Effect of prolonged cooking at low temperatures on the eating quality of Tibetan pork: meat quality, water distribution, and microstructure

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Abstract

Tibetan pigs inhabit the plateau regions and engage in extensive exercise. Their meat is firm and less palatable, and appropriate cooking conditions can improve the flavor of the meat. This study aimed to explore the impact of cooking temperatures and times on the cooking loss rate, moisture content, tenderness, and color of Tibetan pork to elucidate their effects on the eating quality of this meat. The results indicated a negative correlation between temperature and moisture content, which decreased from 72% to 65% as the temperature increased. Meanwhile, the oxidation of myofibrillar proteins substantially increased with cooking temperature and time, resulting in a gradual augmentation of the cooking loss and shear force of the pork. The migration of bound water and the release of free water from the pork were faster after treatment at 80 °C compared with 50 °C. The results of infrared spectroscopy suggested that prolonged cooking led to the conversion of α-helix into β-sheet in the secondary structure of myofibrillar proteins. The microstructures observed through scanning electron microscopy exhibited a gradual degradation of connective tissues in the muscles at high temperatures, and the fiber structure of the muscles became more condensed and granular, blurring the textural appearance of the muscles. These results indicated that prolonged cooking time at a low temperature (steaming for 6 h at 50°C ) was conducive to the optimal tenderness of Tibetan pork. This study provides a theoretical basis for improving the meat quality of Tibetan pork products and developing more rational processing methods.

Keywords: Tibetan pork; Heating temperature and time; Myofibrillar proteins; Microstructure
Graphical Abstract

Vacuum packed

Sous-vide cooked

50°C, 60°C, 70°C, 80°C, 90°C

Five heating treatment

2, 4, and 6h cooking time
1. Introduction

Tibetan porks thrive in a pristine alpine environment, free from pollution, where they are nourished with a comprehensive array of amino acids, which is the closest to the ideal amino acid pattern required by the human body owing to its exceptional nutritional value. Furthermore, Tibetan pork is renowned for its distinctive flavor and superior quality and is thus highly favored by consumers. However, Tibetan porks live on the Western Sichuan Plateau all year round. They have a long growth cycle and engage in heavy exercise. Therefore, the lean meat ratio of Tibetan pork is higher than that of regular pork, which results in firmer meat and poor taste (Ludwiczak et al., 2019). The use of various cooking techniques can induce distinct molecular-level alterations in meat, thereby influencing its physicochemical and sensory attributes. Consequently, it is imperative to explore the impact of diverse cooking techniques on the quality of Tibetan pork meat (Wang et al., 2018; Yu et al., 2017; Zielbauer et al., 2016). The Tibetan region is situated in a high-altitude plateau with a frigid and arid climate. To adapt to local climatic conditions and traditional customs, steaming or boiling is the preferred cooking method in this region.

The cooking process may induce a range of alterations in proteins, including aggregation, crosslinking, oxidation, conformational changes and diminished solubility. These alterations may change the structure and functionality of proteins, thereby impacting the ultimate sensory attributes and nutritional composition of food (Vasanthi et al., 2007). During the cooking process, the variations in the temperature and time of cooking lead to the modification of meat proteins, including disruptions to cell membranes, contraction of connective tissues and muscle fibers, and formation of myofibrils and protein gels within the myoplasm (Berhe et al., 2014). These changes lead to a decline in the protein solubility and water-holding capacity of the meat, which, in turn, affects its tenderness. Connective tissue and muscle fibers are the primary factors influencing muscle tenderness. Studies have demonstrated that connective tissue with a three-dimensional network structure is essential for maintaining muscle structure and function (Bendall & Restall, 1983; da Silva et al., 2020; Scussat et al., 2017). Collagen proteins contract when connective tissue was heated, which affected the tenderness of the meat (N’Gatta et al., 2022).

The utilization of traditional high-temperature cooking methods would result in the excessive contraction of muscle fibers and lead to increased cooking losses and toughness. In recent years, low-temperature prolonged cooking has gained considerable attention owing to its ability to cook meat evenly, retain moisture effectively, and enhance tenderness. The retention of flavor and nutrients in meat is typically enhanced through prolonged cooking at low temperatures compared with conventional high-temperature cooking. As a result, the former method was widely acknowledged as a healthier and more appealing option. Wang et al. (2023) demonstrated that low-temperature cooking can mitigate the impact of heat stress on myofibrillar proteins, reduce protein aggregation, and improve the digestibility of proteins by proteases.
Furthermore, the protein structure and nutrient content were better preserved by cooking at low temperatures.

