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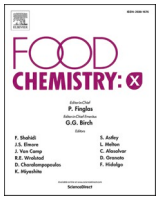

Effects of tea branch liquid smoke on oxidation and structure of myofibrillar protein derived from pork tenderloin during curing

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ABSTRACT

This study focused on how different concentrations of tea branch liquid smoke (TLS) in the curing solution impacted the physicochemical properties and antioxidant properties of pork tenderloin. Five experimental (1.25 mL/kg, 2.5 mL/kg, 5 mL/kg, 10 mL/kg, 20 mL/kg) and blank groups set up over 4 days, and it was found that the physicochemical indexes, antioxidant capacity, thermal stability and protein network structure of the cured meat using 5 mL/kg of liquid smoke were excellent than the other groups used ($P < 0.05$). However, concentrations at 20 mL/kg accelerated protein oxidation. Low frequency nuclear magnetic resonance (LFNHR) revealed that TLS also improved the water holding capacity of the cured meat by increasing the percentage of bound water. Additionally, the correlation analysis demonstrated that the inoxidizability of myofibrillar protein was significantly related to cooking loss and water distribution, which were adjusted by changing the usage of liquid smoke.

Introduction

For the past centuries, traditional methods of smoking food have been used as preservation techniques, especially for meat products. However, the methods not only contribute to pollute the environment (Iko Afé et al., 2020), but the incomplete combustion and pyrolysis of wood can also produce polycyclic aromatic hydrocarbons and other carcinogens (Du et al., 2022). Thus, the use of liquid smoke stands as an alternative. Compared with traditional smoking, the use of liquid smoke requires less time, is more environmentally friendly, has antibacterial properties and may also eliminate polycyclic aromatic hydrocarbons (Simon, de la Calle, Palme, Meier & Anklam, 2005) while still providing the flavor and aroma required by traditional smoking.

There are three liquid smoke production methods, which include smoldering (a slow, low-temperature, flameless form of combustion under a limited oxygen supply); carbonization (a slow pyrolysis using an external heat source in the absence of oxygen); and finally, fast pyrolysis (similar to carbonization except higher heat temperatures are applied). In comparison with smoldering and carbonization, fast pyrolysis has multiple advantages including lower environmental pollution, higher liquid yields and easier control process (Bridgwater & Cottam, 1992). Most of the current research uses negative combustion or carbonation methods to prepare liquid smoke. This application includes preserving and improving the quality of protein-based foods such as meat, seafood and cheese. Therefore, it is of great significance to develop different kinds of green liquid smoke to produce modern meat products.

Although the relationship between meat and human health has been controversial, meat is considered as the best protein-based food to provide essential nutrition for human (Wyness, 2016). For example, pork tenderloin contains low fat and can show less consumer preference due to the lower palatability after cooking (Hwang, Lee & Hong, 2019). Currently, most of the studies on the quality of meat products by commercial liquid smoke have studied the texture, moisture content and protein solubility of meat products (Martinez, Salmerón, Guillén & Casas, 2007). Nevertheless, there is still unlimited space to research the interaction between smoke liquid and protein. China has the largest area of tea plantations in the world and is the second largest exporter of tea in the world. And, in 2014, China's total tea production was 209.2 million tons, up 10.33 % from 2013; this represents 41.6 % of the total global production (Cao et al., 2018). China's abundant tea resources are

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utilized to produce high quality tea branches that are regularly pruned every year. The tea branch liquid smoke (TLS) was prepared by a newly developed dry distillation process to obtain green tea branch smoke liquid. Thus, we prepared liquid smoke product by pyrolysis of tea tree branches with round bottom retort equipment. According to our previous research, TLS has advantages over *Rosa roxburghii* residue liquid smoke, pine and cypress branch liquid smoke regarding phenolic compounds, aldehyde compounds, ketone compounds, alcohol compounds, furan compounds and pyrazines in terms of type and quantity. As many as 36 kinds of volatile flavor substances have been described in the TLS, of which the relative contents of phenols and carbonyl compounds are the highest (29,219 % and 38,825 %, respectively). The total phenolic content was 10.88 ± 0.27 mg/ml, and the carbonyl compound content was 12.39 ± 0.19 g/100 mL, and no harmful substances such as benzopyrene were detected (Gao, 2019). However, the optimal liquid smoke curing conditions of pork tenderloin have yet to be described.

To investigate the effect of different concentrations of TLS on the physicochemical index and antioxidant properties of pork tenderlointhe, liquid smoke of tea twigs was prepared by self-developed dry distillation equipment. The obtained liquid smoke was prepared into different contents of curing solution. The physicochemical indices as well as the antioxidant properties of the proteins were examined after curing of pork tenderloin and demonstrated by examining its microstructure and low frequency nuclear magnetic resonance. Correlations between these indicators were then investigated using Pearson correlation analysis software. This is a preliminary study on the physicochemical properties of myofibrillar protein of pork tenderloin based on different concentrations of TLS, which is beneficial to further promote the exploration of the functional role of TLS in the food field.

Materials and methods

Materials

The fresh tea branches were purchased from Jinsha (Guizhou, China). Pork (Guizhou local pig breed, China) tenderloin was provided by Fuzhiyuan Food Co., ltd.(Guizhou, China), shipped fresh to the lab in ice boxes and used within 24 h for all investigations. The salt was purchased from Chongqing Hechuan Salt Chemical Co., ltd. (Chongqing, China). Dipotassium phosphate (K_2HPO_4) and magnesium chloride ($MgCl_2$) of analytical grade were obtained from Tianjin comeO Chemical Reagent Co., ltd. (Tianjin, China). Analytical grade KH_2PO_4, EGTA, fluorescence dye 8-anilino-1-naphthalene sulfonate (ANS), trichloroacetic acid (TCA), guanidine hydrochloride and 2-nitrobenzoic acid were purchased from Shanghai Yien Chemical Technology Co., ltd. (Shanghai, China). Potassium chloride (KCl) of analytical grade, 2,4-dinitrophenylhydrazine (DNPH), EGTA-2Na and 2.5 % glutaraldehyde for electron microscopy were purchased from Chengdu Jinshan Chemical Reagent Co., ltd. (Chengdu, China), Tokyo Chemical Industry Co., ltd. (Tokyo, Japan), Sinopharm Chemical Reagents Co., ltd. (Shanghai, China) and Beijing solepak Technology Co., ltd. (Beijing, China). Remaining chemicals were of analytical grade and were purchased from Sinopharm Chemical Reagents Co., ltd. (Shanghai, China).

Liquid smoke preparation

The collected tea branches were dried and crushed with a wood crusher (Smj-3kw, Zhengzhou Zhuohui Machinery Equipment Co., ltd, China). Then, the crushed wood was put into the retort for anaerobic combustion (GL-50, Chinese utility model patent, Patent No: ZL 2021 2 2150320. 3, Guiyang Chenyang Machinery Technology Co., ltd, China). The heating temperature adjustable electric stove plate is used to heat the material, and the temperature inside the boiler is observed through the thermometer on the wall of the boiler, so that the distillation temperature can be adjusted for different materials.

The temperature is controlled between 360 and 400 ℃. Liquid smoke stock sample was prepared using the Poligné's method (Poligné, Broyart, Trystram & Collignan, 2002) with some modifications. Briefly, the liquid smoke was purified at low temperature (standing at −10 ℃ for 24 h), then the refined liquid smoke was obtained by filtration. The filtered smoke liquor was divided into five parts and diluted with ultrapure water to obtain the following concentrations: 100, 50, 25, 12.5 and 6.25 %, for backup use.

