Study of the method of water-injected meat identifying based on low-field nuclear magnetic resonance

Jianmei Xu¹, Qing Lin², Fang Yang¹,²,*, Zheng Zheng³, Zhujun Ai³

¹Modern technology education center, Hainan Medical University, Haikou 571199, China.
²College of medical information, Hainan Medical University, Haikou 571199, China
³College of tropical medicine and laboratory, Hainan Medical University, Haikou 571199, China

*Corresponding author e-mail: hy_lab@126.com

Abstract. The aim of this study to apply low-field nuclear magnetic resonance technique was to study regular variation of the transverse relaxation spectral parameters of water-injected meat with the proportion of water injection. Based on this, the method of one-way ANOVA and discriminant analysis was used to analyse the differences between these parameters in the capacity of distinguishing water-injected proportion, and established a model for identifying water-injected meat. The results show that, except for $T_{21b}$, $T_{22e}$ and $T_{23b}$, the other parameters of the $T_2$ relaxation spectrum changed regularly with the change of water-injected proportion. The ability of different parameters to distinguish water-injected proportion was different. Based on $S$, $P_2$, and $T_{23m}$ as the prediction variable, the Fisher model and the Bayes model were established by discriminant analysis method, qualitative and quantitative classification of water-injected meat can be realized. The rate of correct discrimination of distinguished validation and cross validation were 88%, the model was stable.

1. Introduction

The net weight of the meat can be increased by 15%-20%, by forcing the water into the meat before or after slaughter. Some unscrupulous businessmen are driven by interest, and produce and sell the water-injected meat, so that the water-injected meat has always existed in the Chinese market. However, water-injected destroys the original biochemical environment in the meat, which can cause pathogenic microorganism pollution easily, and not only the quality of the water-injected meat is decreased, and the nutrient content of the meat is destroyed, but also produces a lot of bacteria and toxins, harm to the health of consumers [1,2]. The sensory test method can be used to judge the water-injected meat quickly, but the accuracy is poor. The results by physical and chemical detection are accurate, but it is limited by the venue and time. Therefore, it is necessary to study and establish a method for rapid detection of water-injected meat.

Low-field NMR technique was used to study the moisture content of meat and the detection of water-injected meat, because of it has the characteristics of non-destructive, low-volume and fast detection [3-5]. Water is the most important component in meat, Take the porcine tenderloin as an
example, its water accounted for 72.6%, protein accounted for 21.5%, fat accounted for 4.5% [6]. Hydrogen protons in water are excited by the RF pulse to generate NMR signal. The signal amplitude is related to the proton density and the micro-environment. The moisture content and distribution of meat were investigated by studying the transverse relaxation characteristics of water in meat. The relationship between the parameters of transverse relaxation spectrum and the water-injected proportion is the basis for the study the detection of water-injected meat. Many scholars have done the relevant research [7-10] and obtained some consistent conclusion, but there are also inconsistent results, which need to be verified by experiment. In the method of detecting water-injected meat, Wang Shengwei et al. Used principal component analysis and stepwise discriminant method to detect water- injected meat, but did not give the specific model. Wang Xin et al. studied on the detection of water-injected ground meat, using discriminant analysis, the samples were ground meat, the categories contained 16%-40% moderate and severe water-injected ground meat. This sample is no longer non-destructive, and moderate and severe water-injected meat is easily detected by a sense method.

With the strengthening of market supervision, it is necessary to improve the detection capacity to the lower proportion of water-injected meat. In this experiment, pork samples with less than 14% water-injected were selected, and the regularity of the parameters of the transverse relaxation spectrum with the proportion of water- injected was studied by low field NMR. On the basis of this, the significant difference of the parameters of transverse relaxation spectrum under different proportion of water-injected meat was analysed by one-way ANOVA, and distinguishing ability of different parameters to water-injected proportion was determined. The discriminant analysis method was used to establish the identification model of water-injected meat, to realize the qualitative and quantitative classification of the water-injected meat, and to provide the reference for further research on the detection of water-injected meat.

2. Materials and methods

2.1. Experimental instrument

NMR Imaging Analyzing system MesoMR23-060H-I (Suzhou Niumag Co., Ltd. China); Electronic Balance JA16003 (Shanghai Liangping Instruments Co., Ltd, China); Electric Thermostat Temperature Incubator H.SWX-420BS (Shanghai CIMO Instrument Manufacturing Co. Ltd, China); 100μl Micro Injector (Shanghai Gaoge Industry & Trade Co. Ltd, China).

