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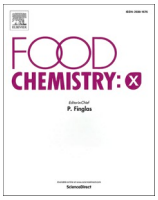

Evaluation of the water state and protein characteristics of Tibetan pork under the storage conditions of modified atmosphere packaging: Effect of oxygen concentration

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ABSTRACT

To explore the changes in water status and protein characteristics of Tibetan pork (TP) under modified atmosphere packaging (MAP) with different oxygen concentrations compared to Duroc×Landrace×Yorkshire pork (DLY), the water holding capacity (WHC), water distribution, protein oxidation, and conformation of both types were determined. Results indicate that under MAP, TP pork and DLY pork exhibited higher water retention and lower protein oxidation compared to air packaging. However, with increased oxygen concentration in the MAP, protein oxidation intensified, leading to reduced WHC in the pork. Compared to DLY pork, TP pork in different packaging conditions maintained the integrity of protein secondary and tertiary structures, reducing protein cross-linking aggregation. The lower content of P_3 in the two-dimensional relaxation spectra, shorter T_1 and T_2 relaxation times, and higher proton density suggest better water retention properties in Tibetan pork. These findings support the development of long-distance preservation and transportation technologies for TP pork.

1. Introduction

The Tibetan pig, which thrives in high-altitude, frigid regions above 3000 m, is a unique local breed in China. As a highland specialty breed, Tibetan pigs possess incomparable advantages compared to numerous exotic and other local pig breeds, such as strong disease resistance and cold tolerance, excellent meat quality, and abundant protein and mineral content (Zhao et al., 2023). However, most of the production areas for Tibetan pork are in remote mountainous regions, where outdated preservation techniques often lead to water loss during storage and transportation. The loss of juice provides favorable conditions for microbial growth and further accelerates the deterioration of Tibetan pork quality (Den Hertog-Meischke et al., 1997), resulting in Tibetan pork not being able to be preserved for long periods of time and transported over long distances. Therefore, developing preservation technologies that delay the quality deterioration of Tibetan pork due to moisture loss during storage and transportation is a key factor in the development of the Tibetan pork industry.

High-oxygen modified atmosphere packaging (80 % O_2 and 20 % CO_2, HiOxMAP) is one of the traditional commercial packaging methods for fresh meat, which typically improves meat colour stability and extends shelf life (Yang et al., 2022). Some studies have shown that HiOxMAP can enhance the water-holding capacity (WHC) of meat during storage. Chen et al. (2015) found that meat under HiOxMAP had higher WHC compared to vacuum-packaged meat. Cayuela et al. (2004) reported similar findings, observing higher storage losses in vacuum-packaged samples compared to HiOxMAP. However, HiOxMAP may lead to the oxidation and denaturation of lipids and proteins. Protein oxidation significantly affects meat quality, typically resulting in decreased WHC, tenderness, and nutritional value (Liang et al., 2024). Qian et al. (2022) reported increased protein denaturation due to decreased solubility and increased surface hydrophobicity, which are unfavorable for maintaining muscle structure and moisture. Pan et al. (2022) indicate that the loss of moisture in muscles is caused by changes in myofibril spacing due to protein oxidation cross-linking. These studies suggest that traditional HiOxMAP, due to excessive protein oxidation, is

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no longer suitable for maintaining meat WHC. It is necessary to explore the effects of different packaging methods, such as appropriately reducing oxygen concentration, on meat WHC and protein characteristics. Furthermore, there are limited reports on the application of MAP in the storage of Tibetan pork. Therefore, to reflect the changes in muscle WHC of Tibetan pork under modified atmosphere packaging, it is necessary to study the effects of modified atmosphere packaging on the changes in protein and moisture content of Tibetan pork and their interactions to further provide a basis for the control of its quality during storage.

Currently, common methods for determining meat WHC include the pressure loss method, near-infrared analysis technology, the drip loss method, and the conductivity method. These methods only qualitatively reflect the WHC of meat and cannot characterise the presence and migration processes of different moisture groups in meat (Oswell et al., 2021). Low-field nuclear magnetic resonance (LF-NMR), as a novel, rapid, non-destructive detection technology, is one of the most effective means to study water distribution and migration in muscles (Song et al., 2021). Lin et al. (2022) used LF-NMR technology to study the WHC of beef under supercooling assisted by a static magnetic field, demonstrating that the static magnetic field treatment can alter the distribution and migration of water in beef muscle, thereby maintaining WHC. Guo et al. (2023) studied the moisture migration during storage of post-slaughter Tibetan yak meat using LF-NMR, providing a theoretical basis for improving the quality of Tibetan yak meat. In recent years, two-dimensional LF-NMR has been developed to better display changes in moisture status. Two-dimensional LF-NMR technology typically uses IR-CPMG sequences to obtain all parameters of transverse and longitudinal relaxation. Compared to traditional LF-NMR, two-dimensional LF-NMR can more detailed and accurately reveal the moisture status in meat (Qian et al., 2022). Song et al. (2021) used two-dimensional LF-NMR technology to study the changes in moisture content of pork under steaming and boiling conditions, showing that the T_1 relaxation time decreased due to steaming heating, weakening the exchange of hydrogen protons with the external environment. Qian et al. (2022) investigated the changes in myowater of beef after thawing at different freezing rates, revealed through two-dimensional LF-NMR that during the thawing process of beef, T_1 and T_2 relaxation times increased, leading to the redistribution of myowater. However, these studies primarily focus on the thermal processing of meat and frozen meat. Research on the application of 2D LF-NMR technology in the storage of chilled meat, particularly for the precise analysis of moisture state changes in Tibetan pork, remains to be explored.

Therefore, this study is based on air packaging and modified atmosphere packaging with different oxygen concentrations (40 % O_2, 60 % O_2, and 80 % O_2). Using two-dimensional LF-NMR technology, it explores changes in moisture state and combines protein characterization to elucidate the relationship between protein and water. This aims to reveal the WHC of Tibetan pork under different storage conditions, provide support for the development of preservation and transportation techniques for Tibetan pork, and address the issue of "cannot be stored, cannot be transported."

2. Materials and methods

2.1. Sample preparation

The experimental materials comprised 10 Tibetan pigs (TP) and 10 Duroc × Landrace × Yorkshire (DLY) pigs at the age of 180 days, provided by a local slaughterhouse in the Linzhi area of Tibet. The *M. longissimus thoracis et lumborum* (LTL) muscles from both sides were removed and aged for 24 h at 4 °C. After ageing, 20 LTL muscle samples from Tibetan pork and 20 pieces from DLY pork were selected and stripped of connective tissue, then trimmed into 100 blocks measuring 8 cm × 2 cm × 2 cm. One randomly selected block from each LTL muscle was assigned to each of five treatment groups, resulting in a total of 20 blocks. These groups were: unpackaged pork (Fresh, day 0), air packaging (Air), modified atmosphere packaging (MAP) with 40 % O_2, 20 % CO_2, and 40 % N_2 (MAP 40), MAP packaging with 60 % O_2, 20 % CO_2, and 20 % N_2 (MAP 60), and MAP packaging with 80 % O_2 and 20 % CO_2 (MAP 80). The samples were stored in MAP boxes with different gas compositions for refrigeration in a high-precision constant temperature test box set at 4 °C ± 0.5 °C. The ratio of sample volume to gas volume in the modified atmosphere packaging boxes was 1:3. The packaging material for MAP was composed of polyamide/polyethylene film (oxygen permeability less than 6 cm^3 /m^2 /d), while the material for air packaging was polyvinyl chloride film for freshness (oxygen permeability of 15,500–17,000 cm^3 /m^2 /d), which was wrapped around the packaging box. After 7 days of meat storage, the gas composition within the packaging boxes was checked to confirm no significant changes. Subsequently, the meat samples were taken out to determine water-retaining property, protein oxidation, and protein conformation indicators.