Meat sample preparation

Fat and fascia of pork loin were removed and each piece of meat was cut into 50 g ± 0.5 g pieces. Then, these were randomly divided into five groups with three parallel pieces in each group. Table salt at 2 % (g/kg) was added to each group, while liquid smoke was added at 2 % (mL/kg). Accordingly, the amount of raw liquid smoke added in the meat sample was 1.25 mL/kg, 2.5 mL/kg, 5 mL/kg, 10 mL/kg, 20 mL/kg, and blank control group (C) without liquid smoke was set. Each group of samples was stored in vacuum at 0 to 4℃. The cured meat pieces were evaluated at four curing time points, and the symbols of D1, D2, D3 and D4 correspond to the day 1, 2, 3 and 4, respectively.

pH values

The pH value was determined according to the method of Huang et al. (2022). The surface's moisture was soaked up with absorbent paper and the sample was grinded. The sample (10 g) was added to distilled water (100 mL), homogenized for 1 min (10000 r/min), and then filtered. The filtrate was used to calibrate the pH meter (Testo 205, Shenzhen Detu Instrument Co., ltd, China) and the measurement was performed five times.

Color

The brightness value L *, the redness value a *, and the yellowness value b * of the sample surface were measured directly using a portable color difference meter (NH350 Agilent, Shenzhen Sanenshi Technology Co., ltd, China). The colorimeter was calibrated using a white board before use. Each meat sample was measured five times in parallel.

Cooking loss

The cooking loss was measured as described by Huang et al. (2022). The meat samples were heated in a constant temperature water bath at 80 ℃ for 5 min. Samples were then removed and rinsed by running water. The cleaned sample was cooled to room temperature (26 ℃) and kitchen paper was used to wipe off the residual liquid left on the surface of the meat after cooking. Cooking loss was calculated using the formula below (1).

$$Cooking\ loss\% = \frac{weight\ of\ raw\ meat - weight\ of\ cooked\ meat}{Weight\ of\ raw\ meat} * 100 \quad (1)$$

Myofibrillar protein extraction

Protein was extracted according to the method reported by Huang et al. (2022) with slight modifications. Fresh pork (50 g) was homogenized (XHF-DY, Ningbo Scientz Biotechnology CO., ltd., China) in 500 mL buffer (100 mmol/L KCl, 11.2 mmol/L K_2HPO_4, 8.8 mmol/L KH_2PO_4, 1 mmol/L EGTA, 1 mmol/L $MgCl_2$) at 0–4 ℃ thrice (9950×*g*, 30 s each time, with an interval of 60 s) and centrifuged for 15 min (4 ℃, 11,000×*g*). Afterwards, 500 mL buffer was added to the precipitate, centri- fuged again under the same conditions and the supernatant was discarded. Next, 125 mL buffer solution was added to the sediment for homogenization, and the suspension was filtered with a 200-mesh nylon sieve to remove the connective tissue to obtain myofibrillar protein solution. Protein concentration was determined by the Biuret method

and adjusted to 0.5 ± 0.05 mg/mL for standby. The myofibrillar protein (MP) extraction method of pickled samples is the same as described above.

Determination of surface hydrophobicity

The surface hydrophobicity of the MP samples was estimated according to the method of (Zhang et al., 2023), with minor modifications. Using ANS as fluorescence probe, the surface hydrophobicity of MP cured with different concentrations of liquid smoke was detected. MP was diluted to a concentration ranging from 0.2 to 1.2 mg/mL using 20 mM phosphate buffer containing 0.6 M NaCl (PBS, pH 7.0). MP solution (5 mL) was mixed with 25 μL ANS (8 mm ANS in 20 mM phosphate, pH 6.0) thoroughly. After mixing, all samples were stored at 4 °C. The samples were then measured with a 970-cathode ray tube fluorescence spectrophotometer (F98, Shanghai Lingguang Technology Co., ltd., China). The excitation wavelength was 374 nm, whilst the emission wavelength was 475 nm, with excitation and emission slit widths of 2.5 nm, and a scan rate of 240 nm/min.

Determination of carbonyl content

The carbonyl content of MP of cured meat was determined using the method described by Huang et al. (2022). Each MP solution (50 μL, 30 mg/mL) was mixed with 2.0 mL of 10 nM 2,4-dinitrophenylhydrazine (DNPH) solution (dissolved in 2.0 N HCl) for 1 h and precipitated with 2.0 mL of 20 % TCA. The precipitate was washed twice using 4.0 mL of ethanol:ethyl acetate (1:1, v/v), blow-dried, and dissolved in 1.5 mL of guanidine hydrochloride (6 M, pH 2.3). The absorbance was measured at 370 nm with a molar extinction coefficient of 22,000 M/cm to calculate the carbonyl content. Each determination was duplicated and performed three times.

Determination of sulfhydryl content

Sulfhydryl content in protein was measured according to the method reported by Huang et al. (2022) with slight modifications. The myofibrillar protein was diluted to 5 mg/mL with phosphate buffer (6.5 mol/L, pH = 7). Then, 1 mL of protein suspension was diluted with 9 mL and 50 mmol/L phosphate buffer containing 8 mol/L urea, 0.6 mol/L sodium

