Study on the effect of storage time on the quality of mutton of different brands by low-field nuclear magnetic resonance

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Abstract: Based on low-field magnetic resonance technology, the quality of Hainan Dong goat meat was studied at the molecular level. With the prolongation of storage time, the pH value of Hainan Dong goat meat showed an overall upward trend, and the T22 peak and T23 peak of transverse relaxation gradually shifted to the left. As the storage time prolonged, the transverse relaxation times of the peaks of samples T22 and T23 showed a decreasing trend. The T22 peak area of the water that is not easy to flow in the sample gradually decreases, while the peak area of the free water T23 gradually increases. The non-flowable water in Hainan East Goat meat was converted into free water. At the same time, the correlation between the transverse relaxation time and the pH value of the mutton quality index was analyzed, and the relationship between the quality of the mutton and the change of storage time was obtained. Finally, this paper compares Hainan Dong goat meat and Guangxi goat meat. The results show that the value of the hard-to-flow water T22 of Hainan Dong goat meat is small and the meat quality is tender. This provides data support for Hainan Dong goat meat to become the brand of lamb.

1. Introduction
Hainan East Goat was mainly produced in the Dongshan Mountains of Wanning, Hainan, so it is abbreviated as the East Goat by the local people. Hainan has abundant conditions of light, temperature, water and heat, and geothermal. Common forage can grow in Hainan for a long time. When sheep eat Baicao Fertilizer, they should choose forage with good growth, high yield, rich nutrition and suitable for tropical areas. Hainan East Goat is famous for its high quality mutton both inside and outside the island. Hainan East goat meat is tender and sweet, with high nutritional value, and has the effect of warming, benefiting qi and tonifying deficiency. Due to the demand of mutton market, Hainan mutton can not meet the dietary supply of local residents, so every day, foreign mutton flows into Hainan Island, mostly from Henan, Guangxi and other places. Due to the high content of placenta in Hainan East Goat, the price of Hainan East Goat meat is higher than that of foreign mutton. At the same time, Hainan East Goat was listed in the first list of protected breeds of livestock and poultry genetic resources by Hainan Provincial Department of Agriculture (2009).

Water is the most abundant chemical component in mutton. Many chemical and physical changes in mutton are related to water. These chemical and physical changes will affect the quality, texture and flavor of mutton¹ [1-2]. At present, the traditional methods of food moisture detection generally use drying method, distillation method and Karl Fischer method [3]. In the process of detection, some proteins in Mutton may change physically or chemically, which destroys the integrity and composition structure of
the tested samples and cannot reflect the physical state information of water in mutton. At the same time, other new technologies (infrared analysis technology \cite{4}) to determine the moisture content of mutton only reflect part of the moisture information in the tested samples, and their application also has some limitations. In contrast, low-field NMR technology has the advantages of non-destructive and non-radiation detection, can effectively obtain the distribution information of water components in mutton, and has obvious non-destructive advantages \cite{5,6}. Based on low-field magnetic resonance technology, this paper studied the quality change rule of Hainan East goat meat at the molecular level, and detected the difference of water distribution and state between Hainan East goat meat and Guangxi goat meat, providing data support for Hainan East goat meat as a brand mutton.

2. Material and Analysis Method

The whole sheep carcass was purchased from the slaughterhouse and transported back to the laboratory in the incubator. The carcass was discharged at 0-4°C for 24 hours. The meat of the front and back legs, longissimus dorsi and other parts were taken out, and the connective tissue such as visible fat and fascia were removed. Meso MR23-060H-I Nuclear Magnetic Resonance Imaging Analyser: Suzhou Newmark Analytical Instruments Co., Ltd. JA1003 Electronic Balance: Shanghai Liangping Instruments and Instruments Co., Ltd. HH.S21 thermostat bath pot: Shanghai Boxun Industrial Co., Ltd. PH-5F flat pen pH meter: Shanghai Sanxin Instrument Factory.

Using CPMG (hard pulse echo) sequence to detect transverse relaxation pattern of meat products has great influence on the intensity and signal-to-noise ratio of sampled signal because of setting different sequence parameters. If the sampling frequency SW is too small, the effective information in the sample may be lost. The larger the SW, the shorter the sampling time, and the larger the sample frequency, the better the complete sample information can be obtained. The shorter the relaxation time of the sample, the larger the SW needed to be set. SW = 100KHz. With the increase of the number of echoes NECH, the transverse relaxation interval of CPMG echo curve becomes larger. Generally speaking, the number of echoes should be set large enough to attenuate the signal completely. However, if the number of echoes is too large, the determination time will be prolonged, the experimental detection efficiency will be reduced, and the signal-to-noise ratio of sampling signal will be reduced; if the number of echoes is too small, the effective information in the sample will be lost. In theory, the number of echoes is the minimum number of echoes to relax the sample. As shown in Figure 1, the signal occupies one third of the window in the image. Set NECH = 5000.