2.2. Sample preparation

There are usually three water-injected ways for the water-injected meat into the market. One way is to pump water directly into the muscles with a syringe, another way is to irrigate water into the stomach and intestines of living pig, the water enter organ tissue through the blood circulatory system. The last way is to soak the slaughtered body for a long time in the water, so that the water into the tissue. This experiment simulated the water-injected meat that produced by the first way. Five porcine Longissimus dorsi were collected, each was prepared into 15 meat samples, the quality of each sample was 10 ± 0.1g, the meat samples cut into square as possible. A group of 3 meat samples, a total of 5 groups. One group was not injected with water as normal meat samples, the other four groups were injected 2%, 6%, 10% and 14% of purified water, as water-injected meat samples. A total of 75 samples were prepared.75 samples were individually loaded into a small self-styled bag, these meat samples were in the 4°C refrigerator for 6 hours. The water into the meat can have time to participate the biochemical processes inside meat.

2.3. NMR measurements and inversion

Remove the samples which had been kept in cold storage for 6 hours from the refrigerator. The samples with self-styled bag were placed into 32 °C thermostatic incubator for 20 minutes to bring the meat temperature to 32 °C. The meat sample was placed on a specially designed animal bed, and were subjected to NMR measurements using a low-field nuclear magnetic resonance imaging analyser.
Each sample measurement was repeated 2 times. The selected radio frequency pulse sequence is the CPMG sequence, sequence parameters are set as follows: PRG=2, TW=6000ms, TE=0.22ms, NECH=18000, SW=100kHz, RFD=0.1ms, RG1=20.0, DRG1=3db, NS=8. The multi-component inversion of the NMR data was carried out by using the magnetic resonance analysis software Ver4.0 to obtain the transverse relaxation spectrum and the related parameter values.

2.4. Statistical analysis
Statistical analyses were carried using with IBM SPSS Statistics 24. Effect of water-injected proportion on the parameters of transverse relaxation spectrum was analyzed by one-way ANOVA, at the 0.05 significance level, S-N-K was used to compare the mean difference between groups.

3. Results and analysis
3.1. The parameters of transverse relaxation spectrum of experimental meat samples
The transverse relaxation of the moisture in the meat decays according to multi exponential laws [11-12]. The transverse relaxation spectrum of each sample is composed of at least three peaks, which correspond to three different states of water, they are the bound water, the immobilized water and the free water [13-15]. The total area of the three peaks (S) reflects the total moisture contained in the meat. The area of bound water peak, the area of immobilized water peak and the area of free water peak, reflects the water content of three state, respectively. The proportion of bound water peak area to total peak area (P_{21}), the proportion of immobilized water peak area to total peak area (P_{22}) and the proportion of free water peak area to total area (P_{23}), indicate the proportion of water in the three states respectively. Because the water of three different states is different in the micro-environment, they have different transverse relaxation time. T_{21}, T_{22} and T_{23} indicate the transverse relaxation time of bound water, of immobilized water and of free water, respectively, they varies within a certain range. The transverse relaxation time corresponding the start, the vertices, and the end of three peaks are denoted by the subscript letters b, m, e, respectively.

3.1.1. Effect of water-injected on the proportion of water in three states and total peak area, according to the pig No., the samples were divided into 5 groups. One-way ANOVA was performed on P_{21}, P_{22}, and P_{23}. For each group of samples, there was no significant difference in P_{21} between the normal meat samples and the meat samples with small water-injected proportion, between the meat samples with close proportion of water-injected. But the overall trend was that P_{21} decreased with the increase of water-injected proportion.
Figure 1. Effect of water-injected proportion on proportion of different states of water.

There was no significant difference in $P_{22}$, $P_{23}$ between normal meat and 2% water-injected meat, for the three groups of samples whose pig No. was 1, 2 and 3. In addition to the above circumstances, for each group of samples, there was a significant correlation between $P_{22}$, $P_{23}$ and the proportion of water-injected, $P_{22}$ decreased significantly with the increase of water-injected proportion, and $P_{23}$ increased significantly with the increase of water-injected proportion. Effect of the water-injected proportion on $P_{21}$, $P_{22}$ and $P_{23}$ of each group of samples as shown in Figure 1, the height of bar graphs represents the average values of $P_{21}$, $P_{22}$ and $P_{23}$, when the water-injected proportion is 0%, 2%, 6%, 10% and 14%. Fig.1 clearly shows that with the increase of the water-injected proportion, $P_{22}$ significantly decreased, while the $P_{23}$ significantly increased.