The use of low-temperature cooking techniques can substantially mitigate the degradation of ingredient quality and water loss, thereby effectively preserving the inherent flavors and nutrients of the ingredients. Consequently, the cooked food has a better flavor and meets the high expectations of contemporary consumers for dietary excellence. Compared with the conventional method, low-temperature prolonged cooking technique substantially increases the moisture content of meat products, remarkably reducing their shear force and cooking loss rate ($P < 0.05$) (Ismail et al., 2022; Yu et al., 2017). Therefore, the use of low-temperature prolonged cooking not only enhances the flavor and tenderness of meat, but also facilitates protein digestion and utilization, which is perfectly in line with the principles of healthy eating.

Therefore, in this study, we explored the impact of varying cooking temperatures and times on quality indicators such as the color, texture characteristics, moisture content, cooking loss, and shear force of Tibetan pork. In addition, we investigated the water distribution status and structural changes of myofibrillar proteins in Tibetan pork using techniques such as low-field nuclear magnetic resonance (LF-NMR), Fourier transform infrared (FTIR) spectroscopy and intrinsic fluorescence measurement. Simultaneously, we examined the muscle tissue texture and muscle fiber structure through SEM. This study can provide a theoretical foundation and technical assistance for enhancing the quality of Tibetan pork and developing more reasonable processing techniques.

2. Materials and Methods

2.1 Preparation of meat samples

Tibetan pork was purchased from Jiuzhaigou (Aba Bowen Agriculture and Animal Husbandry Technology Company Limited, China). Six 12-month-old Tibetan porks (male, castrated, weighing approximately 25–30 kg) were slaughtered according to commercial slaughter procedures. Subsequently, samples of Tibetan pork legs consisting of the superficial gluteus, middle gluteus, deep gluteus, and semitendinosus muscles were collected. Fascia and fatty tissue were removed from the meat, and then the meat was cut into $10 \times 10 \times 5$ cm$^3$ pieces and weighed. Then, the cut meats were placed into polyethylene vacuum bags and packed with a vacuum packaging machine (DZ400, Zhucheng Shengzhong Company Limited, China). The packaging conditions were as follows: vacuum time, 15 s; sealing time, 6 s; and vacuum degree, $-0.1$ MPa. The meat samples were randomly divided into 15 groups. Heat treatment was performed in a thermostatic water bath at 50°C, 60°C, 70°C, 80°C, and 90°C for 2, 4, and 6 h (HH-S6, Zhengzhou Great Wall Technology Industry and Trade Company Limited, China). Three pieces of meat
samples were used in each treatment. The cooked pork was subjected to ice bath for 5 min for subsequent analysis.

2.2 Cooking losses

Cooking loss was determined according to the method described by Wang et al. (2022). The mass of the meat sample before cooking was weighed and recorded as $M_1$. After cooking, the sample was rapidly cooled, and surface moisture was absorbed using filter paper. Then, the meat sample was weighed, and the quality was recorded as $M_2$. Cooking loss was calculated as follows:

$$\text{Cooking losses (\%)} = \frac{M_1 - M_2}{M_1} \times 100\%$$

2.3 Shear force determination

The shear force of the samples was measured according to the method described by N’Gatta et al. (2022) with slight modifications. Five pieces ($1 \times 1 \times 3 \text{ cm}^3$) of meat were removed from the cooked samples, and shear force was determined through texture analysis (TA.TOUCH, Shanghai Baosheng Company Limited, China). The meat pieces were cut using a blade (HDP, Shanghai Baosheng Company Limited, China) perpendicular to the direction of muscle fibers at a speed of 1.0 mm/s. The parameters were as follows: pre and post-test speed, 2.0 mm/s; trigger force, 10.0 g; contact point type, pressure; and contact point value, 20.0 g. Before testing the samples, the instrument was calibrated using a force sensor (capacity: 50 kg) with a calibration weight of 1.0 kg. A weight of 1 kg was placed on calibration platform and was considered to be calibrated if the error was within 1%. The force required to cut the meat samples was expressed as N.

2.4 Texture profile analysis (TPA)

The texture of the sample was determined using a texture analyzer (TA.TOUCH, Shanghai Baosheng Company Limited, China) (Luo et al., 2021). The meat samples were cut into small squares ($10 \times 10 \times 10 \text{ mm}^3$), and each sample was measured five times. The hardness, springiness, chewiness, and cohesiveness of the samples were measured using a cylindrical mandrel with a 36-mm diameter (P/36R). The pretest and test speed was 2.0 mm/s, the post-test speed was 10.0 mm/s, the compression ratio was 40%, and the compression time interval was 5.0 s.