![Fig.1 Sampling signal of mutton](image)

Sampling point TD, in CPMG experiment, software automatically calculates TD according to the set SW and NECH values. The number of repeated sampling is usually 2 times of NS. The larger the NS, the higher the signal-to-noise ratio of the sampled signal, but the longer the scanning time. Set the number of repeated sampling NS = 8. The relaxation time inversion fitting software T-InvfitGeneral was used to
inverse the data. Origin 8.0 is used for data processing and drawing. The Pearson correlation coefficient was analyzed by SPSS24.0.

3. Results and analysis

3.1 Detection of Hainan East Goat Meat and Guangxi Goat Meat by Low Field Nuclear Magnetic Resonance

The quality of Hainan East goat meat and Guangxi goat meat in Fig. 2 and 3 are 20 ± 0.02g. There are three peaks in the lateral relaxation curves of Hainan East goat meat and Guangxi goat meat. These three peaks represent bound water $T_{21}$ (0-10ms), non-flowing water $T_{22}$ (10-100ms) and free water $T_{23}$ (100-1000ms), respectively. Among them, $T_{21}$ represents the water molecular layer on which the polar groups on the surface of protein molecules are closely bound to water molecules; $T_{22}$ indicates the water which is not easy to flow between myofibrils, myofibrils and membranes, and is easily affected by the changes of protein structure and charge; and $T_{23}$ indicates the water which can flow freely in the extracellular space and is maintained by capillary force [8-9].

![Fig.2 Lateral Relaxation Map of Hainan East Goat Meat at Different Storage Time](image1)

![Fig.3 Transverse Relaxation Atlas of Guangxi Goat Meat at Different Storage Time](image2)

It can be seen from the graph that the peak area of $T_{21}$ of Hainan East Goat and Guangxi Goat has little change with the storage time. The peak area of $T_{22}$ and $T_{23}$ shifts to the left gradually [10]. The peak area of $T_{22}$ decreases gradually, while the peak area of $T_{23}$ increases gradually. With the prolongation of storage time, the muscle fibers in meat gradually become loose and compact, and can not absorb more water. There is a flow of water between myofibrils and membranes that is not easy to flow to the interstitial space outside the cell, and it changes into free water, which reduces the flow of water and increases the free water [11]. At the same time, with the prolongation of storage time, the total peak area of Hainan East goat meat and Guangxi goat meat gradually decreased, and the growth of microorganisms in meat consumed part of water, resulting in the decrease of the total moisture content of mutton.

Table 1. The variation of the peak transverse relaxation time of Guangxi goat meat with storage time.

| Time(day) | 1     | 2     | 3     | 4     | 5     |
|-----------|-------|-------|-------|-------|-------|
| T2 (ms)   | 1.054 | 1.113 | 1.083 | 1.054 | 0.848 |
| T2 (ms)   | 59.391| 56.242| 54.731| 53.260| 53.260|
| T2 (ms)   | 601.527| 399.768| 399.768| 378.572| 368.399|
| Time(day) | 6     | 7     | 8     | 9     | 10    |
| T2 (ms)   | 0.895 | 0.971 | 0.971 | 0.920 | 1.276 |
| T2 (ms)   | 51.829| 51.829| 51.829| 51.829| 51.829|
| T2 (ms)   | 368.399| 368.399| 358.499| 358.499| 358.499|
From Table 1 and Table 2, it can be seen that the binding of macromolecular proteins to water in Hainan East goat meat and Guangxi goat meat is relatively close. With the extension of time, the transverse relaxation time of $T_2$ peak shows irregular change and no significant change, which is consistent with the results of Magnolia et al. [12]. The transverse relaxation time of the peaks of $T_{22}$ and $T_{23}$ decreases with the storage time. This is because the content of free water and non-flowing water decreases and the number of hydrogen nuclei decreases, which shortens the time from excited state to equilibrium state, so the relaxation time of samples becomes shorter.

Table 2. The variation of the peak transverse relaxation time of Hainan East Goat meat with storage time.

| Time(day) | 1   | 2   | 3   | 4   | 5   |
|----------|-----|-----|-----|-----|-----|
| $T_1$ (ms) | 0.848 | 0.803 | 0.803 | 0.895 | 0.825 |
| $T_2$ (ms) | 53.2598 | 53.2598 | 51.8286 | 51.8286 | 51.8286 |
| $T_2$ (ms) | 445.787 | 410.807 | 410.807 | 410.807 | 410.807 |
| Time(day) | 6   | 7   | 8   | 9   | 10  |
| $T_1$ (ms) | 0.871 | 0.848 | 0.895 | 1.054 | 0.803 |
| $T_2$ (ms) | 51.8286 | 50.4359 | 50.4359 | 50.4359 | 50.4359 |
| $T_2$ (ms) | 410.807 | 410.807 | 410.807 | 399.768 | 399.768 |