Figure 2. Effect of water-injected proportion on mean of total area.
Figure 2 shows the effect of the water-injected proportion on the total peak area of five groups of samples, the vertical bars represent 95% confidence intervals. For a group of samples of pig No.2, there was no significant difference in S between 6% and 10% of water-injected meat samples. For a group of samples of pig No.3, there was no significant difference in S between the normal meat samples and 2% of water-injected meat samples, there was also no significant difference in S between 6%, 10% and 14% of water-injected meat samples. But on the whole, there was a significant positive correlation between the total peak area and the water-injected proportion. The total peak area of five groups of meat increased with the increase of water-injected proportion.

3.1.2. Effects of water-injected on the start time, peak time and end time of three peaks. As can be seen from Table 1, T21b almost no significant change with the change in the water-injected proportion. The bound water is considered to be a water that is adsorbed by the polar groups of the protein molecule and has a multilayer structure [16]. Infusing water in the meat does not affect the innermost structure of bound water, so that the relaxation time of this partially bound water varies. In combination with the peak time and end time of bound water peak, it shows a certain regularity, on the whole, T21m and T21e increase with the increase of water-injected proportion. It shows that with the increase of water-injected proportion, the multilayer water is likely to increase. This part of bound water is far away from protein macromolecules, and the bound becomes smaller and the relaxation time becomes longer.

As can be seen from table 2, for two group of samples of pig No.2 and pig No.5, there was no significant difference in T22b. For the other three groups of samples, on the whole, T22b stepped increased with the increase of the water-injected proportion. In general, T22m stepped increase with the increase of the water-injected proportion too. At the beginning, T22e increase with the increase of water-injected proportion. When the water-injected proportion continues to increase, T22e may increase or decrease. The immobilized water is the water that is present between the fibrils, the muscle fibers and the membranes. Water-injected may alter the reticular formation of myofibrillar proteins and the net charge of proteins. Thus, the micro environment of the immobilized water is changed, and the corresponding changes of T22b, T22m and T22e are made.

Table 1. The start time, the peak time and the end time of the bound water peak (T21b, T21m, T21e) in different water-injected proportion.

| Pig Number | Water-injected proportion | T21b | T21m | T21e |
|------------|---------------------------|------|------|------|
| 1          | 0.10±0.00⁰                | 0.10±0.00⁰ | 0.10±0.01³ | 0.10±0.00⁰ | 0.10±0.00⁰ |
| 2          | 0.10±0.00⁰                | 0.10±0.00⁰ | 0.10±0.00⁰ | 0.10±0.00⁰ | 0.10±0.00⁰ |
| 6          | 0.10±0.00⁰                | 0.10±0.00⁰ | 0.10±0.01³ | 0.10±0.00⁰ | 0.10±0.00⁰ |
| 10         | 0.10±0.00⁰                | 0.10±0.00⁰ | 0.117±0.035³ | 0.10±0.00⁰ | 0.145±0.042³ |
| 14         | 0.10±0.00⁰                | 0.10±0.00⁰ | 0.132±0.053³ | 0.128±0.043³ | 0.139±0.031³ |
| 0          | 0.769±0.083⁰              | 0.730±0.051³ | 0.736±0.030⁰ | 0.750±0.017³ | 0.827±0.039³ |
| 2          | 0.758±0.039³              | 0.828±0.066³ | 0.695±0.033³ | 0.804±0.042³ | 0.903±0.065³ |
| 6          | 0.929±0.065³              | 0.751±0.043³ | 0.709±0.047³ | 0.860±0.048³ | 0.911±0.040³ |
| 10         | 0.911±0.039³              | 0.860±0.059³ | 0.876±0.043³ | 0.937±0.045³ | 0.975±0.057³ |
| 14         | 0.965±0.042³              | 1.001±0.049³ | 0.869±0.067³ | 1.054±0.074³ | 0.985±0.076³ |
| 0          | 2.69±0.27³                | 2.51±0.18³ | 2.086±0.085³ | 2.210±0.070³ | 2.554±0.093³ |
| 2          | 2.39±0.10⁰                | 2.87±0.21³ | 2.068±0.088³ | 2.53±0.17³ | 2.84±0.12³ |
| 6          | 2.99±0.24³                | 2.73±0.12³ | 2.167±0.052³ | 2.87±0.19³ | 2.923±0.088³ |
| 10         | 3.13±0.10⁰                | 3.13±0.22³ | 2.812±0.083³ | 3.32±0.16³ | 3.067±0.074³ |
| 14         | 3.58±0.16³                | 3.74±0.20³ | 3.07±0.17³ | 3.83±0.24³ | 3.35±0.16³ |
Note: The data in the same column marked with the same superscript letters were not significantly different (P<0.05), there were significant differences in the data with different superscript letters (P>0.05).