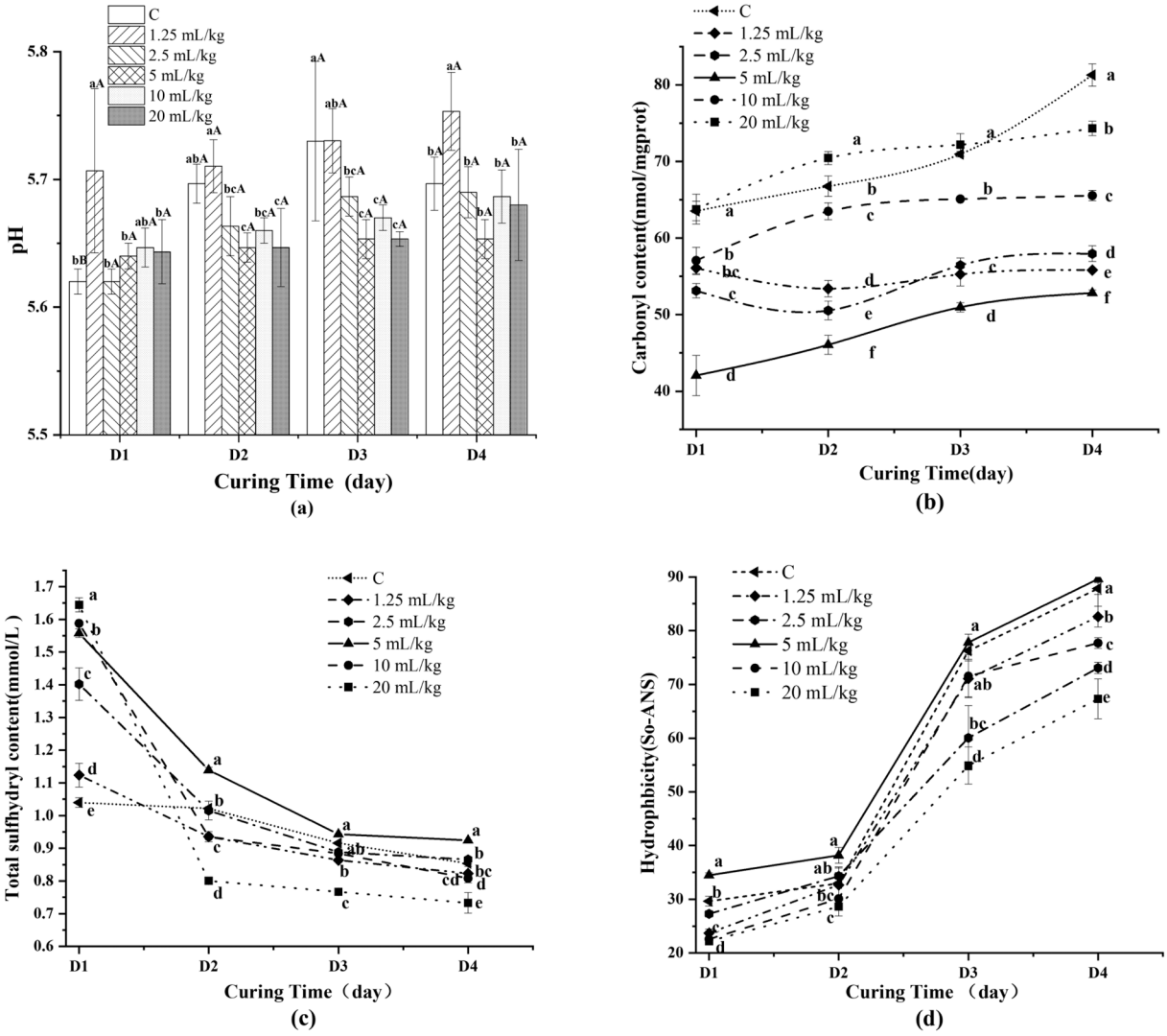

Fig. 1. The effects of different concentrations of TLS on pH of pork tenderloin and the surface hydrophobicity, carbonyl content and total mercapto content of myogenic fibrin with curing time (n = 3). D1: day one; D2: day two; D3: day three; D4: day four; (a): change of pH of pork tenderloin; (b): changes of carbonyl groups in myofibrillin; (c): changes of total sulfhydryl group of myofibrillar protein; (d): changes in surface hydrophobicity of myofibrillar protein; Lower case letters a-f indicate differences between control and the vary concentrations TLS treatment within the same group ($P < 0.05$). Upper case letters A-C indicate differences between control and TLS treat samples during different times ($P < 0.05$).

chloride and 10 mmol/L EGTA-2Na (pH = 7). Then, 3 mL of diluted solution were mixed with 0.4 mL of 0.1 % 2-nitrobenzoic acid and the mixture was left in the dark at 40 ℃ for 25 mins. Finally, the solution was cooled to room temperature and the absorbance was measured at 412 nm using a spectrophotometer. Total sulfhydryl content was calculated by the molar extinction coefficient of 13,600 L/mol.cm. Each determination was duplicated and performed three times.

Differential scanning calorimetry (DSC)

Research methods of differential scanning calorimetry were performed as described by (Wu, Ma, Xian, Liu, Hui & Zhang, 2021a). The changes of protein thermal stability were studied by DSC (AQ20, TA, Co., USA). The specific operation scheme is as follows: each group of cured samples was weighed (1 g) and placed in −80 ℃ refrigerator for backup. To be tested, 10 ± 1 mg of thawed sample was weighed, put into a standard aluminum pot and sealed, and then heated from 25 to 100 ℃ at a scanning rate of 5 ℃/min under nitrogen atmosphere. An empty pan was set as the reference. TA software 5.2.0 was used to analyze enthalpy change (ΔH) and peak temperature (Tm).

Scanning electron microscope (SEM) spectral analysis

SEM samples were prepared as described by (Zhou, Hu & Wang, 2022) using the method conducted with a few modifications. The center part of the salted pork sample was cut into 0.5 cm × 0.5 cm × 0.5 cm and fixed for 24 h in a phosphate buffer containing 2.5 % glutaraldehyde (0.1 mol/L, pH = 7.2). Then, the sample was washed twice with phosphate buffer solution (0.1 mol/L, pH = 7.2) and gradually dehydrated with ethanol at different concentrations (30 %, 50 %, 70 %, 90 %, and 100 %) for 30 min at each concentration. The dehydrated samples were frozen in liquid nitrogen, and dried in a freeze dryer (SCIENTZ-12N, Xinzhi Biotechnology, China). Finally, microstructure of the samples was observed by SEM at a magnification of 200x and 500x.

Low-field nuclear magnetic resonance (LF-NMR)

The analysis of the moisture distribution of meat samples was performed on the LF-NMR analyzer (NMI20-040V-I, Niumag Analytical Instrument Corporation, Suzhou, China) as the method described by (Wu et al., 2021a) with slight modifications. The magnetic field strength was 0.5 T at 32 ℃, and the corresponding proton resonance frequency was 22.4 MHz. The remaining NMR parameters were as follows: SW, 100 kHz; SF, 20 MHz; RFD, 0.080 ms; RG1, 20.0db; P1, 8.00 μs; DRG1, 3; TD,200028; PRG-0; TW, 3000.00 ms; NS, 8; *P*2,15.52 μs; TE, 1.000 ms; and NECH, 2000. The surface moisture of the samples was removed before analysis, and then, 2 g sample was placed in a cylindrical glass tube with a 15 mm diameter. Each sample was preheated for 30 s before testing. Carr Purcell Meiboom Gill (CPMG) sequence was used to measure transverse relaxation time (T2). Relaxation times were recorded and fitted. Each sample was conducted three times.

Statistical analysis

Experimental data were processed on SPSS (IBM SPSS Statistics 22.0, SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to estimate the difference between the means ($P < 0.05$). To determine the correlation among the indexes, the Pearson correlation analysis was performed by using SPSS 22.0, and charts were drawn on the Origin software (Origin 8.6, Origin Lab Corporation, MA, USA), and correlation analysis was conducted.

Results and discussion

pH values, color, and cooking loss

The pH value of the samples gradually increased and differed significantly ($P < 0.05$) with the increase of time curing. As shown in Fig. 1(a), the control and 1.25 mL/kg groups showed a larger variation, with pH values increasing from 5.62 to 5.73 and 5.62 to 5.69, respectively, while the 5 mL/kg group demonstrated the smallest range of variation, only from 5.64 to 5.65. This phenomenon is due to the gradual degradation of lactic acid after acidification of pork tenderloin. The protein in the meat decomposes into ammonia, amino acids and other basic substances, which makes the meat be alkaline, leading to an increase in the pH value (Wu, Ma, Xian, Liu, Hui & Zhang, 2021b). Therefore, when the concentration of TLS is 5 mL/kg, the decomposition rate of protein can be better alleviated and the antioxidant capacity can

Table 1
Effect of different concentrations of TLS on color and cooking loss of pork tenderloin with curing time(n = 3).