2.2. Water holding capacity

2.2.1. Purge loss

The evaluation of purge loss in pork, as described by Wang et al. (2019), involves calculating the proportion of weight lost during storage. At first, the weight of each piece of pork measuring 2 cm × 2 cm × 2 cm was recorded. After being stored, samples taken from trays were promptly dried with a towel before being weighed. The purge loss was determined by the weight before storage (m_1) and the weight after storage (m_2) and calculated by Eq. (1):

$$\text{Purge loss } (\%) = \frac{m_1 - m_2}{m_1} \times 100 \quad (1)$$

2.2.2. Cooking loss

The determination of cooking loss in pork samples under different packaging methods was based on the method by Song et al. (2021), with slight modifications. For the assessment of cooking loss, 10 g of pork samples were employed. These meat specimens were enclosed in polyethylene vacuum bags and immersed in an 80 °C water bath until the inner temperature reached 75 °C, followed by cooling to ambient temperature using running water. Absorbent paper was utilized to eliminate any exudation during the cooking phase. The cooking loss was ascertained by comparing the pre-cooking weight (m_3) to the post-cooking weight (m_4), as calculated using Eq. (2):

$$\text{Cooking loss } (\%) = \frac{m_3 - m_4}{m_3} \times 100 \quad (2)$$

2.2.3. Centrifuging loss

The determination of centrifuging loss in pork samples followed the procedure outlined by Lin et al. (2022). Each meat sample, weighing 2 g, was wrapped in filter paper and subjected to centrifugation (1500*g*, 4 °C) for a duration of 10 min. Centrifuging loss was assessed by comparing the sample's weight before centrifugation (m_5) to its weight after centrifugation (m_6), as calculated using Eq. (3):

$$\text{Centrifuging loss } (\%) = \frac{m_5 - m_6}{m_5} \times 100 \quad (3)$$

2.3. 2D LF-NMR and MRI

The methodology described by Song et al. (2021) was employed for assessing water distribution. Utilizing an LF-NMR Analyzer (PQ-001, Niumag Electric Corporation, Shanghai, China), equipped with specific parameters including a magnetic field strength of 0.5 T, a probe coil diameter of 60 mm, operating at 32 °C, and a spectrometer frequency of 23 MHz, 2D T_1-T_2 relaxation measurements were conducted. Meat blocks subjected to different treatments, weighing approximately 10 g

and measuring 2 cm × 2 cm × 2 cm, were sectioned and positioned within cylindrical glass tubes for NMR scans. Employing an IR-CPMG sequence within a 60-mm glass tube, T_1-T_2 spectra were captured. Each sample was subjected to 21 separate scan repeats, and relaxation data were gathered for periods of 12 and 24 μs using pulses of 90° and 180° from 4000 echoes. The group proportions were computed using an algorithm based on cumulative integration inside the multi-exponential decay model, which was made possible by the data analysis program MultiExp Inv Analysis (Niumag Electric Corporation, Shanghai, China). By inverting just one component, we were able to determine all relaxation times for T_1 and T_2 for 21 scans.

MRI, following the method of Hu et al. (2021) with minor adaptations, was employed to examine the spatial water distribution in meat samples. Obtain proton density images using a multiple spin-echo sequence with TR of 1000 ms, frequency value of 23.128 MHz, and TE of 19.2 ms. After image acquisition, export pseudocolored images using image evaluation software.

2.4. Extraction of myofibrillar protein (MP)

Extraction and concentration of MP were carried out from pork samples under different packaging conditions. Four times the volume of precooled NaCl-PBS (20 mmol/L, pH 7.0) was added to the weighed pork samples, which were then homogenized using an HR-500 shear homogenizer (HUXI Company, Shanghai, China). The homogenate was centrifuged at 3000*g* for 10 min, and the pellet was washed three times with NaCl-PBS solution (40 mL) and twice with NaCl (40 mL, 0.1 mol/L). Purified MP were obtained thereafter. Protein concentration was determined using the bicinchoninic acid assay, and adjusted according to experimental requirements for further analysis.

2.5. Protein oxidation

2.5.1. Carbonyl content

Carbonyl content was determined according to the method of Yang et al. (2024). First, two portions of MP were diluted to 2 mg/mL each, and then each diluted MP was added to separate centrifuge tubes. To both the control and experimental groups, 1 mL of 2 mol/L HCl and 10 mmol/L DNPH were added, respectively. The tubes were oscillated in darkness every 15 min, repeating four times. Subsequently, 1 mL of 20 % (*w/v*) TCA was added to the tubes and allowed to react for 15 min, followed by centrifugation at 10000*g* for 5 min. The precipitate was washed three times with a 1:1 (*v*/v) mixture of absolute ethanol and ethyl acetate. 3 mL of a 6 mol/L guanidine hydrochloride solution was used to dissolve the precipitate in a water bath at 37 °C for 15 min. Finally, the supernatant was collected after centrifugation at 10000*g* for 5 min, and absorbance was measured at 370 nm. Results are expressed as nmol carbonyls/mg protein.

$$\text{Carbonyl content (nmol/mg)} = \frac{A_{370\ \text{sample}} - A_{370\ \text{blank}}}{4.4 \times (\text{protein})} \quad (4)$$

where:$A_{370\text{sample}}$ denotes the absorbance measured at 370 nm for the supernatant of the experimental group, while $A_{370\text{blank}}$ signifies the equivalent measurement for the control group. Protein refers to the concentration of myofibrillar protein, which is maintained at 2 mg/mL.

2.5.2. Total sulfhydryl

The approach described by Wu et al. (2023) was utilized for assessing the overall sulfhydryl content in pork MP. Add 20 μL of Ellman's reagent to 1 mL of 5 mg/mL MP, along with 5 mL of 0.02 mol/L Tris-HCl buffer, and vortex for 1 min. After vortexing, incubate the mixture in a water bath at 25 °C for 1 h. Subsequently, centrifuge at 5000*g* for 15 min. Finally, measure the absorbance of the supernatant at 412 nm and calculate the thiol content using an extinction coefficient of 13,600 M^{-1} cm^{-1}.

2.5.3. Solubility

The method described by Qian et al. (2022) was used to determine the solubility of MP in different packaging modes. The MP samples that were obtained were first diluted with a 20 mmol/L phosphate buffer (pH 7.0) to achieve a concentration of 10 mg/mL. After subjecting the mixture to centrifugal force of 5000 times the acceleration due to gravity for a duration of 20 min at a temperature of 25 degrees Celsius, the salt-soluble and insoluble fractions were successfully separated. Afterwards, the protein content of the liquid remaining after centrifugation was measured using the Biuret technique. The protein solubility was determined by dividing the protein concentration in the supernatant by the starting protein concentration, and expressing the result as a percentage.

2.5.4. Surface hydrophobicity

Surface hydrophobicity was determined following the method of Qian et al. (2022). Firstly, the MP solution was diluted to 2 mg/mL. The diluted MP solution was transferred to a centrifuge tube, and 200 μL of 1 mg/mL bromophenol blue (BPB) were added. After thorough mixing, the mixture was incubated at 25 ± 0.5 °C for 15 min, followed by centrifugation at 4000*g* for 15 min. The control group had identical experimental conditions except for the absence of pork samples. Finally, the 1 mL supernatant after centrifugation was combined with PPDS (9 mL), and absorbance was calculated at 595 nm. The amount of bound BPB estimated from the change between total BPB and free BPB using the following formula determines the surface hydrophobicity of MP samples:

$$\text{Bound BPB } (\mu g) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 40 \mu g \quad (5)$$

with A = absorbance at 595 nm.

2.6. Protein structure

2.6.1. MP secondary structure

Utilizing the Tensor 27 instrument from Germany, the Fourier transform infrared (FTIR) spectrum was obtained to analyse the secondary structure of MP, following the methodology outlined by Wu et al. (2024) with adjustments. First, 20 μL of a 2 mg/mL MP solution is dropped into the FTIR instrument for measurement in ATR mode. The spectral scan range is 4000 to 400 cm^{-1}, scanned at a rate of 0.65 cm/s, with a resolution of 4 cm^{-1}. Finally, infrared spectra deconvolution is performed using PEAKFIT 4.1 software.

2.6.2. Endogenous fluorescence spectra

The following technique was employed to measure endogenous fluorescence spectra, as per Yang et al. (2024). First, the concentration of the MP solution was adjusted to 0.5 mg/mL. The tertiary structure of the sample MP was subsequently determined using fluorescence spectrophotometry. The slit width and excitation wavelength were set at 10 nm and 295 nm, respectively, and the spectra were scanned from 300 to 400 nm.

2.7. Statistical analysis

Data analysis was performed using SPSS (version 22.0). One-way analysis of variance (ANOVA) was used to assess the significance of different packaging methods within the same variety, and *t*-tests were employed to assess the significance among different varieties within the same packaging method, using a significance level of 5 %. Origin 2019 was used for visual analysis. All measurements in the experiment were repeated nine times.

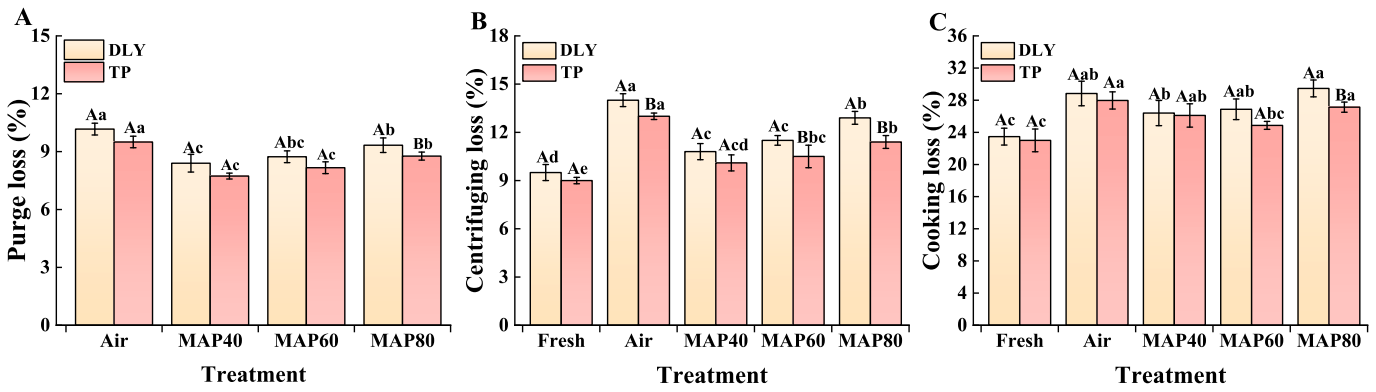

Fig. 1. Mean values of (A) purge loss (%), (B) cooking loss (%), and (C) centrifuging loss (%) of fresh pork meat and pork after chilled storage for 7 days in MAP with different oxygen concentrations and air packaging, respectively. Error bars represent means ± SD (n = 9). The use of different lowercase letters (a-e) to denote indicated significant statistical differences ($P < 0.05$) in the same type of pork with different packaging methods. A-B denote significant statistical differences between TP and DLY pork ($P < 0.05$).