2.5 Chromaticity measurement

The chromaticity of the samples was determined after cooling, and the parameters for chromaticity determination were obtained from the study by Li et al. (2019). The colorimeter (XD-1058, Shenzhen 3nh Technology Company Limited, China) was calibrated with white and black plates before measurement.
The L* (luminance), a* (redness), and b* (yellowness) values of each sample were measured. The measurement parameters were as follows: illuminated D65, with an observation angle of 10°, measurement aperture of 8.0 mm, and closed cone. Five random positions were measured on the surface of each sample.

2.6 Extraction of myofibrillar protein

Myofibrillar proteins were extracted from the Tibetan pork samples according to the method described by Dong et al. (2023) with slight modifications. Ten times volume of homogenization buffer saline (1.35-M NaCl, 47-mM KCl, 100-mM Na₂HPO₄, 20-mM NaH₂PO₄, pH 7.3 ± 0.1) was added to 4-g meat sample. The mixture was homogenized using a high speed homogenizer (XHF-DY, Ningbo Xinzhi Company Limited, China) at 10,610 × g for 30 s. The homogenate was subjected to centrifugation at 4 °C (H175, Hunan Xiangyi Company Limited, China), and the supernatant was discarded. After the precipitate was fully homogenized, a homogenate buffer five times the volume of the homogenization buffer saline was added. Washing with the homogenization buffer saline was repeated once and a layer of gauze was taken to filter out insoluble connective tissues. The filtrate was centrifuged at 4 °C for 10 min, and the residual supernatant was discarded. After washing the precipitate three times by adding 5 times volume of homogenate buffer, myofibrillar proteins were obtained by adding two times the volume of the homogenization buffer saline suspension. The protein content was determined using the BCA protein Assay Kit (P0012, Beyotime Biotechnology Company Limited, China).

2.7 Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy was conducted using an FTIR spectrometer (VERTEX70, Bruker Instruments GmbH Company Limited, Germany) (Liu et al., 2022). Freeze-dried Tibetan pork was pulverized into 200 mesh powder. Then, 2 mg of the sample was accurately weighed and added with 0.1 g of dried potassium bromide (DG82674A, Titan Scientific Company Limited, China). Myofibrillar protein lyophilized powder was ground to a homogeneous powder using a mortar and pressed into thin slices using a tablet press (PMH-B, Bruker Instruments GmbH Company Limited, Germany) for the FTIR analysis. The data acquisition range was 500–4,000 cm⁻¹, and air background spectrum was deducted before each measurement. A total of 32 scans with a resolution of 4 cm⁻¹ were obtained.

2.8 Intrinsic fluorescence measurement

The method was slightly modified according to Dong et al. (2021). Myofibrillar protein from different treatments was dissolved in phosphate buffer (1.35 M NaCl, 47 mM KCl, 100 mM Na₂HPO₄, 20 mM NaH₂PO₄, pH 7.3 ± 0.1) and protein concentration was adjusted to 0.70 mol/mL. PBS buffer was used as a blank solution and fluorescence spectra (FL970, Tianmei Company Limited, China) were performed at an
excitation wavelength of 280 nm, scanned wavelength of 300 - 500 nm, slit widths of 5.0 nm, and scanned speed of 600 nm/min.

2.9 Measurement of low-field nuclear magnetic resonance (LF-NMR)

LF-NMR was measured according to the method described by Purslow et al. (2018) with slight modifications. About 1.50 g of processed intact meat sample was weighed and placed in a 25.00-mm diameter LF-NMR tube (NMI20-060V-I, Suzhou Newmark Electronic Technology Company Limited, China) at room temperature. The sampling frequency was set to 200 kHz, sampling parameter was 21 MHz, and the 90° pulse time was 6.40 μs. The waiting time for repeated sampling was 5,000 ms. Furthermore, the accumulation number was 8; radio frequency delay, 0.02 ms; analog gain, 10.00 dm; and digital gain, 3 dm. After obtaining exponential decay graph, the software Multi-Expln Analysis was used for inversion through joint iterative reconstruction inversion algorithm.