		D1	D2	D3	D4
*L**	C	43.21 ± 2.92^{bAB}	43.58 ± 0.49^{bCD}	48.17 ± 0.44^{aA}	47.62 ± 0.89^{aA}
	20 mL/kg LS	45.31 ± 0.72^{bA}	49.17 ± 1.16^{aA}	44.53 ± 0.9^{bAB}	43.25 ± 1.98^{bBC}
	10 mL/kg LS	44.41 ± 4.26^{aA}	47.21 ± 1.54^{aAB}	41.75 ± 3.95^{aB}	44.24 ± 1.1^{aB}
	5 mL/kg LS	44.17 ± 2.79^{aA}	44.45 ± 1^{aBC}	43.98 ± 2.01^{aAB}	41.29 ± 1.51^{aCD}
	2.5 mL/kg LS	44.83 ± 1.13^{aA}	39.67 ± 2.09^{bD}	43.91 ± 1.92^{abAB}	41.63 ± 1.46^{aBCD}
	1.25 mL/kg LS	38.29 ± 1.56^{bB}	45.25 ± 2.46^{aB}	41.72 ± 2.31^{abB}	40.26 ± 0.86^{bD}
*a**	C	6.74 ± 1.34^{aA}	4.84 ± 0.59^{bA}	4.67 ± 0.83^{bD}	3.77 ± 0.32^{bD}
	20 mL/kg LS	6.8 ± 0.69^{aA}	6.97 ± 1.14^{aA}	7.56 ± 0.33^{aA}	7.77 ± 0.77^{aA}
	10 mL/kg LS	6.2 ± 0.39^{bA}	6.41 ± 1.17^{abA}	6.85 ± 0.17^{abA}	7.76 ± 0.51^{aA}
	5 mL/kg LS	5.71 ± 1.37^{aA}	5.86 ± 0.3^{aA}	6.54 ± 0.46^{aAB}	7.26 ± 0.63^{aAB}
	2.5 mL/kg LS	5.71 ± 1.37^{abA}	5.86 ± 0.3^{bA}	6.54 ± 0.46^{abBC}	7.26 ± 0.63^{aBC}
	1.25 mL/kg LS	6.4 ± 1.91^{aA}	5.19 ± 0.64^{bA}	5.01 ± 0.25^{bC}	4.96 ± 0.13^{abC}
*b**	C	-0.41 ± 0.47^{aA}	-1.86 ± 0.49^{bB}	-4.03 ± 0.36^{dC}	-5.08 ± 0.24^{cD}
	20 mL/kg LS	-1.18 ± 0.17^{aBC}	-1.24 ± 0.6^{aAB}	-1.38 ± 0.49^{bAB}	-1.43 ± 0.59^{aA}
	10 mL/kg LS	-1.3 ± 0.42^{aC}	-1.55 ± 1.1^{aAB}	-2.19 ± 0.45^{aAB}	-2.69 ± 0.38^{aABC}
	5 mL/kg LS	-1.98 ± 0.19^{cD}	-1.89 ± 0.31^{aA}	-1.79 ± 0.09^{bcA}	-1.45 ± 0.24^{bAB}
	2.5 mL/kg LS	-0.53 ± 0.42^{aAB}	-1.14 ± 0.22^{abAB}	-1.45 ± 0.87^{cBC}	-1.92 ± 0.13^{bBC}
	1.25 mL/kg LS	-1.18 ± 0.17^{bBC}	-1.35 ± 0.41^{bAB}	-2.83 ± 0.47^{cB}	-2.15 ± 0.33^{cC}
Cooking loss(%)	C	28.23 ± 1.42^{cC}	30.33 ± 0.93^{bcBC}	33.37 ± 2.11^{abA}	34.6 ± 1.65^{aB}
	20 mL/kg LS	31.07 ± 2.57^{abABC}	29.8 ± 0.26^{bC}	24.03 ± 0.95^{cB}	34.43 ± 3.61^{aB}
	10 mL/kg LS	31.73 ± 1.96^{aAB}	30.23 ± 1.72^{aBC}	20.73 ± 1.71^{bC}	28.8 ± 2.48^{aC}
	5 mL/kg LS	29.03 ± 0.93^{aBC}	28.4 ± 1.01^{aC}	17.9 ± 0.26^{cD}	25.2 ± 2.46^{bC}
	2.5 mL/kg LS	33.07 ± 1.29^{aA}	32.83 ± 2.1^{aAB}	23.63 ± 1.16^{cB}	29.17 ± 0.42^{bC}
	1.25 mL/kg LS	31.77 ± 0.8^{bAB}	33.97 ± 0.99^{aA}	34.7 ± 0.46^{aA}	36.07 ± 1.67^{aB}

D1: day one; D2: day two; D3: day three; D4: day four; Lower case letters a-c indicate differences between control and TLS treat samples during different times ($P < 0.05$); upper case letters A-D indicate differences between control and the vary concentrations TLS treatment within the same group ($P < 0.05$).

increase.

The color of the tenderloins cured with TLS is shown in Table 1. The experimental group containing TLS had significantly higher a^* values compared to the control group ($P < 0.05$). Moreover, results showed that samples had the highest a^* values when the concentration of TLS was at 20 mL/kg. This could have been a result of the browning effect of liquid smoke via Maillard reaction (Lund & Ray, 2017), where the carbonyl compounds in the liquid smoke react with the specific amino acids in the tenderloin, causing the sample to produce brown pigments and, consequently, increase the a^* values. With a higher content of TLS, the change in color is more prominent.

The quality of meat products can also be assessed by its cooking loss. Meat components lost during cooking are mainly water, as well as some sarcoplasmic proteins and intramuscular fat-soluble collagen (Ángel-Rendón et al., 2020). The higher the cooking loss rate, the poorer is considered the food quality. The steaming losses of loin marinated in TLS are shown in Table 1. Results revealed that the steaming loss increased day by day, except for the control and 1.25 mL/kg groups, until finally reaching 34.60 % and36.07 %, respectively. The remaining experimental groups treated with TLS showed a gradual decrease in cooking losses and reached a minimum on the third day followed by an increase. The steaming loss rate of the 5 mL/kg group was the lowest registered (17.90 % on the third day). This value was also significantly lower than that of the other experimental groups ($P < 0.05$), indicating that the loin cured with 5 mL/kg TLS contributed the most for the meat sample's quality. This phenomenon may be because the effective substances in the TLS at that concentration may be the most suitable for creating a spatial binding effect with proteins, which can promote the acyl transfer reaction between proteins (or within proteins) by catalyzing the cross-linking (polymerization) of glutamine and lysine residues, hydrolyze glutamine residues (hydrolytic deamidation), promote the covalent cross-linking interaction of proteins, and improve food texture (Romeih, 2017). Thus, compared with the control group and the 20 mL/kg group, the 5 mL/kg group seems to better induce MP to produce a complex heterogeneous network, meaning that it is difficult to release water during cooking and therefore, there is a higher possibility of low cooking loss (Santhi, Kalaikannan, Malairaj & Arun, 2017).

Protein oxidation

Carbonyl is the main product of protein oxidation, and thus, the content of carbonyl is used to evaluate the degree of protein oxidation (Gan, Li, Wang, Emara, Zhang & He, 2019). As can be observed from Fig. 1(b), there was a significant difference ($P < 0.05$) in the variation of protein carbonyl content between the experimental and control group samples. Under the influence of different concentrations of TLS, the carbonyl content of pork tenderloin on the fourth day was significantly lower than that of the control group (81.29 nmol/mg prot). Moreover, the carbonyl group in the experimental group increased slower than the control group under the influence of TLS. Among them, when the concentration of TLS was 5 mL/kg, the carbonyl content (D1: 42.06, D2: 46.06, D3: 50.96, D4: 52.82 nmol/mg prot) was lower than the other groups on all four days. The carbonyl content in the cured meat samples with high concentration of TLS was the highest among all groups. Some