3. Results

3.1. Water-holding capacity

WHC is one of the most important quality characteristics of meat products. Poor WHC not only led to higher water loss but also accompanied a significant loss of sarcoplasmic proteins and other water-soluble nutrients, ultimately resulting in a decrease in the nutritional value of meat products (Duun & Rustad, 2007). Centrifuging loss was an important indicator commonly used to characterise the WHC of meat. As shown in Fig. 1A, there were significant ($P < 0.05$) differences in drip loss between TP and DLY pork stored in MAP with different oxygen concentrations. With the increase in oxygen concentration, centrifuging loss of both types of pork stored in MAP 40, MAP 60, and MAP 80 groups increased over 7 days of storage. Compared to MAP with different oxygen concentrations, samples in the Air group had the highest centrifuging loss, and the centrifuging loss of TP pork was significantly ($P < 0.05$) lower than that of DLY pork.

Purge loss and cooking loss are also important indicators for measuring muscle water retention (Liu et al., 2022). The water and water-soluble proteins lost from meat are referred to as purge or drip loss. During the process of increasing oxygen concentration from 40 % to 80 % in the MAP group, purge loss continued to increase. The purge loss of DLY pork increased from an initial 8.4 % to 9.3 %, while TP pork increased from 7.73 % to 8.76 % (Fig. 1B). This is consistent with the results of (De Palo et al., 2014) and others, who found a significant increase in moisture loss in beef with 70 % oxygen MAP packaging compared to 46 % oxygen MAP packaging. Additionally, compared to the MAP group, the Air group had the highest purge loss for both DLY and TP pork, at 10.16 % and 9.5 %, respectively. Cooking loss mainly consists of water released during heating from the interstitial spaces of muscle fibers and a small amount of fat, sarcoplasmic proteins, and other components of myoplasmic dissolved from muscle fiber cells (Hwang et al., 2004). Fig. 1C illustrates the effect of different packaging methods on cooking loss of meat samples. The results indicate that cooking loss of DLY pork samples increased with the increase in oxygen concentration in MAP packaging, and when the oxygen concentration was 80 %, the cooking loss of DLY pork (29.46 %) was significantly higher ($P < 0.05$) than that of TP pork (27.13 %). Additionally, DLY pork maintained a lower cooking loss (26.4 %) at an oxygen concentration of 40 %, while TP pork had the lowest cooking loss (24.86 %) at an oxygen concentration of 60 %. Overall, the WHC results indicate that compared to the Air group, the MAP group has higher WHC, but the WHC of pork decreases with the increase in oxygen concentration. Furthermore, TP pork exhibited higher WHC than DLY pork.

3.2. Water status

The proton relaxation properties connected with the water state of muscles were found by means of LF-NMR (Han et al., 2014). Previous studies on the water content of meat have mainly focused on T_2 relaxation spectra. However, there may be minimal differences in T_2

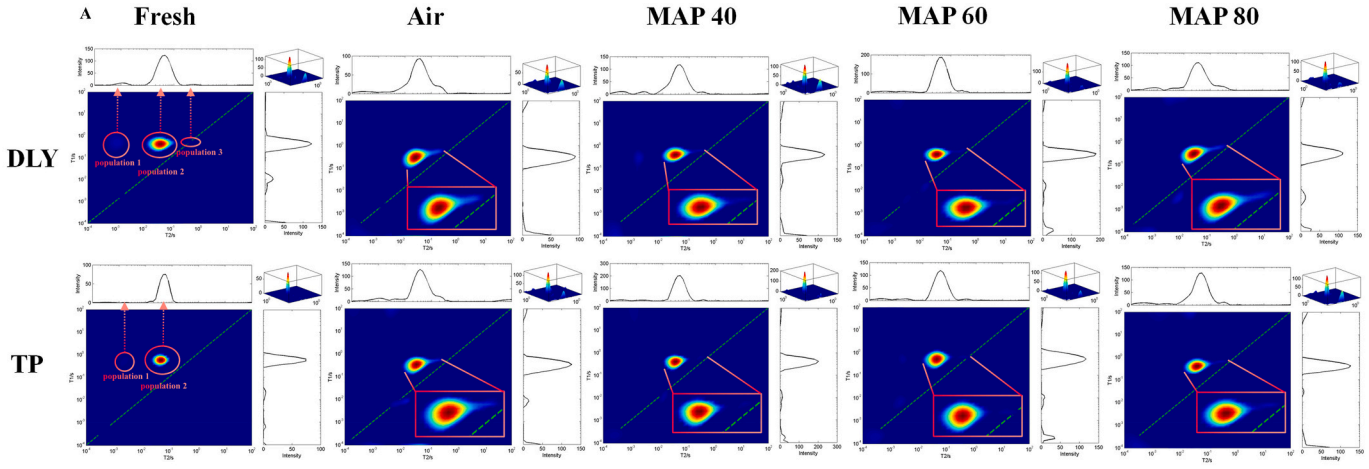

Fig. 2. 2D LF-NMR T_1-T_2 relaxation spectra (A) of fresh pork meat and pork after chilled storage for 7 days in MAP with different oxygen concentrations and air packaging.

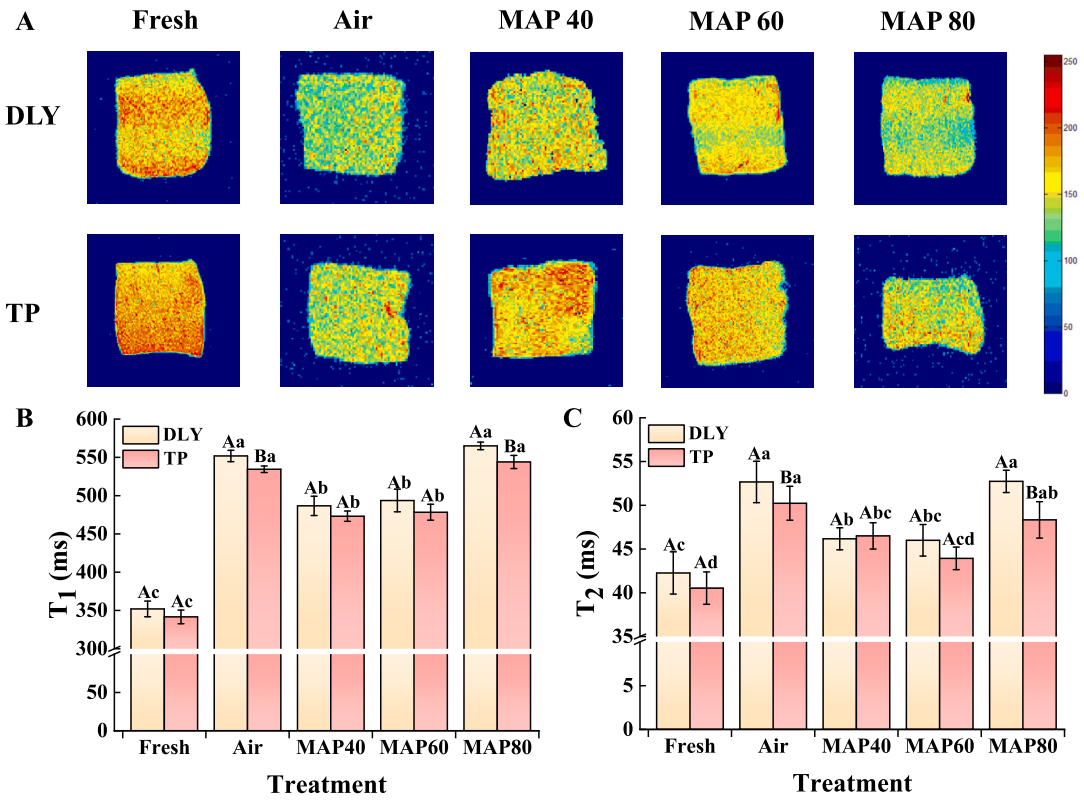

Fig. 3. MRI (A), T_1 relaxation times (ms) (B), and T_2 relaxation times (ms) (C) of fresh pork meat and pork after chilled storage for 7 days in MAP with different oxygen concentrations and air packaging. Error bars represent means ± SD (n = 9). The use of different lowercase letters (a-d) to denote indicated significant statistical differences ($P < 0.05$) in the same type of pork with different packaging methods. A-B denote significant statistical differences between TP and DLY pork ($P < 0.05$).

