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Effects of Brine Heat Treatment Conditions on Pork Myoglobin

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Abstract:

Myoglobin, a pigment protein mainly present in the sarcoplasm, serves as a decisive factor affecting the color of meat and meat products. In order to investigate the effect of heat treatment intensity on meat color during the brine treatment process of meat products, this paper takes pork as the research object, and studies the effects of different cooking time and cooking temperature on the water retention, color and myoglobin of pork. The results showed that, the water holding capacity of pork decreased gradually with the increase of heat treatment intensity; the lightness value (L*) of pork increased first and then decreased, while a* value decreased first and then increased, and b* value fluctuated little with the increase of heat treatment intensity. With the continuous increase of cooking temperature, L* value presented a fluctuating upward trend as a whole, a* value and b* value first increased and then decreased, reaching the maximum at 80°C. Myoglobin content and the ratio of its three forms were greatly affected by the heat treatment temperature. With the increase of heat treatment intensity, the myoglobin content continuously decreased, which changed drastically when the cooking temperature was below 80°C, but tended to stabilize after 80°C. The research results provide a theoretical reference for improving and controlling the pork processing quality in the brine treatment process and improving the quality of braised pork products.

***Keywords**: Heat treatment, Myoglobin, Color.*

I. INTRODUCTION

According to the National Bureau of Statistics, China's meat production in 2020 totaled 77.48 million tons, accounting for about 1/3 of the world's total [1]. With the improvement of living standards, people pay increasingly more attention to the quality of meat and meat products. Color is an important index for people to evaluate the quality and freshness of meat and meat products, which directly affects consumers' purchasing behavior. According to statistical research by Ranaei et al. [2], in 2019, the loss from meat color problems in the United States reached 3 billion US dollars, and the global loss reached 14.2 billion US dollars.

Myoglobin (Mb) belongs to sarcoplasmic protein. As a binding protein composed of globin and heme prosthetic group, it plays a major decisive role in meat color. There are three main forms of myoglobin, namely purple deoxymyoglobin (DeoMb), bright red OxyMyoglobin (OxyMb), and brown MetMyoglobin

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(MetMb) [3]. The three forms coexist in the meat and can be mutually converted. The relative rate of myoglobin oxidation and metmyoglobin reduction affects the meat color changes [4]. During the processing and storage of meat and meat products, factors such as temperature, time, light, pH, and processing mode will reduce the structure stability of myoglobin, change the ratio of DeoMb, OxyMb, and MetMb, and affect the appearance and color of meat and meat products [5]. Most meat and meat products are eaten after heat treatment, while heat treatment will affect the structure and physical and chemical properties of Mb, thus affecting the color and quality of meat and meat products. Zhu Shuran et al. [6] analyzed the effect of heating on myoglobin structure using spectroscopic techniques, finding that heating temperature changes would affect the tissue structure of myoglobin. The higher the heating temperature, the more seriously the secondary and tertiary structures of myoglobin were disturbed. Wang Linke et al. [7] found that in the early stage of heat treatment, MetMb ratio would increase significantly with the heating intensity, while the oxygenation ratio would gradually decrease. The heating temperature 80℃ is the key temperature for the state transition of myoglobin. Such transition was completed after 95℃, reaching an equilibrium state. The temperature range of meat determines the speed of myoglobin denaturation, the most typical of which is the myoglobin denaturation during cooking. This is also a common method for judging the cooking doneness of meat products (raw meat is red inside, cooked meat is brown or grey in appearance). By analyzing color, content and ratio of pork myoglobin in the brine treatment process, this study provides a certain theoretical basis for the Mb sensory judgment of braised pork as well as color regulation in the brine treatment process of pork.

II. MATERIALS AND METHODS

2.1 Test Materials and Instruments

2.1.1 Materials and reagents

Pork was provided by Bengbu Hongye Meat Joint Processing Complex Limited. Five-spice brine is commercially available.

Sodium dihydrogen phosphate and disodium hydrogen phosphate are of analytical grade, from Sinopharm Chemical Reagent Co., Ltd.