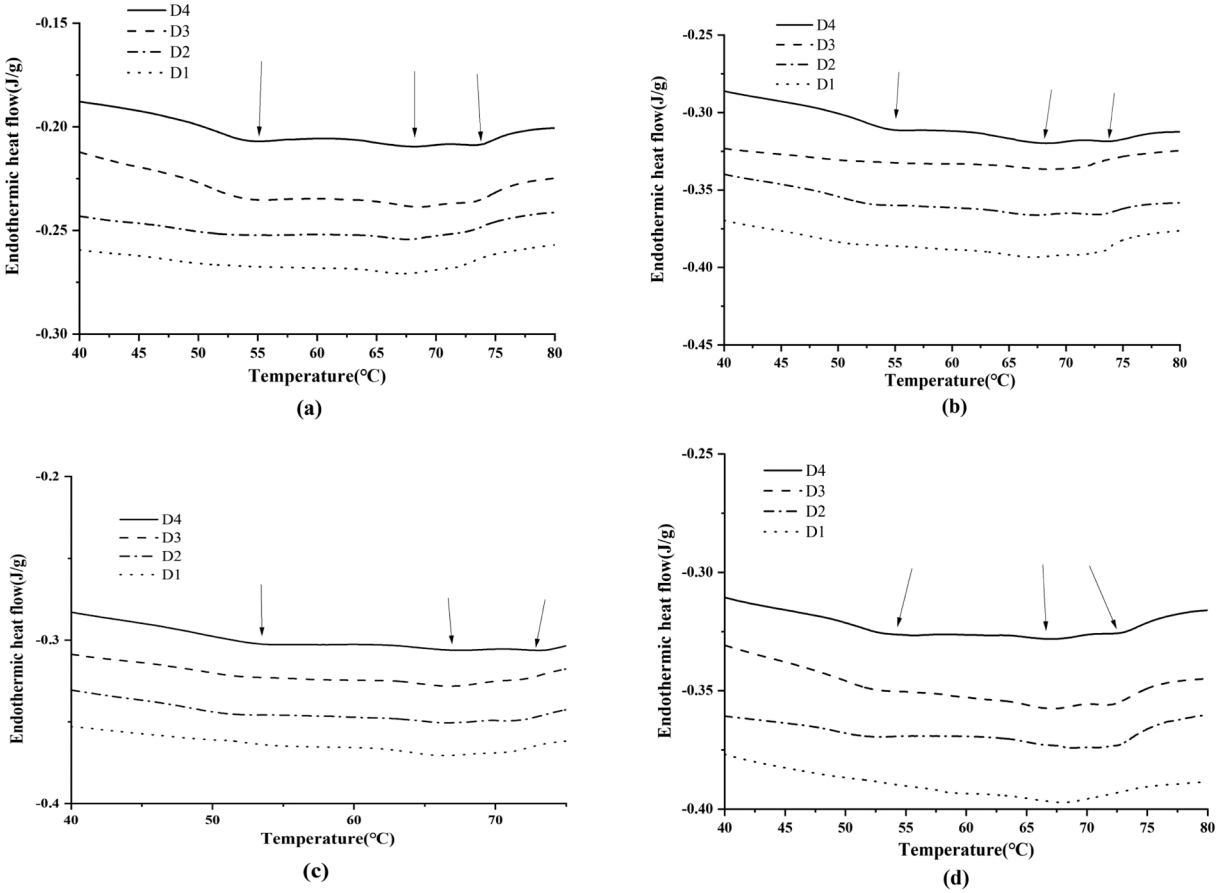

Fig. 2. DSC curves and Tm1 variation curves and ΔH variation trend graphs of tenderloin proteins treated with different concentration of TLS marinade (n = 3). D1: day one; D2: day two; D3: day three; D4: day four; (a): DSC curve of blank control group; (b): DSC curve for 20 mL/kg; (c): DSC curve for 10 mL/kg; (d): DSC curve for 5 mL/kg; (e): DSC curve for 2.5 mL/kg; (f): DSC curve for 1.25 mL/kg; (g): temperature variation curve of myosin denaturation; (h): trend of myosin denaturation ΔH; Lower case letters a-e indicate differences between control and TLS treat samples during different times ($P < 0.05$); upper case letters A-C indicate differences between control and the vary concentrations TLS treatment within the same group ($P < 0.05$).

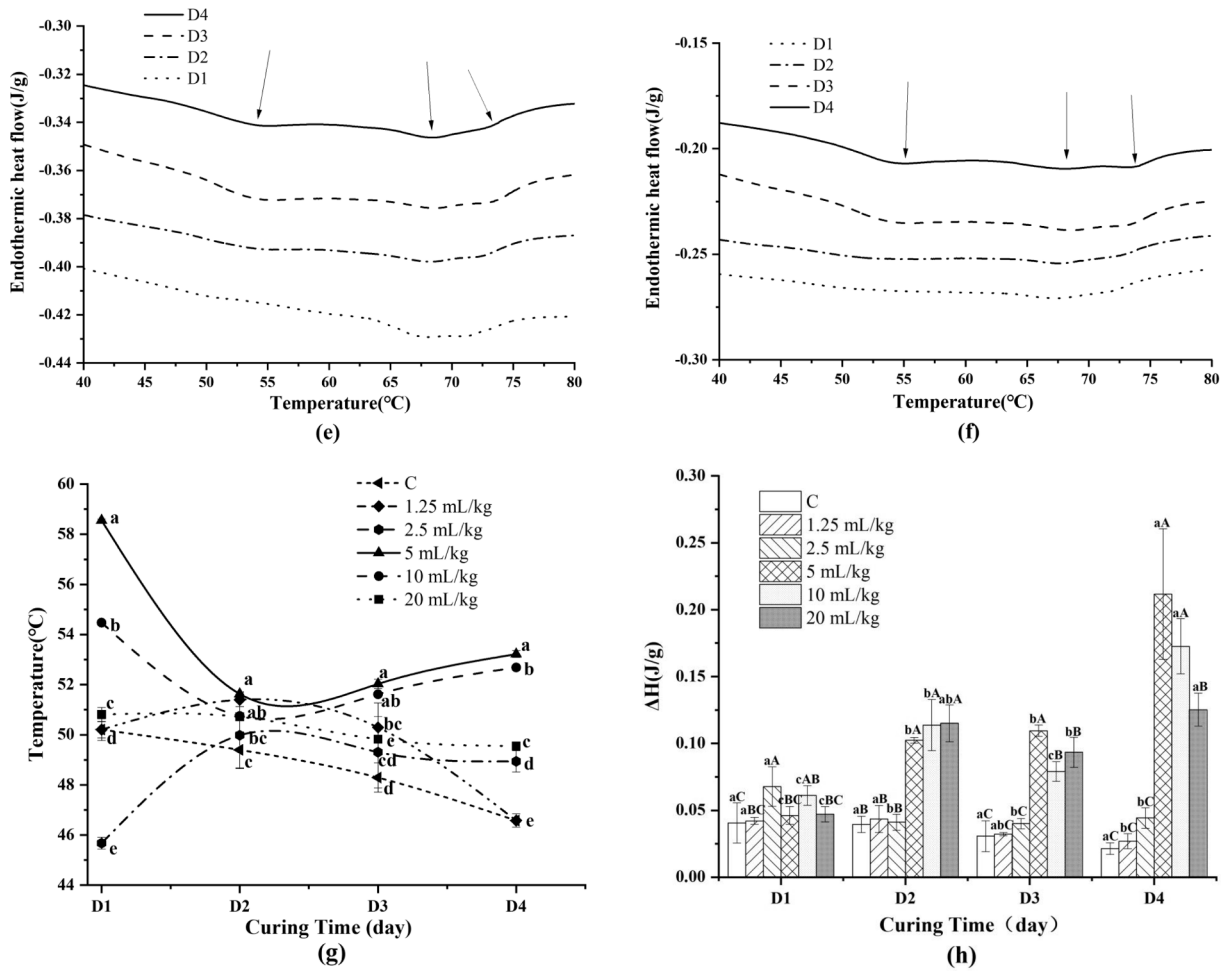

Fig. 2. (*continued*).

basic amino acids (lysine, histidine and arginine) are more vulnerable to free radical attack during the pickling process of high concentration liquid smoke, making them easier to convert into carbonyl derivatives (Soladoye et al., 2017). Thus, the carbonyl compounds in the 5 mL/kg TLS can quench the nucleophilic molecules in the protein, leading to the condensation reaction of carbonyl ammonia. This in turn results in the carbonyl content of 5 mL/kg groups being lower than that of other groups (Huang et al., 2022).

Sulfhydryl content is also an important index to characterize the degree of protein oxidation. MP is rich in sulfhydryl and total sulfhydryl groups include free sulfhydryl groups exposed to the surface of proteins, as well as sulfhydryl groups embedded in molecules. With the progress of oxidation, these sulfhydryl groups are easily converted into disulfide bonds, resulting in the reduction of total sulfhydryl (Lara, Gutierrez, Timón & Andrés, 2011). As can be seen in Fig. 1(c), there was a significant difference ($P < 0.05$) in the change of total sulfhydryl content according to the increase in the marination time of different mass fractions of TLS. Results showed that when marination reached the fourth day, the 5 mL/kg (0.924 mmol/L) sample had the lowest concentration of total sulfhydryl groups and the highest antioxidant capacity when compared with the 20 mL/kg (0.733 mmol/L) and the 10 mL/kg group (0.808 mmol/L). This was consistent with the changes in carbonyl content in the meat samples. However, excessive TLS concentrations during curing led to the oxidation of sulfhydryl groups into disulfide bonds in the meat to exceed the rate of the control group. This phenomenon may be because sulfhydryl residues (cysteine residues) of sulfur-containing amino acids are easier to be oxidized in an exposed environment (Zhang et al., 2020). High concentration of liquid smoke contains a large number of free radicals which promotes the change of protein structure, and some free sulfhydryl groups are produced in the cysteine residues exposed to the protein surface. With the emergence of the S—S bond, it becomes vulnerable to free radical quench (Ge, Han, Zheng, Zhao & Sun, 2020).

