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Effects of ultrasound-assisted thawing on lamb meat quality and oxidative stability during refrigerated storage using non-targeted metabolomics

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ABSTRACT

The aim of this study was to evaluate the changes of ultrasound-assisted thawing on lamb meat quality and differential metabolite profiles during refrigerated storage. Compared with flow water thawing (FW), pH, a^*, C^*, and sulfhydryl content of lamb were significantly increased, while L^*, drip loss and cooking loss were significantly decreased after ultrasound-assisted thawing (UT). On day 1 (UT1 and FW1) and day 7 (UT7 and FW7) in the UT and FW groups, principal component analysis explained 42.22% and 39.25% of the total variance. In this study, 44 (UT1 and FW1) and 47 (UT7 and FW7) differentially expressed metabolites were identified, including amino acids, carbohydrates and their conjugates, nucleic acids, carbonyl compounds and others. The results of this study provide data to clarify the differences between UT and FW, and lay a foundation for the application of ultrasound-assisted thawing in the meat industry.

1. Introduction

The amount of sheep and goat meat imported by China ranks first in the world, mainly from Australia, New Zealand and Uruguay [1]. Freezing is often used in transport to preserve nutritional value and extend the refrigeration time of meat [2]. Frozen meat needs to be thawed before further processing and consumption [3]. However, due to drip loss, enzymatic reaction, and lipid and protein oxidation during thawing, the chemical and physical properties of the meat have unacceptable changes, which eventually causes the meat quality to deteriorate [4]. Therefore, it is crucial to explore efficient and safe thawing methods for the preservation of meat quality.

In recent years, many studies on new thawing technologies have attracted much attention, such as microwave thawing [5], ultrasound-assisted thawing [6], radio-frequency thawing [7] and high-voltage electric field thawing [8], which have the advantages of accelerating thawing speed and reducing thawing loss of meat. Among them, the ultrasound-assisted thawing technology achieves the purpose of thawing by converting vibration energy into heat [9], while the internal temperature of the meat sample remains relatively low [10]. A previous study reported that, compared with microwave thawing, room temperature thawing, and infrared thawing methods, ultrasound-assisted thawing showed a better effect. This was because the ultrasonic thawing was short and the thawing was uniform, which could better maintain the muscle structure and reduced the decline in quality caused by thawing [11]. Studies have confirmed that, compared to water thawing and air thawing, ultrasound-assisted thawing technology reduced thawing time by 71 % and 87 %, respectively [12]. Guo et al. [6] found that the ultrasonic power at 400 W improved the thawing quality of frozen meat better. Sun et al. [13] reported that under the condition of ultrasonic power of 300 W, ultrasound-assisted thawing accelerated the thawing process and maintained meat quality. This is related to the degree of penetration of ultrasound frequency that causes the effect of cavitation. Therefore, selecting appropriate ultrasonic parameters to improve the quality of quick-frozen products without compromising the quality of frozen products is a current research hotspot. Nevertheless, the appropriate application research of the appropriate ultrasonic power in the field of meat preservation needs to be further developed in order to implement it at the industrial level.

As an emerging technology, non-targeted metabolomics can be used to analyze the effects of pre- and post-mortem factors on the metabolomics profile of meat, such as feeding regimes [14], castration [15], geographic origin [16], irradiation treatment [17], post-mortem time [18], and pH [19]. However, no studies have reported the changes in lamb metabolites during refrigeration after ultrasound-assisted thawing.

In this study, we aimed to analyze the effects of ultrasound-assisted

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thawing on changes in lamb metabolites during refrigeration storage using untargeted metabolomics, and to explore the mechanism of ultrasound-assisted thawing affecting the oxidation stability and meat quality of lamb. These results help provide new insights into the application of ultrasound-assisted thawing in the meat industry.

2. Materials and methods

2.1. Chemicals

Methanol, acetonitrile and formic acid were supplied by the Merck Company (Shanghai, China), and ammonium acetate was purchased from Sigma-Aldrich (Shanghai, China), all of them were of high performance liquid chromatography (HPLC) grade. Other reagents used in this study were analytical grade, including ethanol absolute, ethyl acetate, glacial acetic acid, ethyl acetate, and trichloroacetic acid (Sinopharm Chemical Reagent Co., ltd., Shanghai, China).