2.10 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) observations of the sample preparation was performed according to the method described by Wang et al. (2022). Tibetan pork samples were fixed with 2.5 % glutaraldehyde buffer for 4 h (F8016, Adamas Reagent Company Limited, Switzerland). Subsequently, the samples were eluted with 10%, 30%, 50%, 70%, 80%, 90%, and 100 % ethanol for 10 min (73537AQ, Titan Scientific Company Limited, China) and then freeze-dried immediately after flash freezing in liquid nitrogen. The treated samples were fixed to a sample holder using a double-sided tape, sprayed with gold and observed under SEM (Thermo Scientific Apreo 2C, Beijing Opton Optical Technology Company Limited, Czech Republic) at 5 kV and photographed at ×1,000 magnification.

2.11 Statistical analysis

Each measurement was performed at least three times. Data were analyzed using SPSS version 27.0. Statistical differences in the results were assessed through analysis of variance. Duncan’s multiple range test was employed to identify significant differences at a significance level of 0.05. The results were expressed as means ± standard deviation.
3. Results and Discussion

3.1 Effect of cooking process on the water-holding capacity of Tibetan pork

The tenderness and juiciness of meat can be influenced by moisture loss due to heat treatment, which is closely related to the water-holding capacity of meat, mainly through cooking loss. During the cooking process, the muscle bundle gap caused by the transverse contraction of myofibrillar protein enhanced (Ismail et al., 2019; Pakula & Stamminger, 2012), and binding ability to water declined (Ismail et al, 2019). The degradation of myosin and actin as well as the exudation of sarcoplasm due to microstructural changes leads to loss of water and some soluble proteins from the muscle (Okitani et al., 2009). As shown in Figure 1A, the cooking loss of Tibetan pork augmented with the increase of in cooking temperature and time ($P < 0.05$). At a cooking time of 2 h, the cooking loss rates were 7.90%, 14.74%, 32.51%, 37.49%, and 39.78%, with the enhancement of temperature, and the rate of decline was downward. This is consistent with the results of N’Gatta et al. (2022). The moisture content in the Tibetan pork exhibited a decreasing trend with the increase in cooking temperature and time, which corresponds to the change in cooking loss illustrated in Figure 1B. At a cooking time of 2 h, the moisture contents in the Tibetan pork were 72.79%, 69.10%, 67.87%, 66.74%, and 65.05% with the increase in temperature. At a cooking temperature of 50 °C, the moisture contents were 72.80%, 72.26%, and 68.51% with the increase in time. This indicated that high temperatures and long cooking times can seriously damage the water-holding capacity of meat, resulting in reduced moisture (Bıyıklı et al., 2020). Meat has been reported to have high protein solubility and water-holding capacity at a low cooking temperature (60 °C) (Kim et al., 2021; Purslow et al., 2016). This is consistent with the results of the present study, with the lowest cooking loss observed at 50 °C. The cooking loss and moisture content were affected by the combined effects of temperature and time. Simultaneously, as the cooking temperatures increased from 50 to 90 °C, the changes in cooking loss were found to be significant for temperature ($P < 0.05$).

3.2 Effect of cooking process on the tenderness of Tibetan pork

Shear force is one of the common indicators to measure meat tenderness. In the heat treatment process, myofibrillar proteins and connective tissue proteins would be degraded and denatured with the increase in cooking temperature and time, which would affect the tenderness and shear force of the meat. As shown in Figure 2, the shear force of the Tibetan pork exhibits an upward trend and a remarkable decline in tenderness from 50 °C to 90 °C, which is consistent with results of Jiang et al. (2021). The increased cooking temperature caused serious damage to the connective tissues and myofibrils, resulting in decreased tenderness (Latorre et al., 2019; Sánchez del Pulgar et al., 2012). The shear force of the Tibetan pork cooked at 80 °C for 2 h was evidently lower than that cooked at 70 °C ($P < 0.05$) for 4 h. This may be associated...
with the increase in the protein hydrolysis activity and solubility of collagen proteins in cooked meat. The dissolution of collagen protein not only induced connective tissue softening but also improved meat tenderness (Li et al., 2019). The shear force of meat cooked at 80 °C for 4 h was lower than that cooked for 2 h at 80 °C, suggesting that the tenderness of the Tibetan pork had been improve. Although the initial unfolding of collagen protein promoted protein hydrolysis and tenderness, the protein denaturation during prolonged cooking time would inhibit the action of enzymes and thus reduce meat tenderness (Zielbauer et al., 2016). Purslow et al. (2018) explored the effect of shear force on connective tissues and muscle fibers in association with cooking temperatures of 50, 60 °C and above. It was found that collagen protein would decompose under conditions above 60 °C. This suggests that cooking temperature and time combined would affect the tenderness of Tibetan pork.