relaxation between different components, making it difficult to accurately distinguish and identify them. By analysing the transverse and longitudinal relaxation times of samples, protons can be precisely divided into various components (Qian et al., 2022). Therefore, this study utilizes two-dimensional T_1-T_2 relaxation spectra to more easily capture and visualize any minor changes in water migration rates during the storage of pork under different packaging methods, revealing precise information about the distribution of muscle water. T_1 and T_2 relaxation nuclear magnetic resonance spectra are presented on the right and top of the figure, respectively (Fig. 2A). Three forms of water in the muscle correspond to three major populations in the two-dimensional T_1-T_2 relaxation spectra (Population 1: bound water; Population 2: immobilized water; Population 3: free water). The larger the area integral and signal intensity, the redder the colour, and the higher the content of the population (Wang et al., 2020). It can be seen from Fig. 2A that there was no significant change in Population 1 after different packaging treatments, which is attributed to the fact that bound water is located in highly organized muscle fibers and protein structures, making it difficult to migrate (Bertram et al., 2002). It is noteworthy that there were significant changes in Populations 2 and 3 between TP pork and DLY pork under different packaging methods. Population 2 migrated to Population 3 to varying degrees in each treatment, with a higher migration from Population 2 to Population 3 in the Air and MAP 80 groups compared to the MAP 40 and MAP 60 groups, resulting in a larger area for Population 3 and a higher content of free water. Additionally, compared to DLY pork, the area of Population 3 in TP pork under each packaging treatment was lower, and Population 3 was not observed in fresh TP pork.

T_1 and T_2 relaxation times are considered closely related to the WHC of muscles and are commonly used to characterise the mobility of water in muscle tissue (Song et al., 2021). Fig. 3B and C show the effect of different packaging treatments on the relaxation times of pork, with the centroids of T_1 and T_2 determined at the peaks on the right and top of the two-dimensional relaxation spectra, respectively. T_1, as the longitudinal freedom of water, represents the time required for stimulated spin

Table 1
Carbonyl content, total sulfhydryl, protein solubility, and surface hydrophobicity of pork samples from different treatment groups and different breeds.

Treatments	Breed	Carbonyl content (nmlol/mg)	Total sulfhydryl (nmlol/mg)	Protein solubility/%	Surface hydrophobicity (μg)
FR	DLY	0.65 ± 0.02^{Ac}	123.37 ± 0.91^{Aa}	40.33 ± 1.53^{Aa}	1.02 ± 0.10^{Ac}
	TP	0.64 ± 0.02^{Ac}	124.47 ± 2.67^{Aa}	40.50 ± 0.50^{Aa}	1.08 ± 0.13^{Ad}
Air	DLY	3.63 ± 0.15^{Aa}	79.33 ± 1.53^{Bb}	19.69 ± 1.59^{Ac}	4.11 ± 0.10^{Aa}
	TP	3.23 ± 0.21^{Aa}	83.33 ± 1.15^{Ab}	21.83 ± 0.29^{Ad}	3.87 ± 0.12^{Aa}
MAP 40	DLY	2.73 ± 0.21^{Ab}	80.17 ± 1.26^{Ab}	27.00 ± 1.00^{Ab}	2.87 ± 0.06^{Ab}
	TP	2.50 ± 0.10^{Ab}	81.33 ± 2.08^{Ab}	29.67 ± 1.53^{Ab}	2.60 ± 0.10^{Bc}
MAP 60	DLY	2.83 ± 0.15^{Ab}	79.34 ± 1.49^{Ab}	26.33 ± 0.58^{Ab}	2.93 ± 0.12^{Ab}
	TP	2.63 ± 0.12^{Ab}	82.00 ± 2.00^{Ab}	27.50 ± 0.50^{Ac}	2.64 ± 0.05^{Bc}
MAP 80	DLY	3.90 ± 0.10^{Aa}	79.00 ± 1.73^{Bb}	19.83 ± 0.76^{Bc}	3.97 ± 0.06^{Aa}
	TP	3.40 ± 0.10^{Ba}	83.67 ± 1.53^{Ab}	21.50 ± 0.50^{Ad}	3.50 ± 0.10^{Bb}

The use of different lowercase letters (a-d) to denote indicated significant statistical differences ($P < 0.05$) in the same type of pork with different packaging methods. A-B denote significant statistical differences between TP and DLY pork ($P < 0.05$). Results are expressed as mean ± standard deviation (n = 9).

protons to exchange energy with surrounding lattices to reach dynamic equilibrium. The T_1 relaxation time of pork in modified atmosphere packaging increases gradually with the concentration of oxygen (Fig. 3B). The prolongation of relaxation time implies the presence of more mobile water components in the muscle, which may be attributed to the accelerated oxidation of pork protein in a high-oxygen environment, weakening the interaction between water and protein (Utrera & Estévez, 2012). Furthermore, the T_1 relaxation time in the modified atmosphere packaging groups (except for the MAP 80 group) was significantly lower than that in the Air group ($P < 0.05$). This indicates that the gas components in modified atmosphere packaging can delay the oxidative deterioration of muscle, resulting in a tighter binding between water and protein. Similarly, it was found that the T_1 relaxation time of TP pork was significantly lower in the Air and MAP 80 groups than that of DLY pork ($P < 0.05$). T_2, as the transverse freedom of water, represents the time required for stimulated spin-spin protons to exchange energy with neighboring protons to reach dynamic equilibrium. The conclusions drawn from the T_2 relaxation time (Fig. 3C) are consistent with those from T_1. Compared to the Air group, the modified atmosphere packaging groups can better maintain the WHC of pork during storage, and the WHC of pork weakens with the increase in oxygen concentration inside the packaging. This result is consistent with that of Lund et al. (2007), who found that high-oxygen modified atmosphere packaging reduces the WHC of porcine longissimus dorsi.

Based on T_2 relaxation nuclear magnetic resonance spectra, the statistical analysis of the relative contents of three populations of pork under different packaging storage conditions is shown in Table 1. Due to the different degrees of oxidation denaturation of myofibrillar proteins under different packaging methods, differences in the binding capacity of proteins and water occur (Wang et al., 2019). With the continuous increase of oxygen concentration, population 2 showed a gradually decreasing trend, while population 3 showed an increasing trend. Additionally, the percentage of population 3 in high oxygen packaging storage and air storage is significantly higher than that in MAP 40 and MAP 60 groups ($P < 0.05$). Highly denatured proteins result in less water reabsorption, thereby affecting the percentage composition of water in population 3 (Farouk et al., 2012). Furthermore, the percentage of population 3 in air-packaged storage of the two pork types is the highest, with the percentage of population 3 in TP pork (2.67 %) significantly lower than that in DLY pork (3 %) ($P < 0.05$). Overall, the sensitive changes in T_1-T_2 proton distribution of pork under different packaging more intuitively reflect the changes in myo-water dynamics under different oxygen concentration modified atmosphere packaging, revealing that modified atmosphere packaging storage can effectively improve the WHC of pork, but increasing oxygen concentration will gradually lead to a decrease in WHC. Additionally, TP pork exhibits a richer WHC than DLY pork.

MRI is an effective non-contact imaging technique that can visually analyse the moisture content of pork stored in different packaging methods (Guo et al., 2023). In the MRI pseudocolor image, the red area corresponds to high-density H protons, reflecting bound water or immobilized water, while the blue area corresponds to low-density H protons, reflecting free water in skeletal muscle (Wu et al., 2024). As the oxygen concentration increases, the red area of the H proton density map of the two varieties of pork gradually decreases, and the blue area increases, indicating that the immobilized water in pork gradually decreases, and dehydration becomes severe (Fig. 3A). The red area of the H proton image of TP pork in each packaging treatment group is higher than that of DLY pork, indicating a higher water content in TP pork. WHC is usually related to protein structure, and denaturation of myofibrillar proteins will alter the water distribution in pork, leading to an increase in free water content in pork (Yan et al., 2024). Therefore, further verification of the WHC results is needed through changes in protein structure.

Table 2
P_1, P_2, and P_3 of pork (P_1, P_2, and P_3 represents the percentages of population 1, population 2, and population 3, respectively).