2.2. Sample preparation

The *longissimus lumborum* (LL) muscle samples from 8 male small-tailed Han sheep (8 months old, carcass weight: 29.0 ± 1.4 kg) were purchased at 48 h postmortem from a local market in Beijing (Beijing Musenweiye Halal Food Co., ltd.) and transported to the laboratory in ice bags within 20 min. After trimming off visible fat and connective tissue, each loin muscle of each animal was cut into six equal sections (12 cm × 6 cm × 5 cm, 300.0 ± 10.0 g in weight) perpendicular to the fiber direction. Each section of meat was individually packed in a vacuum skin packaging (VSP) and immediately frozen in a freezer (Haier Company, Qingdao, China) at −20 °C for 24 h. VSP samples used a bottom tray (Cryovac polypropylene tray, Sealed Air Corp., Shanghai, China) and a heat-shrinkable vacuum top film (150 μm thick, oxygen permeability rate was 2 cm^3/m^2/24 h/0% relative humidity at 23 °C), water vapor transmission was 4 g/m^2/24 h/90 % relative humidity at 38 °C).

The frozen lamb was thawed using ultrasound-assisted thawing (UT) and flow water (FW) thawing. Thawed meat was refrigerated at 4 °C for 1 d, 4 d, and 7 d. Six sections per animal (n = 8) were randomly assigned to six treatments (two thawing treatments × three refrigeration times; UT1, UT4, UT7, FW1, FW4, and FW7). At each sampling time, meat samples were removed from the VSP to assess pH, meat color, drip loss, cooking loss, protein oxidation, and lipid oxidation in triplicate. About 10.0 g of each meat sample was quickly frozen in liquid nitrogen and stored at −80 °C for metabolomics analysis (six replicates of each group).

2.3. Thawing treatments

2.3.1. Ultrasound-assisted thawing

The samples were ultrasonically thawed using a desktop ultrasonic machine (KQ-700DV, Kunshan Ultrasonic Instrument Co., ltd., Suzhou, China) with real-time temperature measurement system. Based on the previous research basis [6,13], the power of the ultrasound was set to 350 W and the frequency was set to 40 kHz. The sample was immersed in the water bath, and then the temperature of the ultrasonic water bath was maintained at 10° C by adding ice.

2.3.2. Flow water thawing

The thawed samples were placed in a water tank 500 × 300 × 150 mm (20 L), the water temperature was maintained at 10 °C by using ice, and the water flow rate was 120 mL/s.

A digital temperature meter (UT 325, Uni-Trend Technology Limited, Dongguan, China) was inserted into the geometric center of the two spare meat samples to detect the temperature in the thawing process. Thawing was completed when the core temperature of the sample reached 4 °C.

2.4. pH and meat color analysis

The pH of the samples was measured by inserting a portable pH meter (Seven2Go, Mettler Toledo, Greinfensee, Switzerland) equipped with a penetrating electrode into the meat. The pH meter was calibrated with pH 4 and pH 7 standard buffer solutions before use. Each sample was assayed three times.

The surface color of the thawed samples (approximately 80 g) was measured using a colorimeter (CR-400, Minolta Camera Co. ltd., Osaka, Japan) with an illuminant of D65/10°. The LL muscle samples were cut for 45 min after blooming, lightness (L^*), redness (a^*), and yellowness (b^*) of different sites of the exposed surface were randomly selected for three measurements, and the average value was calculated. The Chroma (C^*) was calculated as follows:

$$C^* = (a^{*2} + b^{*2})^{1/2}.$$

2.5. Drip loss and cooking loss measurement

Drip loss was measured according to the modified method of Honikel [20]. At each sampling time, the meat samples were weighed (*w0*), placed in air-filled polyethylene bags, suspended and stored at 4 °C for 24 h. After the sample was taken out, it was tapped with filter paper to absorb water and weighed (*w1*).

$$\textit{Drip loss}\ (\%) = (w0 - w1)/w0 \times 100\ \%.$$

The cooking loss was measured according to the method of Zhang et al. [21]. After the thawed samples were weighed (*M0*), the samples were placed in polyethylene bags and then put in the constant temperature water bath until the central temperature of the samples was 75 °C and taken out. The samples were weighed after cooling (*M1*).