3.3 Textural property analysis

Textural property is an important index to evaluate the quality of meat, in which hardness and springiness are the key factors that ultimately affect consumer acceptance (Malva et al., 2022; Purslow et al., 2016). Table 1 presents the changes in texture (hardness, springiness, chewiness, and cohesiveness) caused by different cooking temperatures and times. During the cooking process, muscle fibers became dense and tight and myofibrillar proteins gradually denatured and solidified, which led to increased meat hardness. As shown in Table 1, higher cooking temperature at the same cooking time led to greater change in hardness ($P < 0.05$). This may be closely related to the degree of degeneration of myofibrillar proteins in the Tibetan pork. The denaturation and aggregation of myofibrillar and collagen proteins increased with the increase in temperature, thus reducing the water-holding capacity and muscle size of the Tibetan pork, leading to increased hardness (Dai et al., 2014). At the same cooking temperature, the hardness of the Tibetan pork exhibited an upward trend with the increase in cooking time. This may be responsible for the hydrolysis of peptide bonds in collagen proteins by some enzymes, resulting in loss of hydration in the connective tissues and an increase in the proportion of denatured proteins (Ayub & Ahmad, 2019).

Springiness refers to as the ability of meat to deform and recover to its original shape under the action of external forces (Pearce et al., 2011). The changes in the springiness of meat during the cooking process are shown in Table 1. Springiness gradually enhanced with the increase in cooking time at 50 °C–70 °C. Sánchez del Pulgar et al. (2012) reported that the springiness of meat was mainly affected by the denaturation of myosin and actin, which generally occurred at around 60 °C. Moreover, the integrity of myofibril structure was disrupted with the increase in temperature, which is consistent with the results of the present study. The springiness of meat cooked at 70 °C for 2 h was prominently higher than that cooked at 80 °C, which could be attributed to denaturation temperature of collagen protein in the range of 60 °C–70 °C. The incomplete transverse fracture of myofibrils in the sample was accompanied by a decrease in
the free water and total moisture content of meat, leading to an increase in fracture stress and springiness (Purslow et al., 2018). The cohesiveness and chewiness of the meat significantly changed with time and temperature parameters individually and in combination. Chewiness refers to the energy required to masticate a solid food product to a state ready for swallowing and is affected by hardness and elasticity (Chang et al., 2011). The chewiness of the Tibetan pork changed with the increase in cooking temperatures (78.78 ± 10.24 at 50 °C; 127.33 ± 29.58 at 60 °C; 586.49 ± 13.32 at 70 °C; 775.78 ± 14.12 at 80 °C; 971.10 ± 21.20 at 90 °C in minimum cooking time). With the increase in temperature, the cohesiveness value exhibited an increasing trend. These results are consistent with those of Roldán et al. (2013).

3.4 Effect of cooking process on the color of Tibetan pork

The color of Tibetan pork is a good intuitive criterion standard to evaluate meat quality. The L*, a*, and b* values of the Tibetan pork under different cooking temperatures and times are shown in Table 2. The L* value of the Tibetan pork gradually increased with the increase in cooking temperature. The L* value was also substantially affected \((P < 0.05)\) by the temperature–time combinations. Furthermore, the effect of temperature on the L* change of the meat samples was significantly greater than that of time (Roldán et al., 2013). As shown in Table 2, the L* value exhibits a decreasing trend at the cooking temperatures of 50 °C and 60 °C, whereas in all the other temperatures, the L* value improved over time. The changes in the L* value were related to protein denaturation and oxidation. Becker et al. (2016) reported that the L* and b* values of meat increased whereas the a* value decreased during the cooking process. This may be due to the denaturation of natural myoglobin by heat and the reduction in the redness of the meat. The decrease in the L* value during cooking at 60 °C was probably caused by factors such as protein and moisture. When cooked at low temperatures, a large amount of moisture was retained and presence of water has a certain hindered effect on propagation of light, resulting in a low L* value (Bıyıklı et al., 2020; Sánchez del Pulgar et al., 2012). There were significant differences in the a* value between the different temperatures and times. The a* value decreased with the increase in temperature. The redness (a* values) of cooked meat was highly dependent on myoglobin denaturation and cooking endpoint temperature. In general, vacuum-cooked meat usually showes higher a* value, and these values decrease with the increase in cooking temperature. The change in the b* value was not remarkable, and the b* value was mainly influenced by the myoglobin oxymyoglobin ratio. Because the meat was under the confined and low-oxygen state during the cooking process, the browning reaction was less, resulting in insignificant changes in the b* value. This result is consistent with that of Dai et al. (2013).
3.5 FTIR analysis