Treatments	Breed	P_1 (%)	P_2 (%)	P_3 (%)
FR	DLY	2.70 ± 0.78^{Aa}	96.65 ± 0.81^{Aa}	0.65 ± 0.05^{Ac}
	TP	2.62 ± 0.99^{Aa}	96.84 ± 1.03^{Aa}	0.54 ± 0.05^{Ac}
Air	DLY	2.93 ± 0.15^{Aa}	94.07 ± 0.12^{Bc}	3.00 ± 0.10^{Aa}
	TP	2.40 ± 0.26^{Ba}	94.93 ± 0.31^{Abc}	2.67 ± 0.15^{Ba}
MAP 40	DLY	1.77 ± 0.35^{Ab}	96.50 ± 0.50^{Aab}	1.73 ± 0.15^{Ab}
	TP	1.97 ± 0.31^{Aa}	96.33 ± 0.29^{Aa}	1.70 ± 0.10^{Ab}
MAP 60	DLY	2.57 ± 0.35^{Aab}	95.63 ± 0.15^{Ab}	1.80 ± 0.20^{Ab}
	TP	2.60 ± 0.46^{Aa}	95.97 ± 0.15^{Aab}	1.43 ± 0.31^{Ab}
MAP 80	DLY	3.00 ± 0.26^{Aa}	94.17 ± 0.31^{Ac}	2.83 ± 0.06^{Aa}
	TP	3.07 ± 0.45^{Aa}	94.43 ± 0.51^{Ac}	2.50 ± 0.10^{Ba}

The use of different lowercase letters (a-c) to denote indicated significant statistical differences ($P < 0.05$) in the same type of pork with different packaging methods. A-B denote significant statistical differences between TP and DLY pork ($P < 0.05$). Results are expressed as mean ± standard deviation (n = 9).

3.3. Protein oxidation

3.3.1. Carbonyl content

Protein oxidation generally occurs during post-slaughter muscle processing or storage, leading to a deterioration in muscle WHC due to irreversible oxidative modifications (Pan et al., 2022). The content of protein carbonyl derivatives is one of the most commonly used indicators to measure the degree of protein oxidation, with higher carbonyl content indicating higher protein oxidation (Xia et al., 2012). As the oxygen concentration in MAP increases, the carbonyl content of meat samples gradually increases (Table 2). The increase in carbonyl content will result in protein cross-linking, affecting protein structure, and ultimately leading to a decrease in muscle WHC (Lin et al., 2022). Compared to the Air group and the MAP 80 group, MAP 40 and MAP 60 significantly delayed the increase in carbonyl content, indicating that modified atmosphere packaging can inhibit protein oxidation, but high oxygen environments will lead to excessive protein oxidation. Additionally, in the MAP 80 group, the carbonyl content of TP pork (3.4 nmol/mg) was significantly ($P < 0.05$) lower than that of DLY pork (3.9 nmol/mg). Under high oxygen modified atmosphere packaging conditions, significant protein oxidation occurred in both breeds of pork, while TP pork was able to effectively delay the high oxygen-induced protein oxidation.

3.3.2. Total sulfhydryl group

Sulfhydryl groups are important functional groups that determine the properties of proteins, with higher total sulfhydryl group content indicating lower protein oxidation (Xu et al., 2024). In this experiment, there was no significant difference in total sulfhydryl group content under different packaging methods ($P > 0.05$, Table 2). Similar findings were also reported by Spanos et al. (2016), who found that increasing oxygen concentration did not affect the free sulfhydryl group content of pork loin during 7 days of storage. It is worth noting that in the Air group and the MAP 80 group, the total sulfhydryl group content of TP pork was significantly ($P < 0.05$) higher than that of DLY pork. The lower total sulfhydryl group content in DLY pork implied more disulphide bond formation, leading to the aggregation of myofibril proteins and disrupting the integrity of protein structure (Yang et al., 2024).

3.3.3. Protein solubility

Solubility reflected the degree of denaturation of myofibrillar proteins, with lower solubility indicating higher denaturation (Qian et al., 2022). Table 2 showed the changes in solubility of myofibrillar proteins from two types of pork under different packaging methods. The solubility of MAP 40 and MAP 60 was significantly ($P < 0.05$) lower than that of MAP 80 and the Air group, with the high oxygen concentration in the MAP 80 group induced greater insoluble protein aggregation. Similarly, in the Air group, microbial growth led to a significant

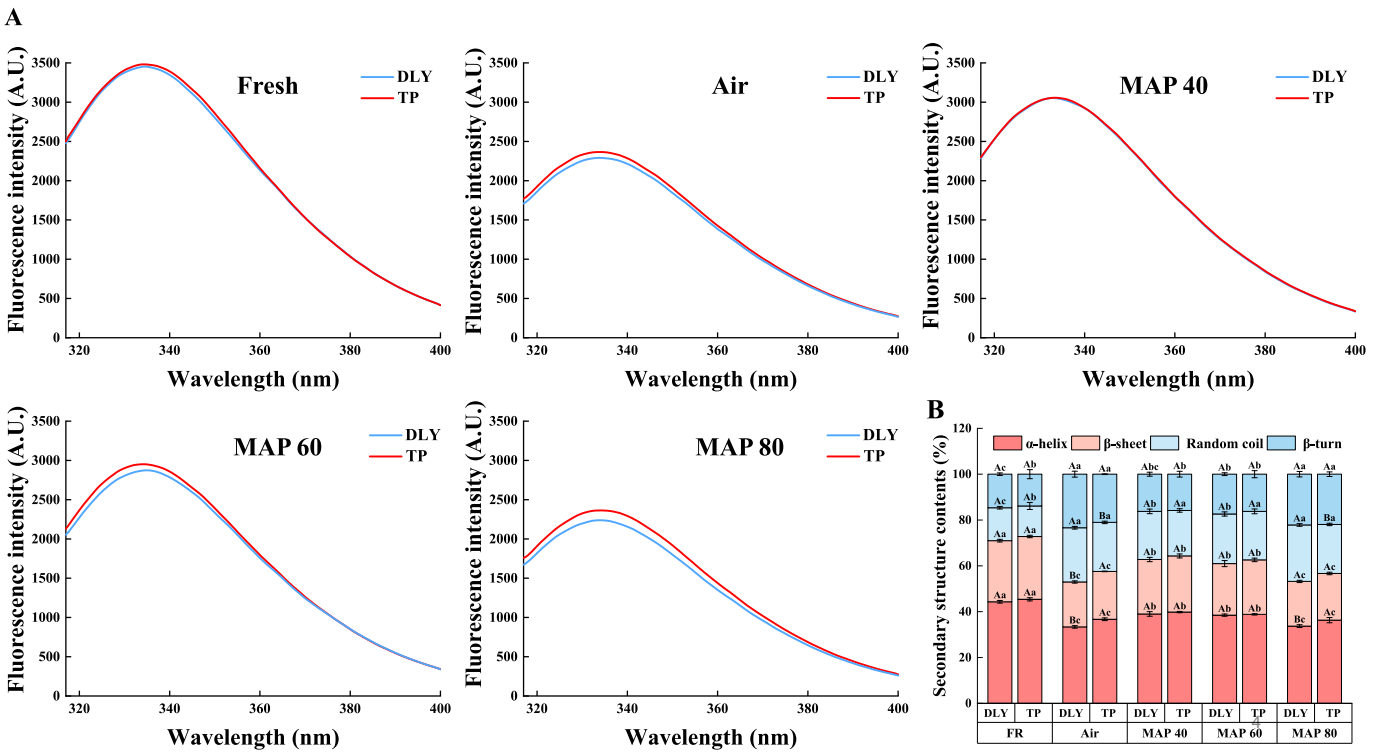

Fig. 4. Changes in endogenous fluorescence intensity (A) and secondary structure (B) of myofibrillar protein from fresh pork meat and pork after chilled storage for 7 days in MAP with different oxygen concentrations and air packaging. Error bars represent means ± SD (n = 9). The use of different lowercase letters (a-c) to denote indicated significant statistical differences ($P < 0.05$) in the same type of pork with different packaging methods. A-B denote significant statistical differences between TP and DLY pork ($P < 0.05$).

aggregation of insoluble proteins and protein denaturation. Furthermore, it was observed that the solubility of TP pork in the MAP 80 group was significantly ($P < 0.05$) higher than that of DLY pork, consistent with the results of carbonyl and total thiol content measurements. The increase in protein carbonyl content and hydrophobic group number in DLY pork under high oxygen environments led to protein structure damage, protein cross-linking, and a decrease in protein solubility.

3.3.4. Surface hydrophobicity

Surface hydrophobicity is considered an indicator of subtle changes in protein structure and is widely used to further evaluate protein conformational stability (Lin et al., 2022). As shown in Table 2, the surface hydrophobicity of the Air group was significantly ($P < 0.05$) higher than that of the MAP 40 and MAP 60 groups, slightly higher than that of the MAP 80 group but not significantly different ($P > 0.05$). This indicates that modified atmosphere packaging can effectively inhibit protein denaturation and maintain protein structure. Additionally, with the increase in oxygen concentration in modified atmosphere packaging, the surface hydrophobicity of each group showed an increasing trend, with the pork surface hydrophobicity in the MAP 80 group being the highest, where DLY pork (3.97 μg) was significantly ($P < 0.05$) higher than TP pork (3.50 μg). The denaturation of myofibrillar proteins may be related to the reduced WHC of myofibrillar proteins, leading to a decrease in water retention (Zhang et al., 2021). The increase in oxygen concentration leads to greater pork oxidation, resulting in loose and unstable protein conformational structures, which further leads to a decrease in pork WHC.

