100 °C (Fig. 1(c)).
Little difference in titin degradation was observed below 50 °C, but titin was completely degraded beyond 60 °C (Fig. 1(c)).

**Muscle ultrastructure**

The final heating temperature had a large influence on the ultrastructure of pork muscles. At 25 °C the structure of myofibrils was regular and aligned and clearly perceived with intact Z lines, I bands and A bands (Fig. 2(a)). As the core temperature increased, changes in the structure of muscle fibres occurred progressively, with the I bands becoming shortened and more condensed (Figs 2(b) and 2(c)). However, the A bands appeared slightly stretched and the M lines became ambiguous between 50 and 60 °C and were difficult to distinguish at 80 °C and above. Z lines were clearly evident throughout the whole heating range, but some breaks were observed near the Z lines at temperatures of 80 °C and above (Figs 2(d) and 2(e)).

**DISCUSSION**

Raw meats are usually cooked to make them acceptable to potential consumers. This is because cooking affects many attributes of eating quality through multiple physicochemical changes, and these attributes usually include tenderness, colour, appearance and cooking loss. Therefore it is important to understand the changes in physicochemical properties and eating quality during heating.

Weight loss through loss of moisture during cooking is an issue of concern to people, because it not only affects a product’s final appearance and juiciness but is also of great economic importance to the catering industry, which primarily sells meat that has been cooked. In our study, cooking loss increased with increasing core temperature, and this trend became more pronounced in the range 60–100 °C. An increase in cooking loss with increasing internal temperature has also been observed in beef13 and rabbit meat,6 but the results of Combes et al.6 showed a constant cooking loss between 80 and 90 °C. This phenomenon was not observed in the present study and may result from a higher water-holding capacity of rabbit meat.14 Cooking loss generally includes a combination of liquid and soluble matter lost from meat during cooking, the main component being water.15 The majority of water in meat is held within the structure of the muscle and muscle cells.16 Therefore the loss of water during cooking is caused by the destruction of structures of muscle cells due to the denaturation of myofibrillar proteins by heat. It has been documented that changes in sarcomere length and cooking loss are inversely proportional.17 In this study, sarcomere length shortened with increasing temperature, particularly when core temperatures increased from 50 to 80 °C, and this is consistent with the loss of water on heating. Results on transverse tissue morphology under optical microscopy also showed that myofibril fibre density increased significantly, doubling after 15 min of heating at 100 °C.18 Although significant shrinkage of myofibrils occurred on heating, unlike the structural changes of myofibrils observed during post mortem tenderisation of meat, there was no occurrence of apparent breaks in myofibrils throughout the whole heating process in our study, which suggested that even though muscle proteins were degraded on heating, they were still located in their original position with no apparent translocation. In addition to temperature, different heating methods (microwave, water bath, etc.) could also result in different cooking losses. Compared with the popular method of microwave heating, heating in a water bath gives rise to low cooking loss.19 This
is likely due to the fact that microwaves cause a fast temperature rise in foods owing to their capacity to generate heat energy inside the food without requiring any medium as vehicle for heat transfer, which does not occur in conventional heating, and a high temperature even for a short time will create a large area of spaces within the myofibrillar mass, which is positively correlated with cooking loss.18 Yarmand and Homayoun20 have also reported that domestic microwave cooking causes more damage to the structure of both the connective tissue and the myofibrillar elements than conventional heating.

In addition, the net charge of muscle proteins may affect the retention of water through interactive forces between charges.16 In our study, pH values increased gradually with increasing temperature (Table 1), which may be attributed to the loss of free acidic groups,21 thus influencing cooking loss. Meanwhile, a significant inverse correlation (P < 0.01) between cooking loss and pH was also observed (Table 2). In our study a significant increase in pH only occurred between 50 and 60 °C and the majority of sarcoplasmic proteins were degraded at the same temperature phase; therefore the change in pH value of meat during heating was likely a result of the dynamic balance of acid–base groups at the surface of sarcoplasmic proteins.