FTIR can be employed to characterize changes in protein structure. Figure 3 (A – E) presents the changes in protein structure under different cooking temperatures and times. The increase in heating temperature and extension of heating time would reduce the intensity of the peaks (Kaur et al., 2014). The result of amide I band (1,600–1,700 cm⁻¹) fitting on protein is shown in Figure 3F. The content of α-helix cooked at 50 °C was remarkably higher than that at other four temperatures (P < 0.05). The result indicated that heating may induce protein denaturation and lead to the transformation of α-helix to β-sheet (Ayub & Ahmad et al., 2019). The stability of α-helix and β-sheet of natural protein was primarily maintained by hydrogen bonds (González-Mohino et al., 2018). The changes in cooking temperature and times led to changes in the hydrogen bonds between protein molecules as well as their degree of aggregation. Meanwhile, the proportion of denatured proteins increased, and hydration of collagen protein molecules with higher covalent crosslinking decreased. The β-sheet notably increased from 36.51% to 44.53% at a cooking temperature of 90 °C compared with that at 50 °C for the same cooking time, whereas α-helix decreased from 27.89% to 23.80%. This may be related to the breakdown of hydrogen or hydrophobic bond within the peptide chain that maintained structure of myofibrillar protein, resulting in reduction in α-helix (Cafferky et al., 2020).

3.6 Fluorescence spectroscopy of myofibrillar protein

Fluorescence spectroscopy was performed to determine the changes in the endogenous fluorescence and the tryptophan residues of proteins as well as to monitor the changes in the tertiary structure of myofibrillar proteins (Song et al., 2021; Wang et al., 2019). During the cooking process, tryptophan residues with microenvironment and natural structure of protein was disrupted and rearranged, causing a gradual decline in fluorescence intensity, whereas fluorescence intensity of samples cooked at 50 °C and 60 °C for 2 h was higher. This implied that the structure of tryptophan residue was not fully exposed on the protein surface. The denaturation temperature of actin was higher than that of collagen protein, so if cooking temperature was between thermal denaturation temperatures of two proteins. The denaturation of collagen proteins may protect the integrity of actin and maximize the water-holding capacity of meat. By contrast, collagen protein has a complex triple-helical structure, and the tertiary structure of protein was not fully unfolded under low-temperature cooking (Dai et al., 2014), resulting in high fluorescence intensity. As shown in Figure 4, the fluorescence intensity of myofibrillar protein gradually decreased with time at 50 °C and 80 °C. The fluorescence intensity of myofibrillar protein decreased with increasing temperature. Fluorescence intensity was mainly associated with the changes in aromatic amino acid residues caused by heating, which is also consistent with the findings (Monago-Maraña et al., 2021). The fluorescence intensity at 60 °C, 70 °C, and
90 °C was the lowest at 4 h. A study (Sahar et al., 2009) reported that there was no obvious difference in the proportion of heat-soluble collagen proteins when cooked at high temperatures for a short time and at 60 °C for a long time. The reason was that intramuscular connective tissue changed fluorescence intensity not by dissolution but through denaturation during prolonged cooking at low temperatures. Tornberg et al. reported that the denaturation temperature of sarcoplasm and collagen protein ranged from 60 °C to 75 °C whereas the denaturation temperature of actin and actin complexes was 90 °C (Li, Tang, et al., 2019; Tornberg, et al., 2005; Wyrwisz et al., 2019). Fluorescence intensity was significantly lower at 90 °C than at 50 °C. The increase in temperature improved the solubility of collagen protein; thus, the number of collagen protein molecules in the unfolded and denatured state increased (Latorre et al., 2019).