$$\textit{Cooking loss}\ (\%) = (M0 - M1)/M0 \times 100\ \%.$$

2.6. Protein oxidation determination

The protein carbonyl content of meat was evaluated following the method of Soglia, Petracci, & Ertbjerg [22]. The protein carbonyl group was oxidized when reacting with 2,4-dinitrophenylhydrazine (DNPH) to form a yellow precipitate. In brief, muscle samples (about 1 g) and 10 mL of cold 0.15 M KCl solution were homogenized after mixing using homogenizer (Ultra-Turrax ® IKA T25, Labotechnik, Germany). One sample (0.1 mL) was mixed with 1 mL of 2 M HCl containing 0.2 %(w/v) DNPH, and the other sample (0.1 mL) was mixed with 1 mL of 2 M HCl and set as the blank. The protein was precipitated with 1 mL 20 % TCA, and was washed with 1 mL ethanol-ethyl acetate (1:1, V:V), and then centrifuged for 5 min at 10,000 g. The washing program was repeated three times. The precipitate was dissolved in guanidine hydrochloride and potassium phosphate buffer, incubated in a 37 °C water bath, and centrifuged for 15 min at 10,000 g. Determine the carbonyl content by reading the absorbance at 370 nm. The protein concentration was determined using the BCA kit (Nanjing Jiancheng Biotechnology Co., ltd., Nanjing, China). The results were expressed as nmol carbonyl per mg protein.

The sulfhydryl groups was determined based on the principle that sulfhydryl compounds react with 5,5′-dithiobis-2-nitrobenzoic acid to produce yellow 5-mercapto-2-nitrobenzoic acid by the method of Wang et al. [23] with minor modifications. Concisely, one gram of meat was mixed with 9 mL of cold 0.9 % saline and homogenized. The homogenate protein concentration was measured using the BCA kit (Nanjing Jiancheng Biotechnology Co., ltd., Nanjing, China). The sulfhydryl content of the homogenates was detected using a total sulfhydryl kit (Nanjing Jiancheng Biotechnology Co., ltd., Nanjing, China). Absorbance values were measured at 405 nm using a microplate reader (Bio-Tek Synergy H4, Bio-Tek, USA). The results were expressed as nmol total sulfhydryl content per mg protein.

Table 1
Effects of different thawing treatments on changes of lamb meat quality traits during refrigerated storage.

Item	Treatments (T)	Days (D)			SEM	Significance		
		1	4	7		T	D	T × D
pH	UT	5.68^{b}	5.76^{a}	$5.8^{a,A}$	0.02	***	***	NS
	FW	5.64^{b}	5.71^{a}	$5.74^{a,B}$				
Drip loss (%)	UT	$0.86^{b,B}$	$0.98^{b,B}$	1.17^{a}	0.05	***	***	NS
	FW	1.05^{A}	1.11^{A}	1.22				
Cooking loss (%)	UT	$29.01^{b,B}$	$31.95^{a,B}$	$33.06^{a,B}$	0.41	***	***	NS
	FW	$30.62^{c,A}$	$33.57^{a,A}$	$34.62^{a,A}$				
*L**	UT	$38.40^{b,B}$	40.47^{a}	$41.24^{a,B}$	0.40	***	***	NS
	FW	$40.80^{b,A}$	41.52^{ab}	$42.51^{a,A}$				
*a**	UT	$13.22^{a,A}$	$12.13^{b,A}$	$12.02^{b,A}$	0.21	***	***	NS
	FW	$12.43^{a,B}$	$10.95^{b,B}$	$10.44^{b,B}$				
*b**	UT	11.39^{b}	12.36^{a}	$12.42^{a,B}$	0.15	**	***	NS
	FW	11.86^{b}	12.47^{a}	$12.77^{a,A}$				
*C**	UT	17.46	17.32^{A}	17.28^{A}	0.17	***	*	NS
	FW	17.19^{a}	$16.60^{b,B}$	$16.51^{b,B}$				

UT, ultrasound-assisted thawing; FW, flow water thawing; SEM, standard error of the mean.
Different capital letters show statistically significant differences between treatments at the same time ($P < 0.05$).
Different lowercase letters show statistically significant differences among different times of the same treatment ($P < 0.05$).
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, no significant differences.