3.4. Protein structure

3.4.1. Secondary structure

Changes in the secondary structure of proteins are typically elucidated by FTIR analysis of the amide I band around 1600–1700 cm^{-1}. Generally, a higher proportion of ordered structures (α-helix and β-sheet) indicates greater structural stability, whereas an increased proportion of disordered structures (β-turn and random coil) often signifies instability and looseness in protein structure. The FR group exhibits the highest α-helix content and the lowest random coil content, indicating the most stable protein structure in the FR group (Fig. 4B). Research by Wu et al. (2024) indicates that hydrogen bonds between peptide chains as well as between carbonyl and amino groups determine the stability of α-helix and β-sheet structures. The α-helix and β-sheet content of myofibrillar proteins in modified atmosphere packaging groups (MAP 40 and MAP 60) are significantly higher than those in the air group ($P < 0.05$), while the β-turn and random coil content showed the opposite trend. Furthermore, as the oxygen concentration increased from 40 % to 80 %, there was a decrease in α-helix and β-sheet contents of myofibrillar proteins, while β-turn and random coil contents increased. The findings of Wu et al. (2023) demonstrated a notable reduction in the levels of α-helix and β-sheet structures, accompanied by a rise in β-turn and random coil structures, in beef samples following a 7-day storage period. These changes reflect a progressive decline in the stability of organized protein secondary structures. Additional findings indicated that the levels of TP pork α-helix and β-sheet were substantially greater in the Air group and the MAP 80 group compared to DLY pork ($P < 0.05$). Conversely, the levels of β-turn and random coil were significantly lower in the Air group and the MAP 80 group compared to DLY pork ($P < 0.05$). This indicates that TP pork maintains a more intact protein secondary structure during storage in the Air and MAP 80 groups.

3.4.2. Tertiary structure - fluorescence intensity (FI)

Endogenous fluorescence spectroscopy is used to reflect the unfolding degree of tryptophan residues and the changes in microenvironments in the tertiary structure of proteins (Yang et al., 2023). The tryptophan fluorescence intensity of MP under different packaging

conditions is shown in Fig. 4A. The maximum emission wavelengths of all treatments were above 330 nm, indicating that the local microenvironment where tryptophan is located is more polar than the aqueous solution outside the protein molecule (Yang et al., 2024). With the increase in oxygen concentration in modified atmosphere packaging, the maximum fluorescence intensity continuously decreased, indicating that the increase in oxygen concentration gradually exposed the side chain groups of tryptophan residues to the aqueous solution, increasing their environmental polarity, thereby facilitating the unfolding of protein tertiary structures. On the other hand, compared with the MAP 40 and MAP 60 groups, the fluorescence intensity of MP in the Air group was lower, indicating that appropriate oxygen concentration in modified atmosphere packaging can effectively protect the protein structure from oxidation-induced unfolding (Xie et al., 2021). Additionally, higher tryptophan fluorescence intensity was observed in TP pork MP than in DLY pork MP in the Air group, MAP 60, and MAP 80 groups, indicating that TP pork maintained the stability of tryptophan residue microenvironments under different packaging conditions, resulting in a more stable protein tertiary structure.

4. Discussion

The WHC during the refrigeration process of different packaging methods is a crucial factor for the profitability and quality of meat products, affecting the development of the meat industry and consumers' purchasing willingness (Zhu et al., 2017). In previous studies, Chen et al. (2015) found that the drip loss of HiOxMAP was significantly lower than that of vacuum packaging after 6 days of storage. Similarly, Delles and Xiong (2014) confirmed that meat stored under high-oxygen MAP had lower WHC compared to vacuum packaging. They attributed this phenomenon to the lower pressure in vacuum-packed samples, which causes meat to lose water (Li et al., 2022). In this study, we fixed the CO_2 levels under different MAP conditions and only varied the oxygen concentration in MAP to further investigate the impact of oxygen concentration on meat WHC. The results showed that MAP with appropriate oxygen concentrations (MAP 40 and MAP 60 groups) effectively reduces purge loss, cooking loss, and centrifugal loss compared to the air group. Conversely, an excess oxygen concentration (MAP 80) worsens WHC (Fig. 1A, Fig. 1B, and Fig. 1C). This may be related to the higher oxidative stress induced by the elevated oxygen concentration in MAP 80. This finding is consistent with the observations of Wang et al. (2019). Additionally, we found that TP pork and DLY pork had poorer WHC under HiOxMAP and air packaging, with TP pork exhibiting relatively higher WHC. These results suggest that appropriately reducing oxygen levels in MAP may be a novel approach to enhancing the WHC of pork.

Previous studies typically use pressure loss, drip loss, and electrical conductivity methods to quantitatively investigate WHC under different packaging conditions (Wang et al., 2019). However, the internal moisture dynamics cannot be characterized. LF-NMR technology, especially two-dimensional LF-NMR technology, can more accurately reflect the changes in water migration under different packaging conditions by combining the generated T_1-T_2 relaxation spectra with MRI proton images (Song et al., 2021). The populations 3 in the Air group and the MAP 80 group had larger areas and higher free water content (Fig. 2A), corresponding to more blue colour in the MRI pseudocolor images. The amount of exudate between fiber bundles increases with the increase of oxygen, due to the migration of intracellular water (Guo et al., 2023). The high oxygen environment promotes the increase of free water, which we speculate is due to the accelerated denaturation of myofibrillar proteins and changes in water distribution caused by excessive oxygen content. Li et al. (2022) also reported that high-oxygen modified atmosphere packaging has higher oxygen levels compared to vacuum packaging. Excessive oxygen concentration intensifies oxidative stress within the meat, diminishing the proteins' ability to form hydrogen bonds and electrostatic interactions with water molecules, thereby increasing moisture loss from the meat. The poorer WHC in the Air group may be due to the absence of antimicrobial gas components, leading to extensive microbial growth and protein degradation, weakening the interaction between protein and water, consistent with the findings of Bao and Ertbjerg (2015). Another finding of the experiment is that the WHC of TP pork is higher than that of DLY pork in both the Air group and the MAP 80 group. The area of population 3 in TP pork is lower under all packaging treatments, with population 3 not detected in fresh TP pork. This conclusion is further supported by two-dimensional T_1-T_2 relaxation LF-NMR spectra and MRI. Furthermore, the red area of the H proton image of TP pork was higher than that of DLY pork, indicating a higher water content in TP pork under different packaging conditions. Generally, a higher intramuscular fat content in pork results in more juice in the meat (Guo et al., 2023). Zhao et al. (2023) study

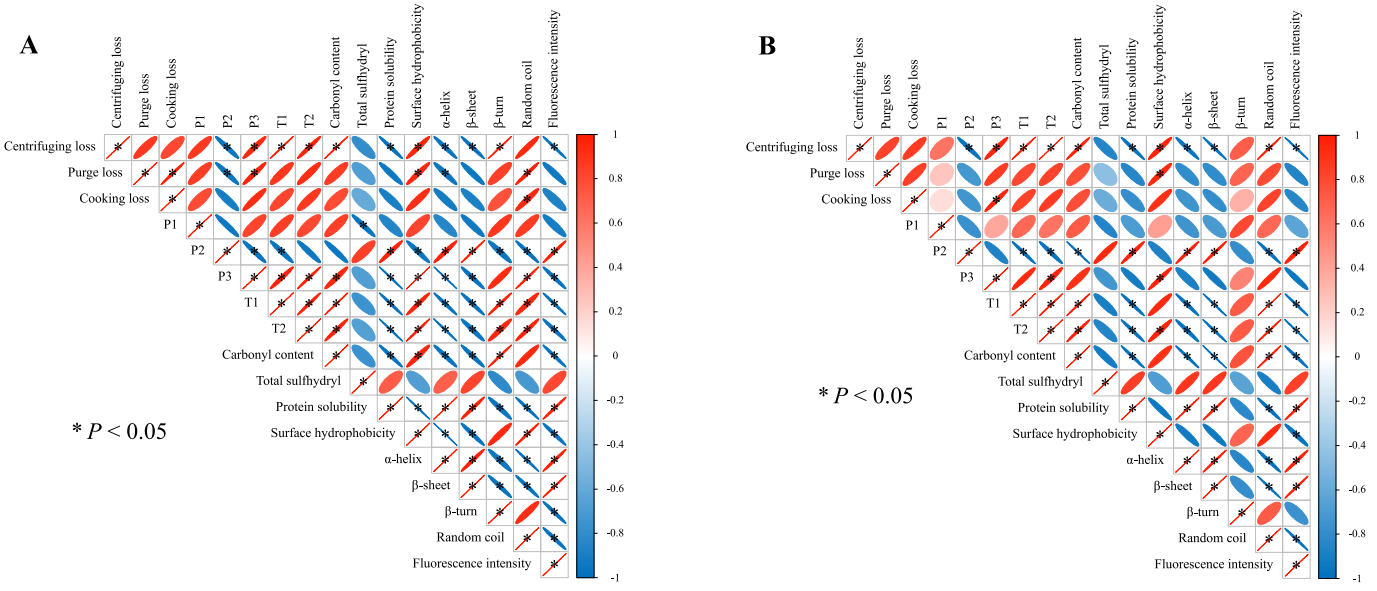

Fig. 5. The water retention capacity of DLY pork (A) and TP pork (B) and protein-related characteristics were correlated, according to Pearson correlation analysis. Red indicates a positive association, and blue indicates a negative correlation. The stronger the association and the deeper the hue, the narrower the ellipse ("*", significant ($P < 0.05$)). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

indicated that Tibetan pork have higher intramuscular fat content compared to Duroc × Landrace × Yorkshire pork. Therefore, we speculate that TP pork possesses more intramuscular fat, thus exhibiting richer WHC.