3.7 LF-NMR relaxation times (T2)

LF-NMR relaxation (T2) measurement can provide direct information on the interactions between water proton and exchangeable proton in proteins. As shown in Figure 5, \(T_{20}(0–5 \text{ ms})\) represented tightly bound water to protein macromolecule (Song et al., 2021). In this study, two peaks appeared in \(T_{20}\), where the peak of 0.8–2 ms was generated by hydrogen in solid proteins; and this peak was from protein-bound water trapped in protein lumen (Anderssen & McCarney, 2022). \(T_{21}(5–100 \text{ ms})\) denotes immobilized water in myofibrillar protein network, and \(T_{22}(100–1,000 \text{ ms})\) was free water between fiber bundles. The increase in cooking temperature and time, myofibrillar contraction, and protein denaturation would reduce binding to water molecules. The water migration from immobilized water to free water, which increased cooking loss and declined tenderness of Tibetan pork. With the increase in temperature, \(T_{21}\) gradually decreased, which may be due to the weakened interaction of myosin and actin with water. Previous studies have demonstrated that myosin denaturation temperature ranged from 40°C to 55 °C, and the transverse contraction induced by myosin denaturation was cooked at 40 °C–60 °C, suggesting a correlation between myosin denaturation and \(T_{21}\) (Cheng et al., 2019; Ghadiri et al., 2017; Y. Li et al., 2022). As shown in Table 3, \(T_{21}\) decreased with time at the same temperature. Zheng et al. (2021) reported that at the same temperature but different cooking times, the protein network structure gradually disrupted with time, and the binding to water molecules was weakened. Contrarily, \(T_{22}\) increased with time, which corresponds to the cooking loss in this study. When the temperature was higher than 60 °C, the free water remarkably improved, and the changes in collagen protein network led to a decline in the bound water of meat. Hydrogen bonds were formed between hydroxyl and hydroxyproline, thermal denaturation of collagen protein led to hydrogen bond breakage with contraction of muscle fibers (Li et al., 2012). When the temperature was increased from 50 °C to 90 °C, \(P_{21}\) decreased from 82.44% to 78.48% and \(P_{22}\) decreased from 9.33% to 2.89% \((P < 0.05)\). Due to the contraction of meat under heat-induced condition, the distance between myofibril declined. During the cooking process of meat, the shrinkage of fibers may occur in transverse and longitudinal directions.
The contraction of collagen protein around muscle fibers resulted in physical restraint on these structures, which forced the water out and led to increased cooking rate.

### 3.8 Changes in the microstructure of Tibetan pork during cooking

High temperature leads to the reorganization of meat microstructure, generating new structures and channels of different sizes, thus affecting the tenderness of meat (Figure 6A - E). When cooked at 50 °C (red circles in Figure 6A), the myofibrillar bundles of the samples were neatly arranged and clearly visible, with a large gap between fibers and the smooth surface. As the cooking temperature was increased (red circles in Figure 6A – E), the muscle fiber gap in the sample became tighter and collagen protein dissolved, which may be due to the thermal denaturation of collagen protein (Li, et al., 2019). When the cooking temperature was increased to 80 °C or 90 °C, the arrangement of muscle fibers was disordered, and the structure of the endomysium was severely damaged. When the cooking time was extended, the perimysium and endomysium became gelatinous, forming gels around the muscle fibers. This is consistent with the findings of Li et al. (2019), who reported that these gaps promoted the formation of waterways and negatively affected the water-holding capacity of the muscles (Van et al., 2017). Simultaneously, lateral and longitudinal contractions caused by myosin denaturation reduced the volume of muscle cells and diameter of muscle fibers (Roldán et al., 2013). The space between myofibrils and their extracellular matrix increased due to the outflow of sap, which causes changes in the disulfide bonds and their polar groups, resulting in increased cooking loss due to sap efflux (Scussat et al., 2017). The results indicated that the microstructure of myofibrillar proteins was gradually destroyed with the increase in cooking temperature and time.

### 3.9 Correlation analysis of temperature and time among the parameters

Correlation heatmap (positive or negative) analysis was conducted to explore the effect of temperature and time interaction and parameters of Tibetan pork (Figure 7). Temperature and time exhibited significant correlations ($P < 0.05$), where cooking loss, shear force, hardness, springiness, and $L^*$ were positively correlated with a correlation index of about 0.6–0.9. These results are consistent with those of Li et al. (2019) and Liu et al. (2022). As described in Sections 3.1 and 3.2, the research results indicated that the changes in temperature and time during the cooking process may affect the tenderness and protein denaturation of Tibetan pork. Moisture content, $a^*$, $P_{21}$, and $P_{22}$ were negatively correlated with temperature and time. The denaturation oxidation of protein and the contraction of myofibrillar protein also led to a decrease in $a^*$ and moisture content. The correlation heatmap showed that temperature and time jointly play a major role in the cooking process of Tibetan pork. High cooking temperature and prolonged cooking time can accelerate the oxidative denaturation of proteins, increase the hardness of the meat, and reduce the
moisture content, among other edible qualities. Therefore, low-temperature prolonged cooking can make Tibetan pork juicy and tender, making it more acceptable to consumers.