2.1.2 Main instruments and equipment

UV-Vis spectrophotometer was from Shanghai Jinghua Technology Instrument Co., Ltd.; high-speed refrigerated centrifuge was from Hitachi; electric constant temperature drying oven was from Jintan Shenglan Instrument Manufacturing Co., Ltd.; colorimeter was from Kunshan Shunnuo Instrument Co., Ltd; high speed homogenizer was from Jiangsu Jintan Medical Instrument Factory.

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2.2 Methods

2.2.1 Sample preparation

Wash the fresh pork, remove the fat and connective tissue, cut it into pieces of about 40g, blanch in water, and then brine. The effects of different brine time and temperature ratio on the myoglobin of braised pork were studied. Each group was repeated 3 times.

2.2.2 Moisture determination

The moisture content determination method refers to the national standard "GB 5009.3-2016 determination of moisture in food".

2.2.3 Determination of color

L^* value, a^* value and b^* value of braised pork were measured by colorimeter.

2.2.4 Determination of myoglobin content

The myoglobin content was determined according to the method of Wang Lishu [8]. 5 g minced pork was added to 25 mL sodium phosphate buffer (0.04 mol/L, pH=6.8). After homogenizing and mixing, stand at 4℃ for 1h, centrifuge at 4 500 r/min for 20 min. Take the supernatant and measure the absorbance values at 525, 545, 565, and 572 nm, respectively. To calculate various types of Mb, the calculation formula is as follows:

$$\text{Total Mb (mmol/L)} = -0.166A_{572}+0.086A_{565}+0.088A_{545}+0.099A_{525}$$

$$\text{DeoxyMb (\%)} = (0.369R_1+1.140R_2-0.941R_3+0.015) \times 100$$

$$\text{MboMb (2 \%)} = (0.882R_1-1.267R_2+0.809R_3-0.361) \times 100$$

$$\text{MetMb (\%)} = (-2.541R_1+0.777R_2+0.800R_3+1.098) \times 100$$

Where, R_1, R_2, and R_3 are the ratios of absorbance A_{572}/A_{525}, A_{565}/A_{525}, and A_{545}/A_{525}, respectively.

2.2.5 Data and analysis

In each group of experiments, measure three times in parallel. The results were expressed as "mean ± standard deviation". The data were processed by SPSS (IBM SPSS Statistics 25) and Origin 2018 for statistical analysis, with significant difference $P < 0.05$.

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III. RESULTS AND ANALYSIS

3.1 The Effect of Different Heat Treatment Conditions on the Moisture Content of Braised Pork

The moisture content changes in pork with heating time and heating temperature during the brine treatment process are shown in Figures 1 and 2.