It is well known that the transfer and loss of moisture are related to the oxidative denaturation of proteins (Lin et al., 2022). An increase in oxygen concentration in MAP can cause higher protein oxidation in meat (Bao & Ertbjerg, 2015). Consistent with previous research findings, this study found that an increase in oxygen concentration led to increased carbonyl content and surface hydrophobicity in the two varieties of pork, with decreased solubility. However, there was no significant difference in thiol groups between different packaging systems (Table 2). Utrera and Estévez (2012) suggested that the decrease in WHC of myofibrillar proteins may be the result of oxidative processes occurring ex vivo. It is worth noting that the Air group and MAP 80 group exhibited the most severe protein oxidation, with significantly lower WHC than the other two groups in MAP. Excessive protein oxidation may disrupt the order and integrity of muscle cells, limiting the ability of myofibrils to absorb water, and reducing the WHC of the meat (Bao & Ertbjerg, 2019). For the two varieties of pork, TP pork exhibited higher protein oxidation in the Air group and MAP 80 group. Studies have shown that the pH of Tibetan pork is lower than that of commercial pork (Zhao et al., 2023). A lower pH may result in more severe protein denaturation, reducing the resistance of myofibrillar lattice to external forces, which may irreversibly alter the spacing between myofibrils, leading to increased drip loss (Zhang et al., 2021). This may explain why TP pork exhibited stronger water retention capabilities than DLY pork. In terms of protein structure, the stability of α-helix and β-sheet structures is maintained by hydrogen bonds, with the stability of α-helix existing between carbonyl and amino groups, while the stability of β-sheet exists between peptide chains of protein molecules. The more severe protein oxidation in the pork, especially DLY pork in the Air group and MAP 80 group, leads to the breaking of hydrogen bonds, disrupting the order of protein secondary structures, resulting in a decrease in the content of α-helix and β-sheet, and an increase in the content of β-turn and random coil (Fig. 4B). With the decrease in the proportion of α-helices, more hydrophobic groups are exposed. This means that the oxidative denaturation of proteins leads to the destruction of the tertiary structure of meat proteins, causing the unfolding of proteins and exposing more hydrophobic amino acids. The fluorescence intensity of myofibrillar proteins (Fig. 4A) confirms this. This is also consistent with the study by Spanos et al. (2016), who reported that protein oxidation can unfold proteins, forcing internal aromatic amino acid residues to appear on the protein surface, leading to the unfolding, cross-linking, aggregation, and denaturation of proteins, reducing their functional properties.

To better elucidate the relationship between WHC and protein oxidation in pork, correlation graphs of WHC and protein-related characteristics were drawn for DLY pork (Fig. 5A) and Tibetan pork (Fig. 5B) using Pearson correlation analysis. The correlation results between Tibetan pork and DLY pork showed similar trends. Specifically, centrifuging loss in both pork varieties was significantly ($P < 0.05$) positively correlated with P_3, T_1, T_2, carbonyl content, and surface hydrophobicity, and significantly ($P < 0.05$) negatively correlated with P_2, protein solubility, α-helix, β-sheet, and fluorescence intensity. Additionally, P_2 and P_3 showed high correlations with T_2 and T_3 regarding protein oxidation. These results confirm that protein oxidation can affect the WHC of Tibetan pork and DLY pork under different packaging and storage conditions, consistent with previous studies (Wang et al., 2019). The findings of this study on the correlation between pork WHC and protein oxidation provide insights for enhancing the quality of pork preservation and freshness during storage.

5. Conclusions

This study utilized two-dimensional LF-NMR T_1-T_2 relaxation technology to investigate the changes in moisture status of TP pork under air packaging and MAP (with oxygen concentrations of 40 %, 60 %, and 80 %) and its relationship with protein characteristics. With the increase in oxygen concentration in the modified atmosphere packaging, the content of population 3 in the two-dimensional relaxation spectrum, as well as T_1 and T_2 relaxation times, showed a regular increase, while WHC gradually decreased. The population 3, T_1, and T_2 proton relaxation values of TP pork were lower than those of DLY pork, effectively inhibiting protein oxidation and maintaining protein conformation stability. In addition, compared to the MAP 40 and MAP 60 groups, the WHC and protein conformation stability of the Air and MAP 80 groups were poorer, with higher protein denaturation levels and higher T_1-T_2 proton relaxation degrees. These results indicate that reducing the MAP oxygen concentration to 40 % or 60 % can mitigate the quality deterioration caused by high-oxygen environments, offering a new approach for maintaining pork WHC. Additionally, this study used two-dimensional LF-NMR technology to elucidate the effects of different packaging methods on TP pork WHC, which is significant for improving TP pork's WHC and quality, and extending its market development and sales cycle. Future research should focus on the molecular connections between protein biochemical reactions and moisture state under different MAP oxygen concentrations, and further explore the application of two-dimensional LF-NMR technology in meat preservation.

CRediT authorship contribution statement

Yong Chen: Writing – original draft, Validation, Methodology, Data curation. **Yiping Yang:** Software, Data curation. **Hengxun Lin:** Methodology, Investigation. **Liye Cui:** Supervision, Investigation. **Zongyuan Zhen:** Supervision, Software, Conceptualization. **Chunhui Zhang:** Software, Methodology, Investigation. **Xia Li:** Writing – review & editing, Project administration, Funding acquisition. **Jingjun Li:** Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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References

Bao, Y., & Ertbjerg, P. (2015). Relationship between oxygen concentration, shear force and protein oxidation in modified atmosphere packaged pork. *Meat Science, 110*, 174–179. https://doi.org/10.1016/j.meatsci.2015.07.022

Bao, Y., & Ertbjerg, P. (2019). Effects of protein oxidation on the texture and water-holding of meat: A review. *Critical Reviews in Food Science and Nutrition, 59*(22), 3564–3578. https://doi.org/10.1080/10408398.2018.1498444

Bertram, H. C., Purslow, P. P., & Andersen, H. J. (2002). Relationship between meat structure, water mobility, and distribution: A low-field nuclear magnetic resonance study. *Journal of Agricultural and Food Chemistry, 50*(4), 824–829. https://doi.org/10.1021/jf010738f

Cayuela, J., Gil, M., Bañón, S., & Garrido, M. (2004). Effect of vacuum and modified atmosphere packaging on the quality of pork loin. *European Food Research and Technology, 219*(4). https://doi.org/10.1007/s00217-004-0970-x

Chen, L., Zhou, G., & Zhang, W. (2015). Effects of high oxygen packaging on tenderness and water holding capacity of pork through protein oxidation. *Food and Bioprocess Technology, 8*(11), 2287–2297. https://doi.org/10.1007/s11947-015-1566-0

De Palo, P., Maggiolino, A., Tateo, A., & Centoducati, P. (2014). Influence of gas mixture on quality and shelf life of veal calf meat. *Italian Journal of Animal Science, 13*(2), 3129. https://doi.org/10.4081/ijas.2014.3129

Delles, R. M., & Xiong, Y. L. (2014). The effect of protein oxidation on hydration and water-binding in pork packaged in an oxygen-enriched atmosphere. *Meat Science, 97*(2), 181–188. https://doi.org/10.1016/j.meatsci.2014.01.022

Den Hertog-Meischke, M. J. A., Van Laack, R. J. L. M., & Smulders, F. J. M. (1997). The water-holding capacity of fresh meat. *Veterinary Quarterly, 19*(4), 175–181. https://doi.org/10.1080/01652176.1997.9694767

Duun, A., & Rustad, T. (2007). Quality changes during superchilled storage of cod (*Gadus morhua*) fillets. *Food Chemistry, 105*(3), 1067–1075. https://doi.org/10.1016/j.foodchem.2007.05.020

Farouk, M. M., Mustafa, N. M., Wu, G., & Krsinic, G. (2012). The "sponge effect" hypothesis: An alternative explanation of the improvement in the waterholding capacity of meat with ageing. *Meat Science, 90*(3), 670–677. https://doi.org/10.1016/j.meatsci.2011.10.012

Guo, Z., Chen, C., Ma, G., Yu, Q., & Zhang, L. (2023). LF-NMR determination of water distribution and its relationship with protein- related properties of yak and cattle during postmortem aging. *Food Chemistry: X, 20*, Article 100891. https://doi.org/10.1016/j.fochx.2023.100891