2.7. Lipid oxidation determination

The method of McDonald and Hultin [24] was slightly modified to assess lipid oxidation by measuring thiobarbituric acid reactive substances (TBARS). Approximately 1.0 g of meat sample was placed in a test tube containing 10 mL of deionized water and homogenized. After mixing 1 mL of muscle homogenate, 3 mL of 2 % butylated hydroxytoluene, and 2 mL of 15 % trichloroacetic acid solution, incubated in a 95 °C water bath for 40 min, and then cooled under running cold tap water. After centrifugation for 10 min, the absorbance was determined at 532 nm using a Unico SQ2800 UV/VIS spectrophotometer (United Products & Instruments Inc., New Jersey, USA). Results were expressed in mg malondialdehyde per kg of meat.

2.8. Non-targeted metabolomics analysis

The muscle tissue was ground by liquid nitrogen and weighed (100.0 mg) into Eppendorf tubes, followed by mixing with 1 mL of a pre-cooled mixture of methanol, acetonitrile, and water (2:2:1, v/v/v). The samples were sonicated for 1 h in an ice bath and left at −20 °C for 1 h, then centrifuged at 14,000 g for 20 min at 4 °C. The supernatant was concentrated to dryness in a termovap sample concentrator. After that, 100 μL of acetonitrile–water solution (1:1, v/v) was added for redissolution, centrifuged at 20,000 g for 40 min at 4 °C. The obtained supernatant was taken for further analysis.

Non-targeted metabolomic analysis of the samples was performed on an ultra-high performance liquid chromatography Q-Exactive Orbitrap mass spectrometer (UPLC Q-Exactive Orbitrap MS) system (Thermo Fisher Scientific, San Jose, CA, USA). Chromatography was performed on an Acquity UPLC BEH Amide column (1.7 μm, 100 mm id × 2.1 mm, Waters Corp., Milford, MA, USA) at a flow rate of 0.3 mL/min and the column was held at 30 °C. The mobile phase consisted of a mixture of mobile phase A (25 mmol/L ammonium acetate and 25 mmol/L ammonium hydroxide in water) and mobile phase B (100 % acetonitrile solution). The injection volume was set to 3 μL. The gradient elution program was as follows: 0–1 min, 95 % B; 1–7 min, 95–65 % B; 7–9 min, 65–35 % B; 9–10.5 min, 35 % B; 10.5–11 min B, 35–95 % B; 11–15 min, 95 % B.

The mass spectrometer (MS) used the heated electrospray ionization (HESI) in positive and negative ion modes for detection. HESI source conditions were set as follows: sheath gas and auxiliary gas flow rates of 30 and 5 arbitrary gases, respectively, spray voltages of 3.8 kV (+) and 3.2 kV (-), capillary temperature and heater temperature of 320 °C and 350 °C, respectively. The MS resolution of the full scan was 70,000, the MS/MS resolution was 17,500, and the mass scan range was 80–1200 *m/z*.

Quality control (QC) samples were prepared by mixing aliquots of each muscle sample and analyzed every-six sample injections to monitor the stability of the analytes in the samples.

2.9. Metabolomics data analysis

Raw MS data were processed using MS-DIAL for peak alignment, retention time correction, and peak area extraction. Metabolites were comparatively identified by Massbank (https://www.massbank.jp), Human Metabolome Database (HMDB) (https://www.hmdb.ca), and an internal database (Shanghai Bioprofile Technology Co., ltd.). Orthographic projection models for Principal component analysis (PCA), Partial least squares-discriminant analysis (PLS-DA) and orthogonal projections to latent structures-discriminant analysis (OPLS-DA) were performed by SMICA-P software (14.1, Umetrics, Umeå, Sweden) to screen for differential metabolites with the variable importance in projection (VIP) > 1 and $P < 0.05$ assessed by analysis of variance (ANOVA). The model parameters R^2Y and Q^2Y were used to check the quality of the prediction model. Metabolic pathway analysis was determined online using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (https://www.genome.jp/kegg). The results were visualized using the Pheatmap package (v.4.0.3) in R for agglomerative hierarchical clustering.