Table 2. The start time, the peak time and the end time of the immobilized water peak ($T_{22b}$, $T_{22m}$, $T_{22e}$) in different water-injected proportion.

| $T_{22}$ Water-injected proportion | Pig Number |
|-----------------------------------|------------|
|                                  | 1          | 2          | 3          | 4          | 5          |
| $T_{22b}$ 0                      | 18.43±0.44a| 20.90±0.63a| 18.25±0.00a| 18.25±0.00c| 18.98±0.56a|
| 2                                  | 19.72±0.60b| 21.30±0.63a| 18.61±0.56a| 17.91±0.53b| 18.43±0.44b|
| 6                                  | 19.34±0.00b| 21.30±0.63a| 19.16±0.44a| 19.72±0.60b| 18.43±0.44b|
| 10                                 | 19.73±0.00b| 21.30±0.63a| 19.34±0.00b| 19.5±0.4b| 18.81±0.89b|
| 14                                 | 20.30±0.47d| 21.71±0.00a| 19.34±0.00b| 18.80±0.60c| 18.98±0.56a|
| $T_{22m}$ 0                      | 44.33±1.3d| 47.91±1.4b| 43.47±0.00c| 43.47±0.00d| 45.2±1.3b|
| 2                                  | 45.6±1.1b| 48.80±0.00b| 43.47±0.00c| 44.3±1.3d| 46.06±0.00b|
| 6                                  | 46.06±0.00b| 48.80±0.00b| 43.47±0.00c| 47.9±1.4b| 46.06±0.00b|
| 10                                 | 46.06±0.00b| 48.80±0.00b| 46.06±0.00b| 46.06±0.00b| 46.06±0.00b|
| 14                                 | 47.91±1.4f| 51.71±0.00e| 46.06±0.00f| 46.5±1.1f| 47.0±1.4f|
| $T_{22e}$ 0                      | 117±11d| 110.8±2.7e| 97.71±0.00d| 118.5±3.6e| 117.1±1.3d|
| 2                                  | 114±11d| 125.6±3.8f| 110.8±2.7f| 141±1.1f| 130.4±0.9f|
| 6                                  | 140±4.3b| 135.7±4.0f| 117.5±6.6f| 160±2.4f| 141±4.3f|
| 10                                 | 146±27d| 146.5±0.00e| 133±4.0f| 15±18e| 161.4±4.8e|
| 14                                 | 131±7.4h| 174.26±0.00b| 122.1±5.3h| 165±15g| 152±4.5g|

Note: The data in the same column marked with the same superscript letters were not significantly different (P<0.05), there were significant differences in the data with different superscript letters (P>0.05).

It can be seen from Table 3 that $T_{23b}$ sometimes decreases with the increase of water-injected proportion, sometimes decreases first, then increases, sometimes decreases first, then increases and decreases. While $T_{23m}$ and $T_{23e}$ showed a very strong regularity, with the increase of water-injected proportion, first decrease and then increase. The mechanism of this phenomenon is unknown.