Han, M., Wang, P., Xu, X., & Zhou, G. (2014). Low-field NMR study of heat-induced gelation of pork myofibrillar proteins and its relationship with microstructural characteristics. *Food Research International, 62*, 1175–1182. https://doi.org/10.1016/j.foodres.2014.05.062

Hu, F., Qian, S., Huang, F., Han, D., Li, X., & Zhang, C. (2021). Combined impacts of low voltage electrostatic field and high humidity assisted-thawing on quality of pork steaks. *LWT - Food Science and Technology, 150*, Article 111987. https://doi.org/10.1016/j.lwt.2021.111987

Hwang, I. H., Park, B. Y., Cho, S. H., & Lee, J. M. (2004). Effects of muscle shortening and proteolysis on Warner–Bratzler shear force in beef longissimus and semitendinosus. *Meat Science, 68*(3), 497–505. https://doi.org/10.1016/j.meatsci.2004.04.002

Li, S., Guo, X., Shen, Y., Pan, J., & Dong, X. (2022). Effects of oxygen concentrations in modified atmosphere packaging on pork quality and protein oxidation. *Meat Science, 189*, Article 108826. https://doi.org/10.1016/j.meatsci.2022.108826

Liang, R., Zhang, W., Mao, Y., Zhang, Y., Li, K., Luo, X., & Yang, X. (2024). Effects of CO_2 on the physicochemical, microbial, and sensory properties of pork patties packaged under optimized O_2 levels. *Meat Science, 209*, Article 109422. https://doi.org/10.1016/j.meatsci.2023.109422

Lin, H., He, X., Liu, C., Meng, J., Guan, W., Hou, C., Zhang, C., & Wang, W. (2022). Static magnetic field-assisted supercooling preservation enhances water-holding capacity of beef during subzero storage. *Innovative Food Science & Emerging Technologies, 80*, Article 103106. https://doi.org/10.1016/j.ifset.2022.103106

Liu, J., Liu, D., Zheng, A., & Ma, Q. (2022). Haem-mediated protein oxidation affects water-holding capacity of beef during refrigerated storage. *Food Chemistry: X, 14*, Article 100304. https://doi.org/10.1016/j.fochx.2022.100304

Lund, M. N., Lametsch, R., Hviid, M. S., Jensen, O. N., & Skibsted, L. H. (2007). High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine longissimus dorsi during chill storage. *Meat Science, 77*(3), 295–303. https://doi.org/10.1016/j.meatsci.2007.03.016

Oswell, N. J., Gilstrap, O. P., & Pegg, R. B. (2021). Variation in the terminology and methodologies applied to the analysis of water holding capacity in meat research. *Meat Science, 178*, Article 108510. https://doi.org/10.1016/j.meatsci.2021.108510

Pan, J., Li, C., Liu, X., He, L., Zhang, M., Huang, S., Huang, S., Liu, Y., Zhang, Y., & Jin, G. (2022). A multivariate insight into the organoleptic properties of porcine muscle by ultrasound-assisted brining: Protein oxidation, water state and microstructure. *LWT - Food Science and Technology, 159*, Article 113136. https://doi.org/10.1016/j.lwt.2022.113136

Qian, S., Hu, F., Mehmood, W., Li, X., Zhang, C., & Blecker, C. (2022). The rise of thawing drip: Freezing rate effects on ice crystallization and myowater dynamics changes. *Food Chemistry, 373*, Article 131461. https://doi.org/10.1016/j.foodchem.2021.131461

Song, Y., Huang, F., Li, X., Han, D., Zhao, L., Liang, H., Rui, M., Wang, J., & Zhang, C. (2021). Water status evolution of pork blocks at different cooking procedures: A two-dimensional LF-NMR T_1-T_2 relaxation study. *Food Research International, 148*, Article 110614. https://doi.org/10.1016/j.foodres.2021.110614

Spanos, D., Tørngren, M. A., Christensen, M., & Baron, C. P. (2016). Effect of oxygen level on the oxidative stability of two different retail pork products stored using modified atmosphere packaging (MAP). *Meat Science, 113*, 162–169. https://doi.org/10.1016/j.meatsci.2015.11.021

Utrera, M., & Estévez, M. (2012). Oxidation of myofibrillar proteins and impaired functionality: Underlying mechanisms of the carbonylation pathway. *Journal of Agricultural and Food Chemistry, 60*(32), 8002–8011. https://doi.org/10.1021/jf302111j

Wang, C., Wang, H., Li, X., & Zhang, C. (2019). Effects of oxygen concentration in modified atmosphere packaging on water holding capacity of pork steaks. *Meat Science, 148*, 189–197. https://doi.org/10.1016/j.meatsci.2018.10.001

Wang, S., Lin, R., Cheng, S., & Tan, M. (2020). Water dynamics changes and protein denaturation in surf clam evaluated by two-dimensional LF-NMR T_1-T_2 relaxation technique during heating process. *Food Chemistry, 320*, Article 126622. https://doi.org/10.1016/j.foodchem.2020.126622

Wu, G., Yang, C., Bruce, H. L., Roy, B. C., Li, X., & Zhang, C. (2023). Effects of alternating electric field assisted freezing-thawing-aging sequence on longissimus dorsi muscle microstructure and protein characteristics. *Food Chemistry, 409*, Article 135266. https://doi.org/10.1016/j.foodchem.2022.135266

Wu, G., Yang, C., Lin, H., Hu, F., Li, X., Xia, S., … Zhang, C. (2024). To what extent do low-voltage electrostatic fields play a role in the physicochemical properties of pork during freezing and storage? *Journal of Agricultural and Food Chemistry, 72*(3), 1721–1733. https://doi.org/10.1021/acs.jafc.3c08470

Xia, X., Kong, B., Liu, J., Diao, X., & Liu, Q. (2012). Influence of different thawing methods on physicochemical changes and protein oxidation of porcine longissimus muscle. *LWT - Food Science and Technology, 46*(1), 280–286. https://doi.org/10.1016/j.lwt.2011.09.018

Xie, Y., Chen, B., Guo, J., Nie, W., Zhou, H., Li, P., Zhou, K., & Xu, B. (2021). Effects of low voltage electrostatic field on the microstructural damage and protein structural changes in prepared beef steak during the freezing process. *Meat Science, 179*, Article 108527. https://doi.org/10.1016/j.meatsci.2021.108527

Xu, C., Chen, G., Chen, S., Xu, J., Chen, C., Xia, Q., Sun, Q., Wei, S., Han, Z., Wang, Z., & Liu, S. (2024). Effect of linoleic acid-induced oxidation on the water retention of golden pompano: Myoglobin and myofibrillar protein oxidation. *LWT - Food Science and Technology, 192*, Article 115719. https://doi.org/10.1016/j.lwt.2023.115719

Yan, X., Li, H., Wang, X., Hu, Z., Li, J., Zheng, H., Wang, J., & Zhen, Z. (2024). From amino acid analysis to improved gel properties: The role of dl-valine in Landaise goose myofibrillar protein. *Food Chemistry: X, 21*, Article 101123. https://doi.org/10.1016/j.fochx.2024.101123

Yang, C., Wu, G., Li, Y., Zhang, C., Liu, C., & Li, X. (2023). Effect of low-voltage electrostatic field on oxidative denaturation of myofibrillar protein from lamb-subjected freeze–thaw cycles. *Food and Bioprocess Technology, 16*(9), 2070–2081. https://doi.org/10.1007/s11947-023-03041-0

Yang, C., Wu, G., Liu, Y., Li, Y., Zhang, C., Liu, C., & Li, X. (2024). Low-voltage electrostatic field enhances the frozen force of −12 °C to suppress oxidative denaturation of the lamb protein during the subsequent frozen storage process after finishing initial freezing. *Food Chemistry, 438*, Article 138055. https://doi.org/10.1016/j.foodchem.2023.138055

Yang, J., Yang, X., Lin, H., Liang, R., Niu, L., Zhu, L., … Zhang, Y. (2022). Investigation of the relationship between microbiota dynamics and volatile changes in chilled beef steaks held under high-oxygen packaging enriched in carbon dioxide. *Meat Science, 191*, Article 108861. https://doi.org/10.1016/j.meatsci.2022.108861

Zhang, Y., Puolanne, E., & Ertbjerg, P. (2021). Mimicking myofibrillar protein denaturation in frozen-thawed meat: Effect of pH at high ionic strength. *Food Chemistry, 338*, Article 128017. https://doi.org/10.1016/j.foodchem.2020.128017

Zhao, L., Erasmus, S., Yang, P., Huang, F., Zhang, C., & Van Ruth, S. (2023). Establishing the relations of characteristic aroma precursors and volatile compounds for authenticating Tibetan pork. *Food Chemistry, 427*, Article 136717. https://doi.org/10.1016/j.foodchem.2023.136717

Zhu, H., O'Farrell, M., Hansen, E. W., Andersen, P. V., Berg, P., & Egelandsdal, B. (2017). The potential for predicting purge in packaged meat using low field NMR. *Journal of Food Engineering, 206*, 98–105. https://doi.org/10.1016/j.jfoodeng.2017.03.008