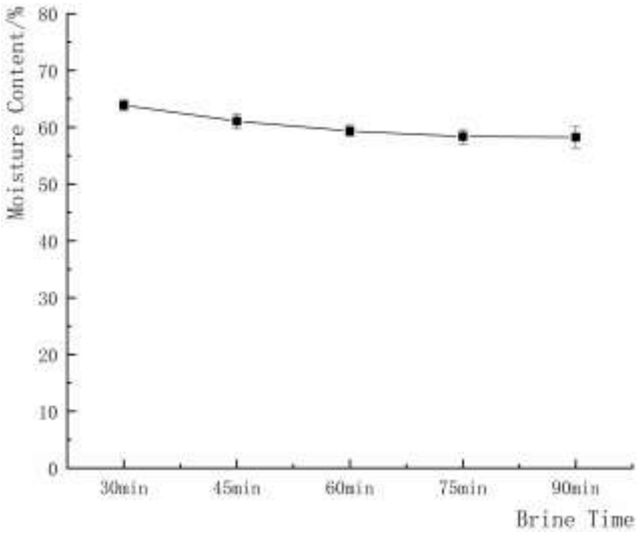

Figure 1 Effect of heat treatment time on pork moisture content

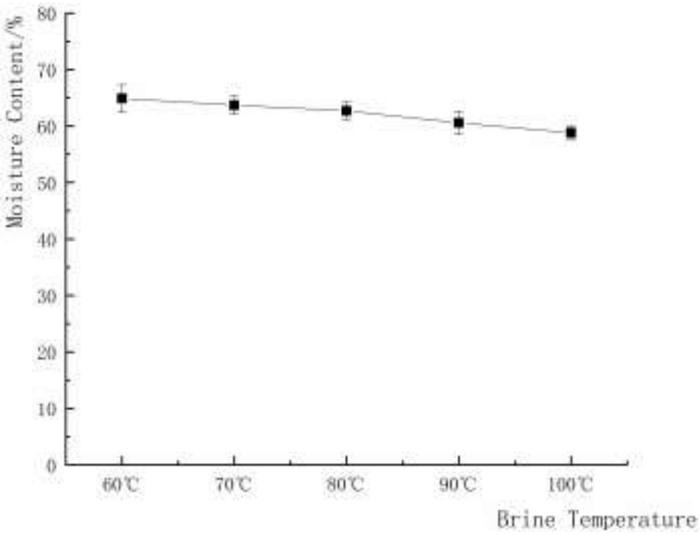

Figure 2. Effect of heat treatment temperature on pork moisture content

Figures 1 and 2 show that with the increase of brine temperature or the prolongation of brine time, the moisture content of the braised pork continuously declines, and the moisture content of the braised pork is inversely proportional to the heat treatment intensity during the brine treatment process. For its reason, with the increase of heat treatment intensity in treatment time or treatment temperature, protein

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denaturation occurs, which destroys the structure, resulting in decreased hydrophilic groups originally distributed on the molecule surface, while a large number of hydrophobic groups inside the molecule are exposed on the molecule surface, leading to lower water holding capacity of the braised pork [9]. Long-term heat treatment will not only cause muscle fiber contraction and free water loss, but also damage the cytoplasm and muscle structure, resulting in the dissolution of proteins, which further reduces the product's water holding capacity [10]. On the other hand, the increase in heat treatment intensity will promote the oxidative decomposition of fat in meat, and the fat will be oxidized to generate glycerol and fatty acid, which will also be fully oxidized to generate carbon dioxide and water. The content of polyunsaturated fatty acids in meat and fat-soluble vitamins, etc. is reduced in this process, and the structural integrity of cell membrane, etc. is destroyed, resulting in the outflow of intracellular fluid and the reduction of water holding capacity [11].

3.2 The Effect of Different Heat Treatment Conditions on the Color of Braised Pork

The color changes of pork during heating and brine treatment are shown in Figures 3 and 4.

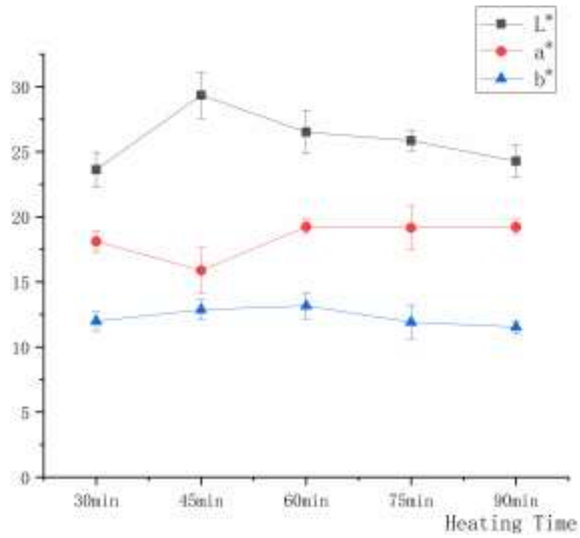

Figure 3. Effect of heat treatment time on pork color

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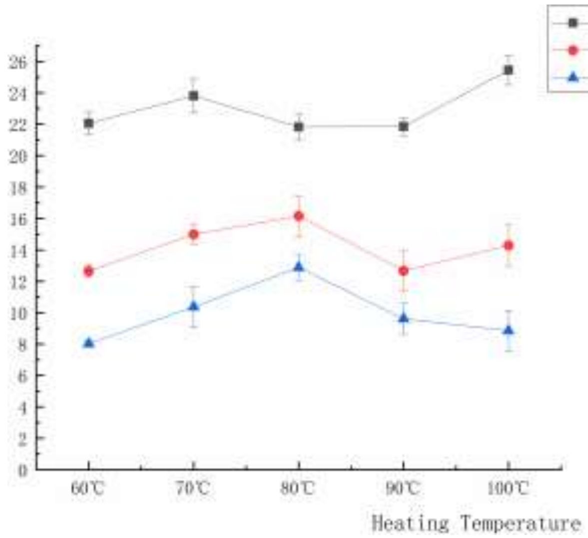