Table 3. The start time, the peak time and the end time of the free water peak ($T_{23b}$, $T_{23m}$, $T_{23e}$) in different water-injected proportion.

| $T_{23}$ Water-injected proportion | Pig Number |
|-----------------------------------|------------|
|                                  | 1          | 2          | 3          | 4          | 5          |
| $T_{23b}$ 0                      | 220±19a| 265±22a| 261.27±0.00a| 192.1±9.2a| 231±24a|
| 2                                  | 222±18a| 222±15a| 203.4±6.0a| 171.0±5.1a| 188.3±5.7a|
| 6                                  | 184.6±4.00b| 220±11a| 221±31b| 200±16a| 184.7±6.8a|
| 10                                 | 210±20a| 228.4±6.8b| 216±15b| 220±10b| 190.1±6.7b|
| 14                                 | 258±11b| 219.64±0.00b| 260±30b| 199.6±9.3b| 213±6.7b|
| $T_{23m}$ 0                      | 416±24d| 453±28d| 493.70±0.00c| 370±19d| 429±42d|
| 2                                  | 416±24d| 407±12d| 377±11d| 323.1±9.6d| 377±11d|
| 6                                  | 348.9±1.00c| 407±12d| 402±53d| 392±20b| 377±11d|
| 10                                 | 389±3.2d| 449±27d| 404±29d| 424±25d| 395.6±9.5d|
| 14                                 | 466±24d| 461±12d| 473±49d| 440±14d| 471±20d|
| $T_{23e}$ 0                      | 629±43e| 624±47e| 698.59±0.00b| 544±33b| 650±64e|
| 2                                  | 600±35f| 571±35f| 544±33f| 480±15f| 560±24f|
| 6                                  | 523.1±1.00e| 576±17e| 579±64e| 599±28e| 576±17e|
| 10                                 | 572±40f| 667±46f| 635±38g| 675±71f| 611±18g|
| 14                                 | 734±49g| 716±36g| 735±61g| 741±27m| 748±32g|
Note: The data in the same column marked with the same superscript letters were not significantly different (P<0.05), there were significant differences in the data with different superscript letters (P>0.05).

3.2. Establishment of water-injected meat identification model
The result of the data analysis is that, except for $T_{21b}$, $T_{22e}$ and $T_{23b}$, the other parameters of the $T_2$ relaxation spectrum change regularly with the change of water-injected proportion. Whether meat was injected with water or not, and how much water was injected can be reflected by the change of these parameters. However, because the pig has a biological difference, the normal meat samples taken from the same parts of different pigs was measured, the measured parameters of the value is also different. It is difficult to determine the threshold of normal meat on the parameter. A statistical methods are needed for the study. The discriminant analysis in multivariate statistical method is adopted. The meat samples were classified according to the water-injected proportion, some parameters of the transverse relaxation spectrum are used as the prediction variables, through the training of the known samples, the rules of classification was summarized, discriminant functions was established, to realize the judgment and classification of water-injected meat. In this experiment, meat samples were divided into five categories, according to 0%, 2%, 6%, 10% and 14% water-injected proportion. The first category was the normal meat without water-injected, the second to fifth categories were water-injected meat with 4 different water-injected proportion.

3.2.1. Select the predictive variables to establish the discriminant function. Different parameters of transverse relaxation spectrum can reflect the changes of different properties in the meat samples with different proportion of water-injected, these parameters differ in their ability to distinguish between the water-injected proportions. The average values of parameters of 5 group of samples at different water-injected proportion were calculated, and one-way ANOVA was carried out at 95% confidence probability. The results show that $S$, $P_{22}$ and $P_{23}$ have a stronger ability to distinguish between the water-injected proportions. However, for the normal meat and 2% of water-injected meat, the difference of some $S$, $P_{22}$, $P_{23}$ were small and mixed together, resulting in distinguishing between the normal meat and 2% of water-injected meat difficulties. Taking into account the peak time and the end time of free water peak with the water-injected proportion decreased first and then increased, so that $T_{23m}$ and $T_{23e}$ of the normal meat mixed together with 14% of water-injected meat, and was separated from 2% of water-injected meat. $T_{23m}$ and $T_{23e}$ can provide a basis for distinguishing normal meat from the water-injected meat with low proportion.

In summary, the two times of the free water peak and the several parameters with strong ability to distinguish between water-injected proportions were selected as the prediction variables of the discriminant function. According to the assumption of discriminant analysis, each prediction variables cannot be a linear combination of others. In other words, the predictive variables are independent of each other and have no multicollinearity. Otherwise it will affect the stability of the discriminant function. It is necessary to remove the variables that produce multiple collinearity in the prediction variables. The multicollinearity of parameters of the same type is analysed preliminarily, by the cross-scatter diagram, the results show that there is a very significant linear relationship between $P_{22}$ and $P_{23}$, and only one can be selected as prediction variables. There is also a significant linear relationship between the two times of the free water peak, and only one of the two times is chosen as the prediction variables. Finally, $S$, $P_{22}$, and $T_{23m}$ were selected as a prediction variables.