Tenderness is rated as the most important eating quality characteristic of meat by consumers.22 Therefore influences of cooking on meat tenderness have received considerable attention. It is known that heating can strengthen myofibrillar protein networks, which leads to toughening, but at the same time may solubilise connective tissue, leading to tenderisation. The decrease in meat tenderness is generally observed in two distinct phases: between 40 and 60 °C and between 65 and 80 °C.8,22 Bouton and Harris23 attributed the first increase in meat toughness to connective tissue denaturation and the second to denaturation of myofibrillar proteins. However, Davey and Gilbert6 offered the opposite interpretation and attributed the first increase in meat toughness to denaturation of myofibrillar proteins and the second to connective tissue denaturation. In our study, increase in toughness was also observed in two separate phases from 25 to 50 °C and again from 60 to 100 °C. It is widely accepted that shrinkage of sarcomeres due to denaturation of myofibrillar proteins could cause an increase in toughness. In our study, there was no change in toughness at temperatures between 50 and 60 °C, which differs from the findings of Christensen et al.,24 who observed a decrease in meat toughness for the same temperature interval and attributed it to collagen solubilisation. This is possibly because the longissimus dorsi muscle used in our study has a relatively low collagen content compared with the semitendinosus muscle used by Christensen et al.24 In addition, heating method is also an important factor affecting the tenderness of meat. For example, the tenderness of meat heated in a water bath is better than that of microwave-heated meat,19 which may be due to the higher loss of water from muscle during microwave heating resulting in greater hardening of myofibrillar proteins.

Meat colour is an important trait of eating quality in that consumers use colour in their decision process to purchase meat as it is considered to be an indicator of meat freshness. Meat colour is also used by consumers as an indicator of the degree of ‘doneness’.25 During cooking, meat becomes progressively browner from its initial red colouration. Objective colour measurements confirmed the visual findings: L*, a* and b* values increased significantly as the core temperature increased between 25 and 50 °C; at core temperatures above 50 °C, with increasing core temperature, L* values did not change significantly, a* values decreased and b* values increased consistently. These results differed somewhat from previous findings.26,27 However, a* and b* values changed more significantly at temperatures below 50 °C, which may be due to the higher pH values at internal temperatures greater than 50 °C, since higher pH decreases denaturation of myoglobin and thus change in colour. Although meat colour is closely related to temperature, visual appraisal of cooked colour by consumers, who use brown colour of meat as

Figure 2. Representative transmission electron micrographs showing changes in muscle ultrastructure at internal temperatures of (a) 25, (b) 50, (c) 60, (d) 80 and (e) 100 °C. Scale bar: 1 μm.
an indicator of ‘doneness’, is not a completely reliable indicator. For example, premature browning was found to occur owing to the presence of oxymyoglobin and metmyoglobin in meat at the time of cooking,26 moreover, some researchers observed that beef patties remained pink when cooked to 74 °C or higher.29

CONCLUSION
In summary, the heating temperature has a significant effect on pH, CL, WBSF, colour, degradation of muscle proteins and ultrastructure of muscle fibres. With increasing internal temperature, CL increased gradually (P < 0.01), while WBSF increased in two separate phases from 25 to 50 °C and again from 60 to 100 °C (P < 0.05), with a stable phase from 50 to 60 °C (P > 0.05); conversely, a significant increase in pH (P < 0.05) occurred between 50 and 60 °C. Strong correlations (P < 0.01) among CL, pH, L* and b* were observed during the cooking process. With increasing temperatures, degradation of many muscle proteins occurred gradually. However, myosin remained unchanged below 80 °C and then was degraded rapidly, whereas actin showed no visible change at any of the temperatures investigated. Meanwhile, the visual appearance of the muscle fibre structure changed significantly on heating, with sarcomeres contracting transversely and longitudinally and becoming condensed, but there was no occurrence of breakage within fibres.

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