Figure 4. Effect of heat treatment temperature on pork color

Figure 3 shows the effect of heating and brine treatment on pork color. With the increase of heat treatment time, the L^* value of pork increases first and then decreases. The increase of L^* at 30-45min may be due to the increase of treatment time, in which, most of the proteins were denatured, and the chemical bonds between protein molecules were distorted or broken, so the structure of muscle fibers changed, with the agglutination degree increased, ferroheme in the muscle replaced or released, which in turn increased the brightness value of the meat [12]. At 45-90min, L^* decreases with the increase of processing time. For its reason, in the long-term brine treatment, the muscle loses water and shrinks, the meat color deepens, and the water retention of the meat decreases, so the tissue fluid dissolves and adheres to the meat, which reduces the surface reflectance, affecting the optical properties of the meat [13]. a^* value first decreases and then increases with the increase of heat treatment time. Within 30-45 min heat treatment, the protein was deformed, the myoglobin structure in the meat was destroyed, the muscle lost water, and the protein contracted, nutrients such as tryptophan were lost, reducing the a^* value. After 45min, the a^* value rebounded, possibly due to flesh color deepening after serious protein denaturation and shrinkage. After 60min, although the redness a^* value fluctuates, the change is small and tends to stabilize. Compared with the L* value and a^* value, b^* value fluctuates little with the increase of brine time, mainly due to fat oxidation and spices.

Figure 4 shows the effect of heating and brine temperature on pork color. With the increase of heat treatment temperature, the L^* value of pork fluctuates, increasing at 60-70℃. At the temperature 60℃, there is low protein denaturation in the meat. When the temperature rises to 70℃, the degree of protein denaturation increases, resulting in increased L* value. Under increasing temperature, the L* value first decreases and then increases. The decrease of the brightness at 80℃ and the flattening of the brightness at 90℃ may be due to the combined effect of the slow denaturation of proteins and the loss of muscle water. After 90℃, the brightness value increases significantly, possibly due to the denaturation and swelling of protein at higher temperature. The a* value and b* value first increased and then decreased with the increase of cooking temperature as a whole, reaching the maximum value at 80℃. Under the low

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temperature state, with the continuous increase of the heating temperature, the muscle loses water, the protein contracts, and the flesh color deepens, so that the measured a^* value and b^* value increase. Under increasing temperature after 80℃, there are myoglobin denaturation, increased ratio of metmyoglobin and continuously decreased ratio of bright red oxymyoglobin, so protein degrades, the meat color lightens, which makes a^* value and b^* value decreased on the whole.

3.3 The Effect of Different Brine Conditions on the Myoglobin Value of Braised Pork

The changes in the total myoglobin and the ratios of the three forms of myoglobin during the heating and brine treatment of pork are shown in Figures 5-8.