At present, nuclear magnetic resonance (NMR) refers to the splitting of nuclear energy levels in a magnetic nucleus under the action of a static magnetic field. When the external radio frequency electromagnetic wave acts on the nuclear system under certain energy conditions, the atomic nucleus absorbs the incident electromagnetic wave energy and transits from low energy level to adjacent high energy level. When the RF pulse is turned off, the time for the hydrogen nucleus to recover from the excited state to the equilibrium state is related to the state of the hydrogen nucleus and its physical and chemical environment. Therefore, the transverse relaxation map can be used to reflect the properties, distribution and dynamic information of water in meat products. Water content in Mutton accounts for more than 75% of meat, and physical and chemical changes in mutton are related to water. By studying the water distribution and state change in mutton, we can know the influencing factors of mutton quality change. Figure 4 shows the transverse relaxation $T_2$ inversion results of Hainan East goat meat and Guangxi goat meat at the same time. It can be seen from the figure that the area of Hainan East goat meat $T_{22}$ peak is larger than that of Guangxi goat meat $T_{22}$ peak, indicating that Hainan East goat meat exists between myofibrils and membranes and is not easy to flow water. The transverse relaxation time of the peak value of $T_{22}$ in Hainan East goat meat is smaller than that in Guangxi goat meat. The shorter the relaxation time, the tighter the combination of water and substrates, and the longer the relaxation time, the freer the water [13]. That is to say, Hainan Dongshan goat meat is not only not easy to flow more water, but also closely bound with myofibril, so the meat quality of Hainan Dongshan goat meat is tender. Therefore, Hainan East Goat has the characteristics of thin skin, tender meat, fat but not greasy.
3.2 Changes of PH Value of Hainan East Goat Meat with Storage Time

Hainan East Goat has excellent meat quality, uniform fat distribution, tender skin, thick meat, delicious and tasteless. At present, the pH value is an important index to detect the freshness of mutton. When the pH value drops to near the protein isoelectric point or denatures the protein, it will directly affect the meat quality [14]. The change of pH value of Hainan Donggoat meat with storage time is shown in Fig. 5. The change of mutton pH value in the first three days was not significant, because the physiological metabolism terminated after slaughter, aerobic respiration changed into anaerobic respiration, glycolysis occurred, and the accumulation of lactic acid in the final product decreased the pH value [15]. The pH value of mutton increased continuously from 4 days to 7 days, which may be due to the degradation of protein, glycogen and other macromolecule substances into polypeptides and amino acids under the decomposition of bacteria and enzymes, and the release of alkaline groups and other alkaline substances, thus increasing the pH value of mutton [16].

3.3 Preson correlation analysis between transverse relaxation parameters and pH value of Hainan East goat meat

Table 3 is the Pearson correlation data of transverse relaxation parameters and pH value of Hainan East Goat Meat. The Pearson correlation of T21, T22, T23 and pH value of quality index was analyzed. The results showed that there was a significant negative correlation between transverse relaxation time T22 and mutton pH value, and the correlation coefficient was -0.755. The correlation coefficient between transverse relaxation time T23 and mutton pH value was -0.522. By comparing the correlation analysis between T21, T22, T23 and pH value, it was found that the relaxation time of non-flo wing water T22 was more significantly related to mutton quality. It can be concluded that the change of transverse relaxation time of mutton is closely related to the change of mutton quality. With the change of water content and distribution of mutton, the pH value of mutton changes accordingly, which affects the quality of mutton.

| Variable | T21 | T22 | T23 | pH  |
|----------|-----|-----|-----|-----|
| T21      | 1   |     |     |     |
| T22      | 0.239 | 1   |     |     |
| T23      | 0.151 | 0.901** | 1   |     |
| pH       | 0.065 | -0.755* | -0.522 | 1   |

Note: * P < 0.05, ** P < 0.01

4. Conclusion

In this paper, the effect of storage time on the quality of mutton of different brands was studied by low field nuclear magnetic resonance technology. The results showed that with the prolongation of refrigeration time, the peak value of T22 and T23, the lateral relaxation time and the total peak area of Hainan East Goat meat showed a downward trend. It was not easy for flowing water to be converted into free water, which resulted in the decrease of the content of flowing water and the increase of the content of free water. Compared with Guangxi goat meat, Hainan East goat meat is not easy to flow more water, and closely bound with myofibril, so the meat quality of Hainan East goat meat is tender. According to the Pearson analysis of relaxation parameters and pH value of quality index, there was a significant correlation between relaxation time of T22 and pH value of Hainan Dongshan goat meat. Therefore, low-field nuclear magnetic resonance technology can fully reflect the changes of water content and distribution of meat, and characterize the changes of meat quality. In this paper, the difference of water distribution and its status between Hainan Donggoat meat and Guangxi goat meat was detected to provide data support for Hainan Donggoat meat as a brand mutton.

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