2.10. Statistical analysis

All analyses were performed in triplicate. Meat physicochemical composition data were analyzed by two-way ANOVA as a completely randomized design using the PROC-MIXED program of the SAS 9.2 program (SAS Institute, Cary, NC, USA). Thawing treatments (T, ultrasound-assisted thawing and flow water thawing), refrigeration time (D, 1 d, 4 d and 7 d), and the interaction (T × D) were considered fixed effects, while each piece of meat was considered a random effect. The least-square means program was used to calculate the treatment mean. *P* values below 5 % were determined to be significantly different. Figures in the study were generated with Origin software (v. 9.0, MicroCal Software Inc., Northampton, MA, USA).

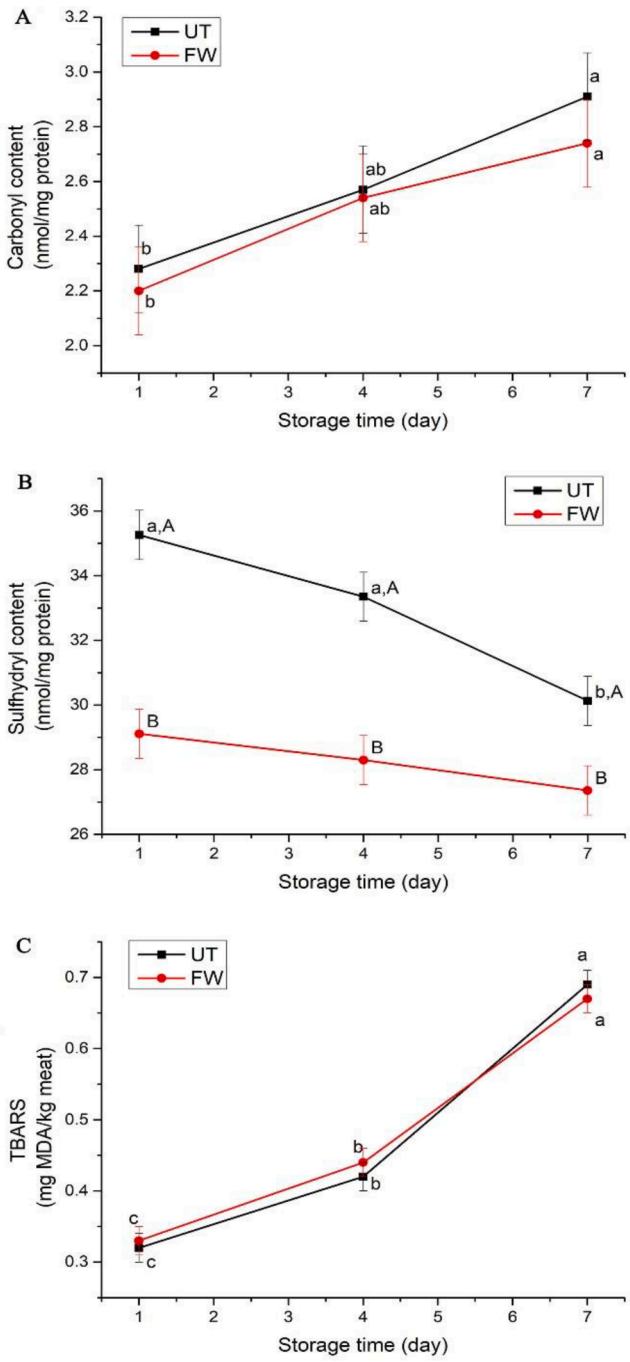

Fig. 1. Effects of different thawing treatments on carbonyl content (A), sulfhydryl content (B) and TBARS (C) of lamb during refrigerated storage. UT and FW represent ultrasound-assisted thawing and flow water thawing, respectively. Different capital letters show statistically significant differences between treatments at the same time ($P < 0.05$). Different lowercase letters show statistically significant differences between different times of the same treatment ($P < 0.05$).