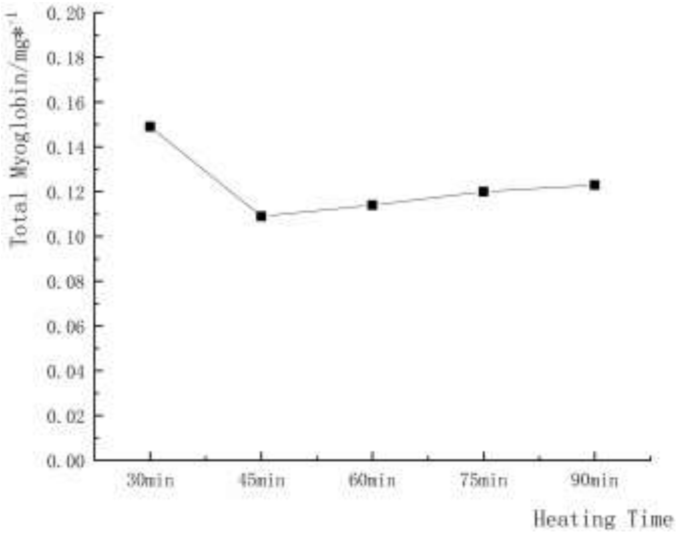

Figure 5. Changes of total Mb after brine treatment for different time

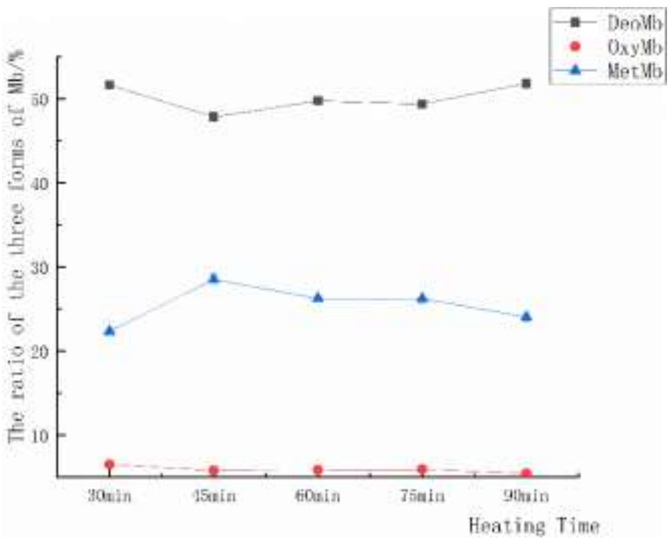

Figure 6. Changes in the ratio of the three forms of Mb after brine treatment for different time

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Figures 5 and 6 respectively show the changes of the total myoglobin, the ratio of three different forms of myoglobin in braised pork with cooking time: deoxymyoglobin, oxymyoglobin and metmyoglobin. Figure 5 indicates that Mb content decreases at first and then increases slightly with the extension of the treatment time under the same temperature. The significant decrease in the Mb content within 30-45 min is due to the gradual complete denaturation of the protein under continuous heating, which reduces solubility and changes the heme iron structure [14]. Long-time cooking reduces the water holding capacity of the muscle and causes the protein to contract, so that the myoglobin content in the meat sample rises, but the amplitude is low, which is consistent with the research of Wang Linke et al. [7]. Determination of the ratios of three different forms of myoglobin revealed that the OxyMb content was the lowest, which was less affected by cooking time. The DeoMb content first decreased and then increased with the prolongation of cooking time, which was opposite to MetMb. In the early stage of cooking, heating leads to protein denaturation, which prompts the rapid conversion of OxyMb to tan MetMb and purple-red DeoMb. When cooking continues, due to the decomposition of myoglobin and the loss of muscle water, MetMb and DeoMb fluctuate continuously, but are relatively stable, suggesting that cooking time exerts little effect on the morphology of myoglobin.