The antioxidant capacity of liquid smoke depends largely on its components, especially phenolic compounds (Soldera, Sebastianutto & Bortolomeazzi, 2008). When the concentration of liquid smoke is moderate, its antioxidant effect is greater, which can improve the stability of tenderloin (Soares et al., 2016). Therefore, when the concentration of TLS is 5 mL/kg, the degradation of sulfhydryl in MP can be delayed.

Surface hydrophobicity

Surface hydrophobicity can directly reflect the state of protein functional properties and the changes of protein conformation and structure, such as its tertiary structure. Fig. 1(d) shows the effect of TLS with different mass fractions on the surface hydrophobicity of MP. With the increase of curing time, the surface hydrophobicity of pork tenderloin myofibrillar protein in each group gradually increased and showed a significant difference between group C and experimental group ($P < 0.05$). At the fourth day of curing, the surface hydrophobicity of group C (87.85) was higher than that of 20 mL/kg (67.29), 10 mL/kg (77.70), 2.5 mL/kg (73.07), and 1.25 mL/kg (82.61), but lower than that of 5 mL/kg (89.63). Higher surface hydrophobicity indicates stronger hydrophobic interaction of protein molecules (Li, Zhang, Lu & Kang, 2021). Therefore, when the concentration of TLS was 5 mL/kg, the

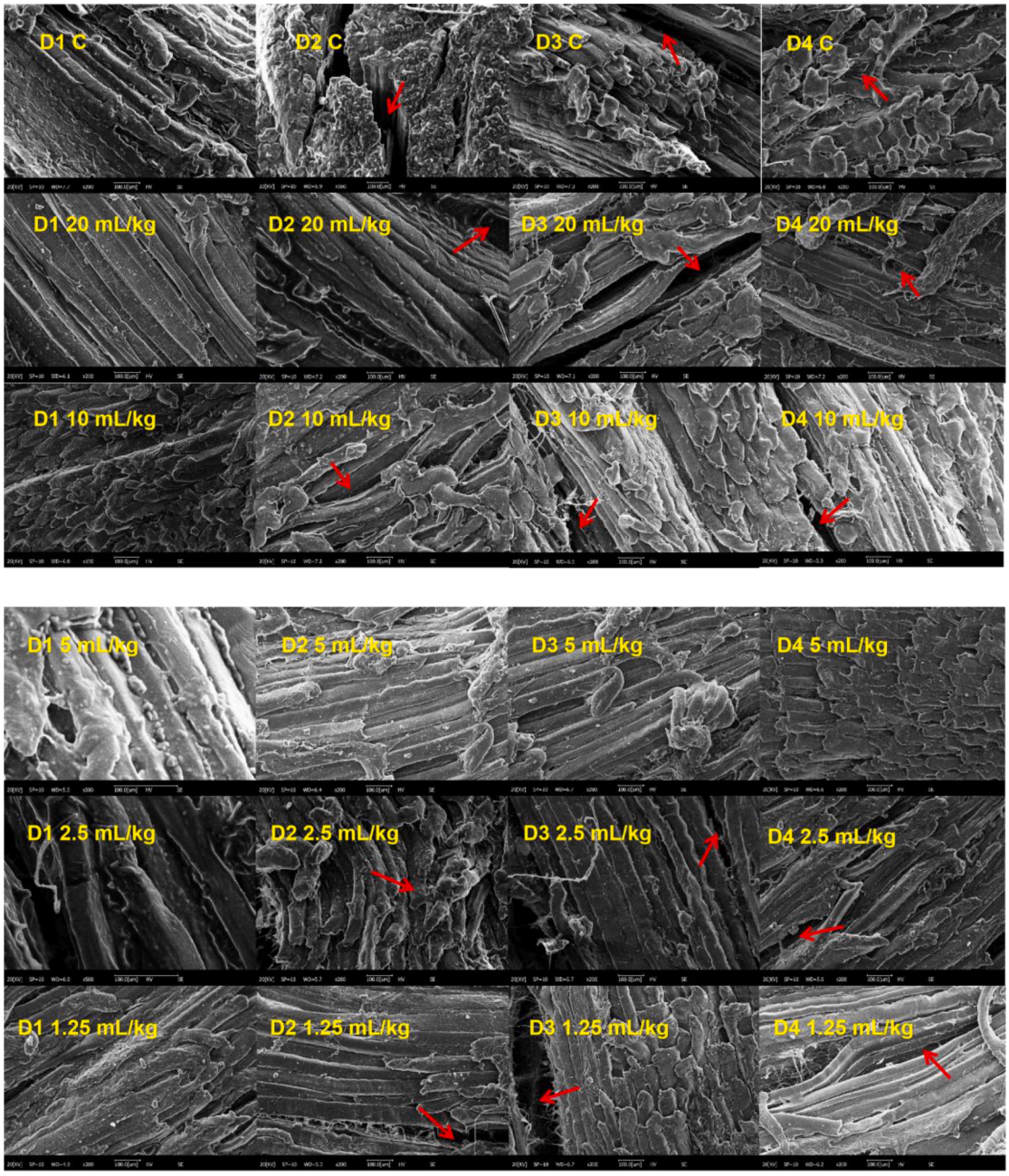

Fig. 3. The effect of TLS with different concentration on the microstructure of tenderloin cured at ×200 or ×500 times magnification. D1: day one; D2: day two; D3: day three; D4: day four.

surface hydrophobicity increased significantly. However, the high concentration of TLS inhibited the unfolding of protein tertiary structure ($P < 0.05$).

On a similar note, there are a large number of phenols in the TLS. The interaction between polyphenols and MP changes the conformation of protein, resulting in the unfolding of protein structure and the exposure of hydrophobic amino acids encapsulated therein. However, when the concentration of liquid smoke is too high, more polyphenols bind to MPs, resulting in excessive cross-linking and aggregation between proteins, hydrophobic amino acids were re embedded (Huang et al., 2022).

Differential scanning calorimetry

Protein denaturation involves endothermic dissociation and cleavage of covalent and non-covalent bonds within molecules. Tm and ΔH reflect the stability of protein structure, where Tm represents the temperature of protein denaturation and ΔH corresponds to the energy required to induce protein denaturation (Kaushik et al., 2016). As shown in Fig. 2(a-f), during thermal analysis of muscle protein, three absorption peaks representing myosin denaturation (Tm1), sarcoplasmic protein denaturation or connective tissue (Tm2), and actin denaturation (Tm3) were found between 25 ℃ and 100 ℃ (Soyer, Özalp, Dalmış & Bilgin, 2010). As myosin directly reflects the physical and chemical properties of meat and meat products (Huang, Zhang, Zhang, Fang & Zhou, 2021), this research paper will focus on the denaturation curve of myosin.

It was showed significant differences ($P < 0.05$) between the variation of MP thermal stability under the influence of TLS, as seen in Fig. 2 (g). The thermal stability of myosin was higher on the first day, when TLS was at a concentration of 5 mL/kg (58.55 °C) and 10 mL/kg