3. Results and discussion

3.1. Changes in meat quality traits

The effects of different thawing treatments on the changes in lamb meat quality traits during refrigerated storage are illustrated in Table 1. All meat quality traits were not affected by the interaction effect of thawing treatment and storage time ($P > 0.05$). As an important indicator for evaluating meat quality, pH value affects the sensory quality and nutritional value of meat [25]. The pH of lamb stored for 7 days in the UT group was higher than that in the FW group ($P < 0.05$). This was due to the longer thawing time in the FW group, and the loss of minerals with the aqueous solution disrupted the ionic balance in the meat and resulted in a decrease in pH [26]. The pH increase due to storage time may be related to the formation of alkaline compounds in the meat [27].

Drip loss and cooking loss are key indicators for evaluating meat quality, reflecting the water retention properties of muscles. The UT group had lower drip loss and cooking loss ($P < 0.05$) compared with the FW group. In addition, drip loss and cooking loss showed a significant increasing trend with the prolongation of storage time ($P < 0.05$). Prolonged flow water thawing treatment could increase the degree of damage to the muscle fiber structure, which reduced the water-holding capacity of the muscle and lead to increased drip loss and cooking loss [28–29]. These results were consistent with those obtained by Guo et al. [6], who demonstrated that appropriate ultrasound power treatment reduced water loss in muscle. All these results indicated that the ultrasound-assisted thawing treatment improved the water retention capacity of the muscle.

Consumers' purchasing preferences in the market are determined by the color of meat [30]. The color of lamb meat changed to varying degrees after thawing by the two different methods. The L^* and b^* values in the FW group were higher ($P < 0.05$) compared with the UT treatment group, on the 7th day of storage. The increase in L^* was due to the moisture within the meat cells diffused to the meat surface causing more light to be reflected after FW treatment [13]. The elevated b^* value caused by FW treatment may be related to browning reactions [31]. The L^* value increases gradually with the storage time, which also corresponds to the result of drip loss. The a^* and C^* values of the UT-treated group were significantly higher than those of the FW-treated group ($P < 0.05$). In general, the a^* value was related to myoglobin and hemoglobin concentrations, and these results were similar to those of Guo et al. [6], who found that appropriate ultrasound-assisted thawing could slow down the oxidation of chromoproteins. The a^* and C^* values showed the same trend over time, indicating that the loss of water and the oxidation of myoglobin could lead to darker meat color [28].

3.2. Protein and lipid oxidation stability

The changes in carbonyl content, sulfhydryl content and TBARS in lamb meat are shown in Fig. 1. The formation of carbonyl compounds and the loss of total sulfhydryl groups were often used to reflect protein oxidation [23]. Thus, higher carbonyl content and lower sulfhydryl groups represent a higher degree of protein oxidation in the meat samples. Compared with the FW group, the UT group had no significant effect on the carbonyl content of lamb during the refrigerated storage period ($P > 0.05$). However, the content of sulfhydryl groups in lamb was significantly increased in the UT-treated group ($P < 0.05$), indicating a higher level of protein oxidation in the FW-treated group. Protein oxidation can lead to the oxidation of partially active sulfhydryl groups to form disulfide bonds, resulting in a decrease in the content of total sulfhydryl groups [32]. In the present study, the protein carbonyl content of meat increased with storage time after both thawing methods (Fig. 1A), and the carbonyl content of meat on day 1 was significantly lower than that on day 7 ($P < 0.05$). This result was similar to the observations reported by Xu et al. [33] for lamb during refrigerated storage. Sulfhydryl content was found to decrease significantly with storage time only in the UT-treated group (Fig. 1B), where the sulfhydryl content at 1d and 4d was significantly higher than that at 7d ($P < 0.05$). This indicated that the degree of protein oxidative denaturation gradually increased with the increase of storage time. These results could be attributed to the cavitation effect of ultrasound, which effectively preserved the integrity of cells during thawing, thereby inhibiting the oxidation of proteins [34].