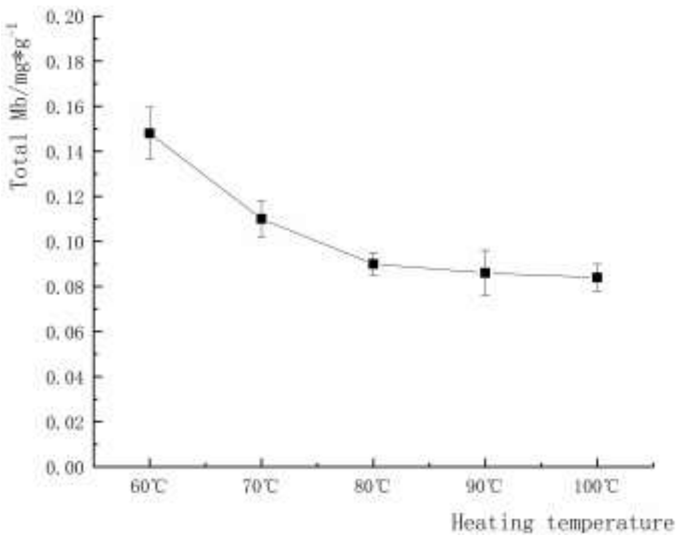

Figure7. Changes of total Mb after brine treatment at different temperatures

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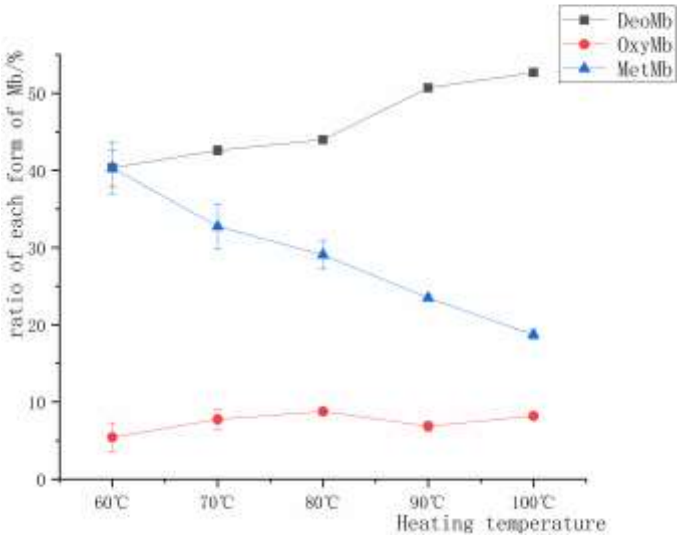

Figure 8. Changes in the ratios of the three forms of Mb after brine treatment at different temperatures

Figures 7 and 8 show the changes in Mb, the ratios of OxyMb, MetMb, and DeoMb with the cooking temperature, respectively. Compared with cooking time, brine temperature affects Mb and its morphology more. The total Mb decreases with the increase of cooking temperature. Mb content exhibits more significant change when the cooking temperature is ≤80℃, which tends to stabilize when the cooking temperature is≥ 80℃. This may be because when the temperature is ≤80℃, the protein is gradually denatured, and the structure, solubility and heme iron structure of the protein change rapidly, resulting in a rapid decrease in the detected content [15]. When the temperature is ≥80℃, the protein or myoglobin is completely denatured, tending to stabilize, so the detected content also tends to be constant. As for MetMb, due to the partial globin denaturation in Mb during heat treatment, it has reduced binding capacity against the heme prosthetic group, resulting in the exposure and oxidation of the heme prosthetic group, thereby generating MetMb [16]. With the increase of cooking temperature, the secondary and tertiary post-nodes of myoglobin are disrupted and destroyed, and heme is gradually dissociated, so that the ratio of MetMb gradually decreases, the ratio of DeoMb increases accordingly, and the ratio of OxyMb also increases slightly.

IV. CONCLUSION

Heat treatment is the main way in meat and meat products processing. Heat treatment at different intensities will change the product color to varying degrees, which affects the product color state by affecting the myoglobin content in the meat and the ratio of its various forms. In this experiment, pork was subject to brine treatment under different heat intensities to study its effect on pork color and quality. The study found that with the increase of heat treatment intensity, the protein in the meat was gradually denatured, with muscle contracted, tissue structure destroyed and water holding capacity gradually reduced. The color of meat (L^*, a^*, b^*), myoglobin content, and the ratio of the three forms of myoglobin all changed with the increase of heat treatment intensity. With the prolongation of cooking time, L* value first increased and then decreased, a^* value first decreased and then increased, and b* exhibited no significant fluctuation. The myoglobin content and the ratio of deoxymyoglobin in myoglobin tended to

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first decrease and then slightly increase, which was opposite to the ratio of metmyoglobin, while ratio of oxymyoglobin was low. When the cooking temperature was increased, more significant change was observed in the meat color and myoglobin. When the cooking temperature was l≤ 80℃, L^* continuously decreased, a^* value and b^* value increased, and the myoglobin content decreased significantly. When the cooking temperature was ＞ 80℃, the L^* value increased, a^* value and b^* value showed a decreasing trend, and the myoglobin content decreased slightly, showing gentle changes. The ratio of metmyoglobin continued to decrease with the increase of cooking temperature, while the ratio of oxymyoglobin and deoxymyoglobin exhibited an increasing trend.

This study analyzes the changes in physical and chemical properties such as moisture, color and myoglobin content of pork during the brine treatment process, aiming to provide theoretical reference for improving and controlling the processing and meat quality of braised pork. However, heat treatment is only the most widely used method in broiler meat processing, and there are many factors that can affect the color and myoglobin of braised meat, demanding further study.

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