3.2.2. Establishing the discriminant function. First, testing whether $S$, $P_{22}$, and $T_{23m}$ are appropriate as prediction variables. Table 4 is the result of tests of equality of class means. Wilks' lambda value is between 0~1, and the smaller the value, the greater the difference between the class means. The mean of $S$ is the largest difference among the categories, and $P_{22}$ is the second, the $T_{23m}$ is the least. The difference between categories reached a very significant and significant level, because of $P$ of the three
items were less than 0.05, indicating that the three parameters are suitable as the prediction variables of the discriminant analysis, which is consistent with the previous analysis.

**Table 4. Tests of Equality of Class Means.**

| Predictive variables | Wilks’ Lambda | F    | df1 | df2 | P     |
|----------------------|---------------|------|-----|-----|-------|
| S                    | 0.094         | 48.083 | 4   | 20  | 0.000 |
| P_{22}               | 0.155         | 27.311 | 4   | 20  | 0.000 |
| T_{23m}              | 0.436         | 6.459  | 4   | 20  | 0.002 |

The Fisher criterion is chosen as the criterion, and the Fisher discriminant functions is established according to the Canonical discriminant function coefficients. Significance level of the discriminant functions is tested according to the Wilks’ Lambda test results shown in table 5. Three discriminant functions will appear due to the selection of three prediction variables. The third function of \( P = 0.665 > 0.05 \), indicating that the difference of the function between the five categories did not reach a significant level, the third function is not statistically significant. Therefore, only the first two functions are taken as Fisher discriminant functions. The Fisher discriminant function is

\[
y_1 = 0.002S - 46.642P_{22} + 0.015T_{23m} - 4.093
\]

\[
y_2 = -0.001S - 10.805P_{22} + 0.031T_{23m} + 13.184
\]

Identification model of water injection meat based on Fisher discriminant criterion was established. The first function has the ability to distinguish more than the second function, and the cumulative contribution rate of the two functions is 99.8%.

**Table 5. Tests of Wilks’ Lambda.**

| Test of Function | Wilks’ Lambda | Chi-square | df | Sig. |
|-----------------|---------------|------------|----|-----|
| 1 to 3          | 0.019         | 78.883     | 12 | 0.000 |
| 2 to 3          | 0.479         | 14.724     | 6  | 0.023 |
| 3               | 0.960         | 0.816      | 2  | 0.665 |

According to the Fisher discriminant function, the discriminant score at the mean value of the five categories of meat samples was calculated, as the score value of each class of centroids. The abscissa represents the first discriminant function, and the ordinate represents the second discriminant function, the centroid of each category can be plotted in the figure. Then substitute the measurement value of the prediction variable of each meat sample into the two discriminant functions, to calculate the score, respectively, and in the Figure marked the meat sample position. Drawing the diagram of discrimination score of the meat samples with different injection proportion. It can be seen from the Figure 3, the centroids of five categories is far away. There are significant differences between the various categories. Five categories of meat samples with different water-injected proportion in the Figure can be basically differentiated.
Figure 3. Discriminant score plot of the training meat samples with different water-injected proportion.

The Bayes criterion is chosen, the classification function coefficients is used to establish the Bayes discriminant function for distinguishing five categories of meat samples with different water injection proportion. The Bayes discriminant function is

\[
y_1 = 0.088S + 2462.565P_{22} + 0.066T_{23w} - 1828.083
\]

\[
y_2 = 0.091S + 2440.819P_{22} + 0.016T_{23w} - 1842.291
\]

\[
y_3 = 0.1S + 2311.685P_{22} + 0.052T_{23w} - 1879.096
\]

\[
y_4 = 0.107S + 2118.803P_{22} + 0.122T_{23w} - 1856.477
\]

\[
y_5 = 0.116S + 1926.576P_{22} + 0.22T_{23w} - 1899.264
\]

Bayes discriminant requires homogeneity of the covariance of the sample. The results of the hypothesis test show that the statistic of Box 'M' is 45.833>0.05, and the covariance matrices of various category are equal at the significance level of 0.05, and the Bayes discriminant function is valid. The prediction variable values of each meat sample are substituted into the five discriminant functions, and the function value is obtained. Comparing these functions values, the largest value of the function are the categories in which the meat sample is classified. According to the Bayes discriminant function and the prediction variable value of the measured meat, the meat samples can be classified.