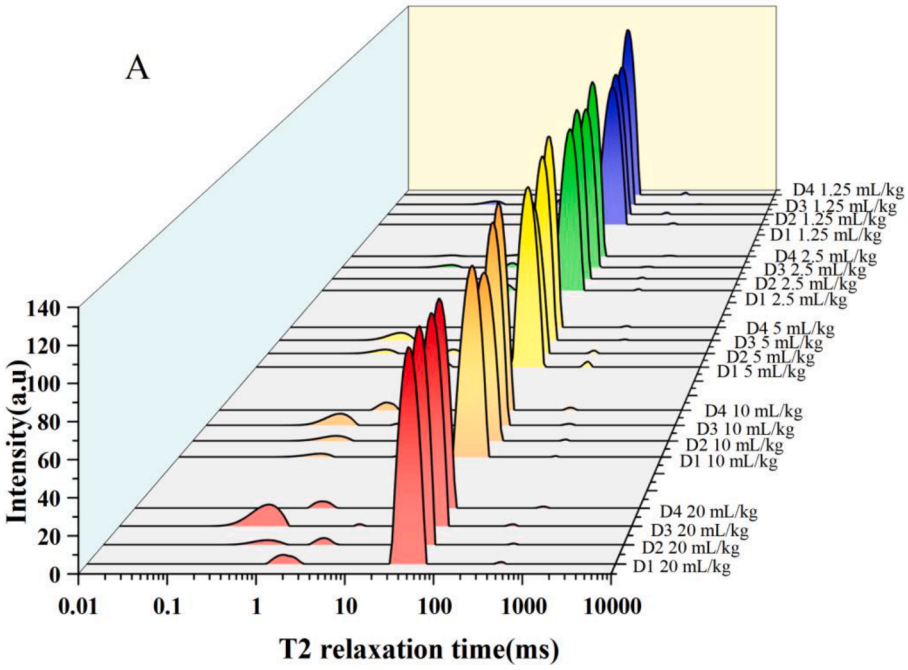

Fig. 4. Moisture state of tenderloin under the influence of different concentration of TLS. D1: day one; D2: day two; D3: day three; D4: day four; (A): indicates the change of moisture status with time under different concentrations of TLS curing treatment; (B): denotes the change in water percentage with time under different concentrations of TLS curing treatment; (C): denotes the time variation of the peak apex with time under different concentrations of TLS curing treatment.

(54.47 °C), with the 5 mL/kg group having better thermal stability. Subsequently, their thermal stability decreased regularly on the second day, gradually increased with the increase of curing time, and finally reached 53.22 °C and 52.68 °C on the fourth day. In contrast to this phenomenon, the DSC of the two groups with low concentration of TLS (2.5 mL/kg and 1.25 mL/kg), showed a tendency to rise first and then to decrease after the denaturation temperature reached 49.98 °C and 53.39 °C on the second day. This may be due to the enhanced intercellular contact of functional substances or free radicals in TLS with the protein's ionic strength and the formation of new covalent bonds due to the aggregation of proteins. Thus, it caused conformational changes and particle size increase, inducing protein denaturation more easily (Li et al., 2020). After that, the thermal stability of meat samples may improve again and it may also continue to increase with the degradation of groups, as well as with the occurrence of chemical reactions such as carbonyl condensation. Still, the mechanism of muscle protein unfolding during heating is very complex and cannot be explained only from the DSC results. Thermal denaturation of different muscle proteins occurs in a wide temperature range, and other secondary muscle proteins (such as titin and tropomyosin) may affect the peak value of DSC to a lesser extent. The phenomenon of meat samples in 2.5 mL/kg and 1.25 mL/kg groups may be due to the fact that the low concentration of TLS maintains thermal stability at the beginning, but since the concentration of TLS is low and there is excessive water, there is a lack of antibacterial effect, leading to the development and growth of microorganisms. The microorganisms harbored may grow by ingesting protein, this can result in the destruction of protein's structure (Soares et al., 2016).

The variation of ΔH and its significant difference ($P < 0.05$) in porcine *linea musculus* under the influence of TLS can be seen in Fig. 2 (h). The results showed that ΔH increased regularly with increasing curing time for samples with TLS concentration at 5–10 mL/kg. Moreover, it increased to 0.211 J/g and 0.172 J/g on the fourth day. ΔH performed best in the samples of 5 mL/kg group. This demonstrates that the thermal stability of myosin in the samples treated with TLS is enhanced. This may be due to the increased resistance of myosin to structural changes, such as aggregation and side chain polar group modification. However, the ΔH of the samples treated with TLS in the 2.5 mL/kg and 1.25 mL/kg groups gradually decreased. This could be explained by the low concentration of TLS that did not have a strong antibacterial effect, and thus, the excess moisture contributed to the growth of microorganisms, consequently making proteins more susceptible to denaturation. This phenomenon is also consistent with the change of denaturation temperature.

Scanning electron microscope

A scanning electron microscope (SEM) was used to observe the pork tenderloin tissue treated with different concentrations of TLS under the magnification of 200 or 500 times. The results showed that with the prolongation of curing time, muscle microstructure treated with different concentrations was significantly different ($P < 0.05$). It can be seen from Fig. 3 that with the increase in curing time, the 5 mL/kg muscle microstructure group had a tight arrangement of muscle fibers and a small gap between fibers.. In addition, compared with the 5 mL/kg group, the gap between the 20 mL/kg group and the 10 mL/kg and 5 mL/kg group was increased, as muscle fibers were not closely arranged, being loose instead. For group C and 1.25 mL/kg LS, large pores appeared on the surface of the sample due to the disordered arrangement of muscle fibers and increased gaps. These gaps may promote the formation of water channels and negatively affect the water holding capacity of muscles (Li et al., 2022), which is consistent with the results of cooking loss. Myofibrils of pork tenderloin cured with 5 mL/kg TLS were swollen and closely connected when compared with other groups, as the gap between the fibers was smaller and more orderly arranged. This indicates that a 5 mL/kg concentration of TLS can delay more successfully the damage to muscle tissue structure caused by oxidation.

Lf-NMR

The water mobility and distribution of pork tenderloin cured with different concentrations of TLS with the passage of curing time were studied by LF-NMR transverse relaxation method. The T2 relaxation

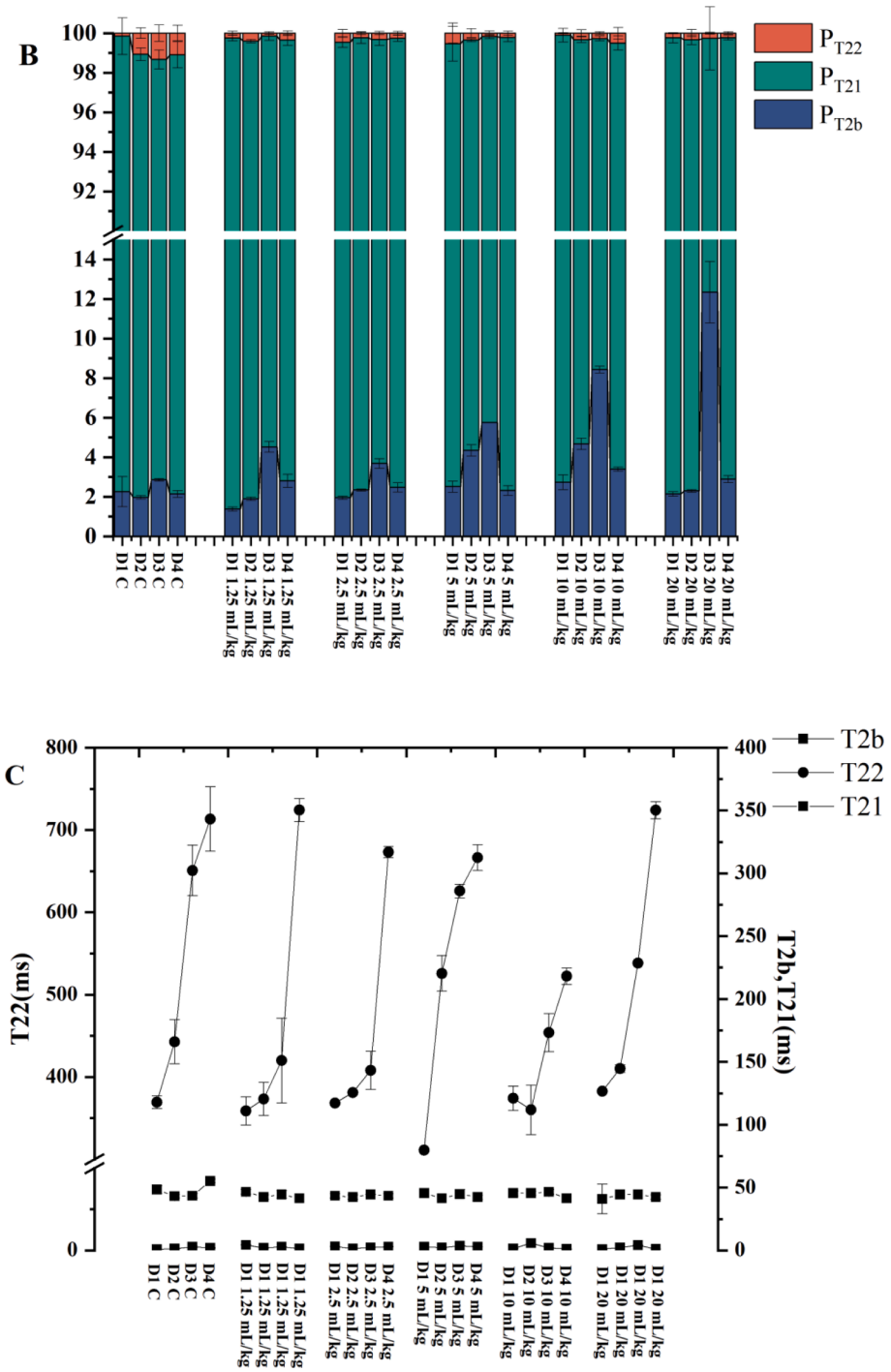

Fig. 4. (*continued*).