4. Conclusions

The tenderness of Tibetan pork significantly increases when cooked at a lower temperature and longer cooking time. The optimal tenderness of Tibetan pork is achieved when cooked at 50°C for 6 hours. In this study, the tenderness and solubility of myofibrillar and collagen proteins remarkably decreased with the increase in cooking temperature. The protein exhibited that α-helix transformed into β-sheet, the fluorescence intensity decreased, and the original uniform and dense network structure was destroyed. Meanwhile, protein oxidation occurred during the cooking process, which reduced the stability of immobilized water. The increase in temperature accelerated the migration of immobilized water to free water. The SEM results indicated that connective tissue gradually disintegrated with the increase in temperature and that the arrangement of muscle fibers became dense. The correlation analysis revealed that the effect of temperature was more prominent compared with time. Therefore, high cooking temperature and prolonged cooking time should be avoided that effect of protein thermal denaturation. In practice, low-temperature cooking can make Tibetan pork juicy and tender, and this conclusion can provide a reference and theoretical basis for the research and development of new Tibetan pork products. However, the palatability and acceptability of meat after various cooking durations still require further investigation and analysis.
Author Contributions: JML: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing—original draft preparation, Writing—review and editing. XFL: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing—original draft preparation, Writing—review and editing. XH: Methodology. FG: Methodology. ZL: Formal analysis, Data curation. PS: Project administration, Investigation. ZDL: Investigation, Writing—review and editing, Funding acquisition, Project administration, Supervision. QH: Investigation, Writing—review and editing, Funding acquisition, Project administration, Supervision.

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Conflicts of Interest: The authors declare no conflict of interest.
References


Figure 1. Effects of different temperature and time treatments on cooking loss (A) and moisture content of Tibetan pork (B). Capital letters indicate a significant ($p < 0.05$) difference in cooking temperature. Lowercase letters indicate significant ($p < 0.05$) differences in different cooking times.
Figure 2. Effects of different temperature and time treatments on shear force of Tibetan pork. Capital letters indicate a significant ($p < 0.05$) difference in cooking temperature. Lowercase letters indicate significant ($p < 0.05$) differences in different cooking times.
Figure 3. FTIR of Tibetan pork treated at different temperature and time. A - E (50, 60, 70, 80, 90 °C); F: Protein secondary structure of Tibetan pork treated at different temperature and time.
**Figure 4.** Intrinsic fluorescence measurement of tibetan pork treated at different temperatures and times. A - E (50, 60, 70, 80, 90 °C).
Figure 5. Relaxation time $T_2$ of Tibetan pork treated with different temperature and time, in the direction of the arrow are: 50 °C (2, 4, 6 h); 60 °C (2, 4, 6 h); 70 °C (2, 4, 6 h); 80 °C (2, 4, 6 h); 90 °C (2, 4, 6 h).
Figure 6. Effect of microstructure of Tibetan pork treated with different temperature and time. A - E (2 h: 50, 60, 70, 80, 90 °C); F - J (4 h: 50, 60, 70, 80, 90 °C); K - O (6 h: 50, 60, 70, 80, 90 °C).
Figure 7. Correlation between temperature time and parameters of Tibetan pork.
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<th>Temperature (℃)</th>
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<th>Springiness</th>
<th>Chewiness</th>
<th>Cohesiveness</th>
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* Capital letters indicate a significant ($p < 0.05$) difference in cooking temperature. Lowercase letters indicate significant ($p < 0.05$) differences in different cooking times.
Table 2. Effect of different temperature and time treatments on color of Tibetan pork.

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<th>b*</th>
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*Values indicate the mean ± standard deviation and those without the same letter differ significantly,  \( p < 0.05 \), \( n=3 \). Capital letters indicate a significant (\( p < 0.05 \)) difference in cooking temperature. Lowercase letters indicate significant (\( p < 0.05 \)) differences in different cooking times.
Table 3. Relaxation time and peak area percentage change of Tibetan pork treated at different temperatures and times.

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*Capital letters indicate a significant ($p < 0.05$) difference in cooking temperature. Lowercase letters indicate significant ($p < 0.05$) differences in different cooking times.