3.2.3. Judgment effect and stability verification of Bayes discriminant model, the discriminant effects of Bayes discriminant models have been tested by means of distinguished validation and cross validation. Distinguished validation: the training meat samples is reclassified according to the established discriminant function. The closer the classification is to the actual classification, the better the discriminant effect. Cross-validation method is to first remove a primitive meat sample, re-establish the discriminant function, and then use the discriminant function of the sample classification. Continue to repeat until the completion of the identification of all the original meat samples, calculate the accuracy of discrimination [17].
The results of validating the model using two methods are shown in Table 6. As can be seen from Table 6, the accuracy of the distinguished validation and the cross validation is 88%. The results of the two methods are the same, indicating that the established discriminant model is stable [18]. There were three wrong judgment, one of the normal meat samples belonging to category 1 was misjudged to category 2, and the other 2% of the injected-meat belonging to category 2 was misjudged to category 1 and 6% of the injected-meat belonging to category 3 was misjudged to category 2. Misjudgment occurs between the meat samples which there was a small difference in water injection.

**Table 6.** Results of distinguished validation and cross validation statistics.

| Method                  | Groups | Prediction Group Member |
|-------------------------|--------|-------------------------|
|                         |        | 1 | 2 | 3 | 4 | 5 | Total |
| Counting                | 1      | 4 | 1 | 0 | 0 | 0 | 5   |
|                         | 2      | 1 | 4 | 0 | 0 | 0 | 5   |
|                         | 3      | 0 | 1 | 4 | 0 | 0 | 5   |
|                         | 4      | 0 | 0 | 0 | 5 | 0 | 5   |
|                         | 5      | 0 | 0 | 0 | 0 | 5 | 5   |
| Redistinguished Validation Counting(%) | 1 | 80 | 20 | 0 | 0 | 0 | 100 |
|                         | 2 | 20 | 80 | 0 | 0 | 0 | 100 |
|                         | 3 | 0 | 20 | 80 | 0 | 0 | 100 |
|                         | 4 | 0 | 0 | 0 | 100 | 0 | 100 |
|                         | 5 | 0 | 0 | 0 | 0 | 100 | 100 |
| Counting                | 1      | 4 | 1 | 0 | 0 | 0 | 5   |
|                         | 2      | 1 | 4 | 0 | 0 | 0 | 5   |
|                         | 3      | 0 | 1 | 4 | 0 | 0 | 5   |
|                         | 4      | 0 | 0 | 0 | 5 | 0 | 5   |
|                         | 5      | 0 | 0 | 0 | 0 | 5 | 5   |
| Cross Validation        | 1      | 80 | 20 | 0 | 0 | 0 | 100 |
|                         | 2 | 20 | 80 | 0 | 0 | 0 | 100 |
|                         | 3 | 0 | 20 | 80 | 0 | 0 | 100 |
|                         | 4 | 0 | 0 | 0 | 100 | 0 | 100 |
|                         | 5 | 0 | 0 | 0 | 0 | 100 | 100 |

Notes: 88.0% of the samples in prediction group were classified correctly.

4. Conclusion

When the proportion of water-injected does not exceed 14%, $S$, $P_{23}$, $T_{21m}$, $T_{21e}$, $T_{22b}$ and $T_{22m}$, increases with the increase of water-injected proportion. $P_{21}$ and $P_{22}$ decreases with the increase of water-injected proportion. $T_{23m}$ and $T_{23e}$ decreases first and then increases with the increase of the water-injected proportion.

The ability of different parameters to distinguish water-injected proportion is different. The parameters whose ability of distinguishing water-injected proportion is strong are $S$, $P_{22}$ and $P_{23}$.