time distribution obtained is shown in Fig. 4(A). There are three different states of water in the sample. T2b (0–10 ms) corresponds to the bound water tightly bound to macromolecules; T21 (10–100 ms) represents the fixed water captured in the myofibrillar protein network, and finally, T22 (100–1000 ms) refers to the free water existing in the space between fiber bundles (Xu, Jin, Zhang & Chen, 2017).

The changes in the peak areas of T2b, T21 and T22 are shown in Fig. 4 (B). It is visible that the percentage of T2b increased gradually in each group along with the marination time extension and finally reached the highest percentage on the third day. This was particularly noticeable in the 20 mL/kg group, where the percentage of bound water accounted for 12.3 % of total water, but the percentage of bound water decreased in each group on the fourth day. Furthermore, the proportion of T22 gradually decreased with the extension of curing time. These results indicate that TLS could limit the ability of free movement of water molecules in muscle fibers, creating a denser network structure and stronger water binding ability. This occurrence may be due to the compounds in TLS that enhance the ability of proteins to bind to water, thus reducing the damage to protein structure and inhibiting oxidative denaturation of proteins (Li et al., 2021).

As can be seen in Fig. 4(C), the relaxation time of T2b and T21 had little effect as the curing time changed, but the relaxation time of T22 gradually increased. When the curing reached the fourth day, the relaxation times of the 10 mL/kg (673.197 ms), 5 mL/kg (666.537 ms),

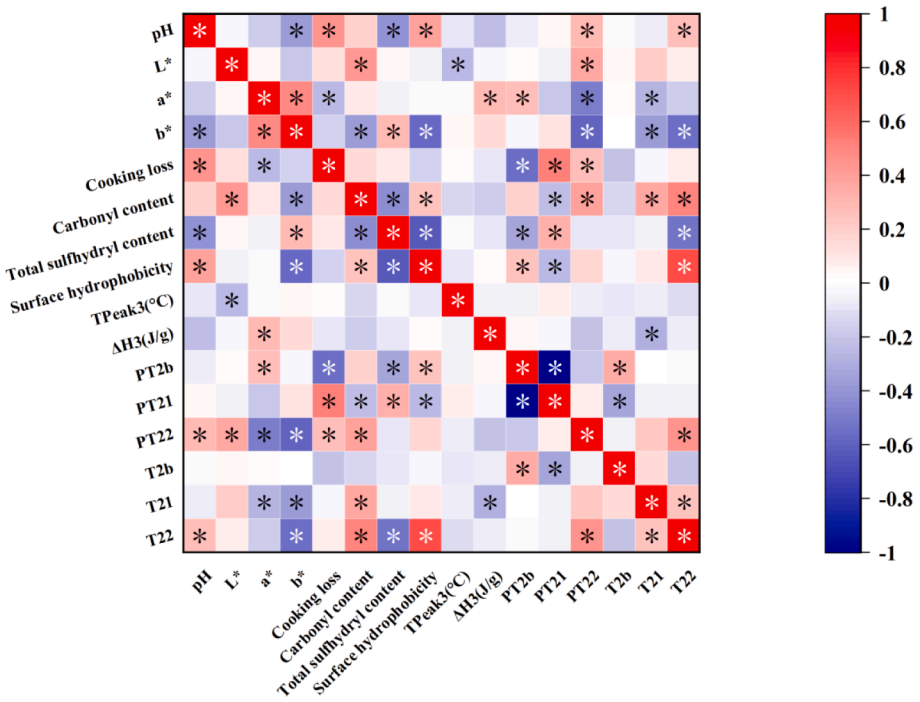

Fig. 5. Pearson's correlation analysis. Heat map of correlations between data on physical and chemical indicators, oxidation indicators, DSC and moisture distribution changes (*, $P < 0.05$).

and 2.5 mL/kg (522.473 ms) groups were shorter when compared with the control group (713.367 ms). The high concentration of TLS in the sample causes a decrease in the relaxation time of bound and solidified water, resulting in stronger binding between water and macromolecules. This phenomenon is consistent with the previous findings.

Correlation analysis

As shown in Fig. 5, correlations between physicochemical parameters, protein oxidation, surface hydrophobicity, DSC, and changes in water distribution were analyzed. The correlation between cooking loss and pH values was significant and positive ($P < 0.05$), while surface hydrophobicity was significant and positive with pH values and carbonyl content and negative with color ($P < 0.05$). PT2b was significant and negative with cooking loss and total sulfhydryl group and significant and positive with surface hydrophobicity ($P < 0.05$); PT21 was significant and positive with cooking loss and total sulfhydryl group and significant and negative with PT2b, carbonyl group and finally, PT21 was significantly and positively correlated with cooking loss and total sulfhydryl group, and negatively correlated with PT2b, carbonyl group and surface hydrophobicity ($P < 0.05$). The results indicated that protein oxidation indexes were closely related to moisture distribution, color and steaming loss. While adding different concentrations of TLS, the antioxidant properties of tenderloin were altered by affecting the changes in the indexes of moisture distribution, pH values, and steaming loss.

Conclusion

This study demonstrated the effect of different concentrations of TLS on the physicochemical properties, microstructure, and moisture distribution of pork loin during the curing process. When the concentration of TLS in pork tenderloin was 5 mL/kg, the cured tenderloin had a best performance of pH values and WHC, and there was improved hydrophobicity, reduced carbonyl value and higher total mercaptan base, and antioxidant capacity was also displayed. Combining the results of DSC and microstructure, the addition of a 5 mL/kg concentration of TLS improved the thermal stability of loin meat proteins and could have a better protein network structure. According to the results of LF-NMR, the TLS of the tea-tree branch increased the percentage of bound water with a denser network structure and stronger water binding ability, which reduced the damage to protein structure and inhibited oxidative denaturation of proteins. The results of the correlation analysis indicated further that protein oxidation indexes were closely related to moisture distribution, color, and steaming loss. Therefore, the antioxidant capacity of tenderloin had a close correlation with the indexes of moisture distribution, pH values, and steaming loss, which were adjusted by the altered concentration of TLS.

CRediT authorship contribution statement

Yanpei Huang: Conceptualization, Visualization, Formal analysis, Writing – original draft. **Ying Zhou:** Conceptualization, Formal analysis, Writing – review & editing. **Yuanyuan Liu:** Conceptualization, Formal analysis, Writing – review & editing. **Jing Wan:** Conceptualization, Formal analysis, Writing – review & editing. **Ping Hu:** Conceptualization. **Linggao Liu:** Conceptualization, Writing – review & editing. **Mingming Li:** Writing – review & editing. **Yeling Zhou:** Methodology. **Sha Gu:** Project administration. **Dan Chen:** Writing – review & editing. **Bokai Hu:** Resources. **Ke Hu:** Writing – review & editing. **Qiujin Zhu:** Conceptualization, Supervision, Writing – review & editing, 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

Data will be made available on request.

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