Based on the parameters of transverse relaxation spectrum, the identification model of water-injected meat was established by discriminant analysis, and the classification of water-injected meat can be realized theoretically. Based on the total peak area, the proportion of immobilized water and the peak time of free water peak as the predictive variables, the Fisher model and the Bayes model were established by discriminant analysis method. The Fisher model was used to draw the discriminant score graph, and the water-injected meat samples was qualitatively classified. Bayes model can be used for quantitative classification of the water-injected meat samples, the rate of correct discrimination of distinguished validation and cross validation were 88%, the model was stable.

In order to further improve the reliability and accuracy of the discriminant model, more samples should be trained in the follow-up research, and new predictive variables should be found to reduce the false positive rate.
Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (No.11364004), Science research project of Hainan higher education institutions (Grant No. Hnjg2017-41), education research project of Hainan Medical University (Grant No. HYZX201603) and Innovative training program for Chinese University Students (Grant No.201611810076). J. M. Xu and Q. Lin contributed equally to this work. J.M. Xu, F. Yang and Q. Lin participated in experimental design. J.M. Xu, Z. Zheng and Z.J. Ai collects data. J.M. Xu and F. Yang analyzed the data. All authors discussed the results and commented on the manuscript.

References

[1] Ni Zecheng. The reasons and countermeasures for water-injected meat. Journal of Agricultural Sciences 2016,37 (4): 89-92.
[2] Cheng Linghao, Jiao Yongliang. Inspection Methods of the PSE Meat, Water-Injected Meat and Gel-Injected Meat. China Animal Health Inspection 2015,32 (4):28-31.
[3] D Fan, S Ma, L Wang, et al. 1H NMR studies of starch-water interactions during microwave heating. Carbohydrate Polymers, 2013,97 (2):406-412.
[4] FL Chen, TM Wei, B Zhang. Characterization of water state and distribution in textured soybean protein using DSC and NMR. Journal of Food Engineering, 2010, 100 (3): 522–526.
[5] M Li, H Wang, G Qiao, et al. Determining the drying degree and quality of chicken jerky by LF-NMR. Journal of Food Engineering 2014, 139 (139):43–49.
[6] Sui Jixue, Zhang Yiming. Frozen food technology. Beijing: China Agricultural University press, 2015 (10):18.
[7] ZL Pang, HE Xu-Xiao, LI Chun-Bao. A Method for Detection of Water Content in Pork Using Low-Field Nuclear Magnetic Resonance (LF-NMR). Food Science, 2014,35 (4):142-145.
[8] LL Chen, LI Xia, CH Zhang, CH Tang. Determination of Different State Moisture Content in Five Kinds of Meat Using Low-Field NMR. Journal of Analytical Science, 2015,31 (1):90-94.
[9] Wang SW, Mu YC, Zhao X, et al. Study on discrimination of gum-injected, water-injected mutton based on the LF-NMR relaxometry characteristics. Food Ind, 2015, (6): 184-188.
[10] Wang X, Wang Z Y, Chen L H, et al. LF-NMR Relaxation Characteristics and Discriminant Analysis of Water-injected Ground Meat. Modern Food Science & Technology, 2016, (5): 79-84.
[11] CF Hazlewood, BL Nichols, NF Chamberlain. Evidence for the existence of a minimum of two phases so ordered water in skeletal muscle. Nature, 1969, 222 (5195): 747-750.
[12] RJ Brown, F Capozzi, C Cavani, et al. Relationships between 1H NMR relaxation data and some technological parameters of meat: a chemometric approach. Journal of Magnetic Resonance, 2000, 147 (1): 89-94.
[13] Fjelknermodig S, Tornberg E. Water distribution in porcine M. longissimus dorsi in relation to sensory properties. Meat Science, 1986, 17 (3):213-31.
[14] Tornberg E, Andersson A, Göransson A, et al. Water and fat distribution in pork in relation to sensory properties. Pork quality: Genetic and metabolic factors, 1993: 239-256.
[15] Bertram H C. Field gradient CPMG applied on postmortem muscles. Magnetic Resonance Imaging, 2004, 22 (4):557-563.
[16] Ruan RS. Application of nuclear magnetic resonance technology in food and biological system. Beijing: China Light Industry Press, 2009.
[17] Zhao Naqing, Yin Ping. Medical data analysis. Shanghai: Fudan University Press,2014 (07):192.
[18] Chen H J, Xi-Bing L I, Liu A H, et al. Identifying of mine water inrush sources by Fisher discriminant analysis method. Journal of Central South University, 2009, 40:1114-1120.