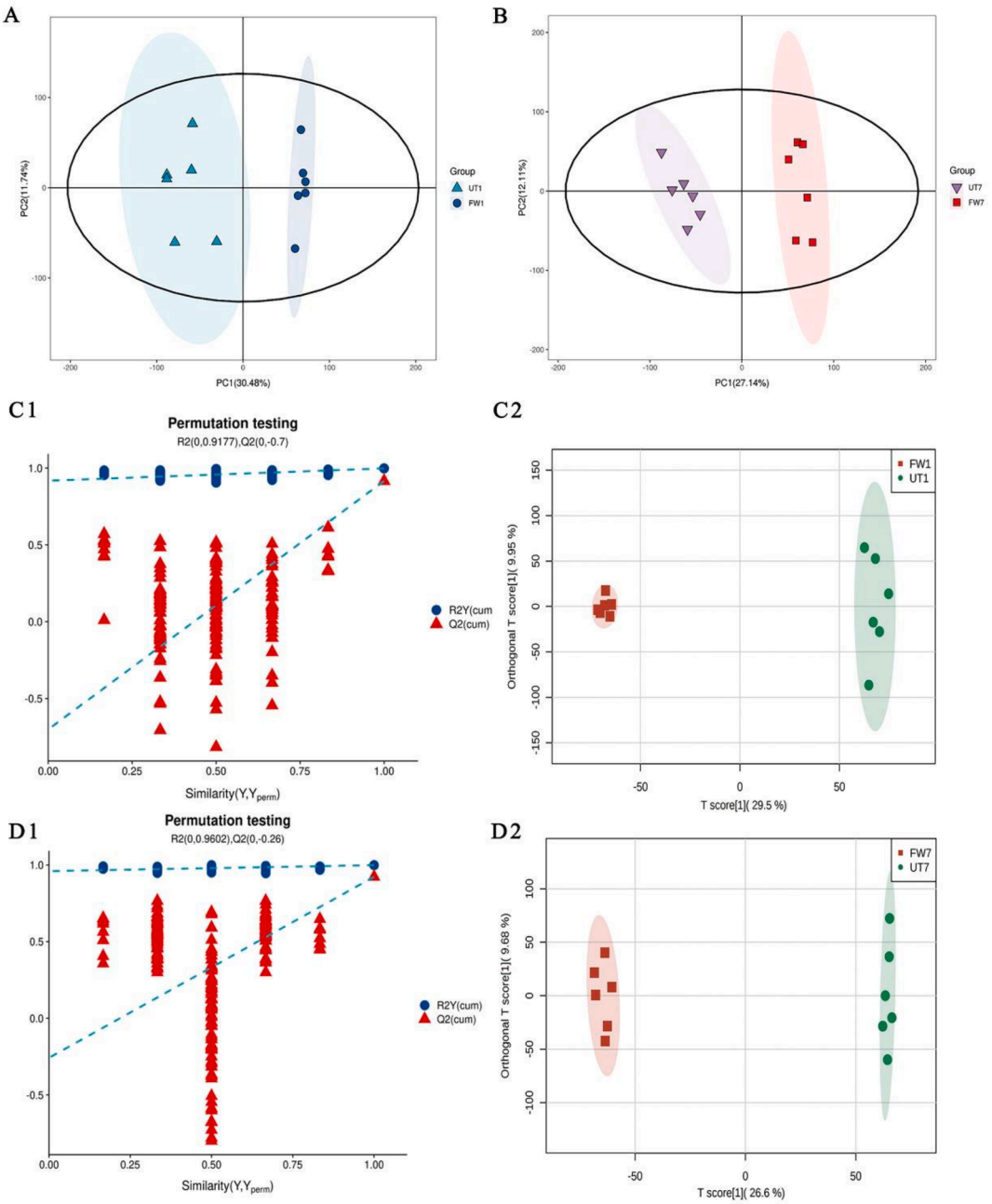

Fig. 2. Multivariate statistical analysis of lamb samples under different thawing methods: PCA score plots (A and B), OPLS-DA permutation test results (C1 and D1) and OPLS-DA score plots (C2 and D2). UT1, day 1 of refrigerated storage after ultrasound-assisted thawing; UT7, day 7 of refrigerated storage after ultrasound-assisted thawing; FW1, day 1 of refrigerated storage after flow water thawing; FW7, day 7 of refrigerated storage after flow water thawing.

As shown in Fig. 1C, no significant effects were observed on TBARS in lamb during refrigerated storage between the two thawing methods ($P > 0.05$). In this study, regardless of the thawing method, the reason for no difference in TBARS might be related to the fact that ultrasound ensured that the thermal effect energy was stabilized near the freezing point during the thawing process [35], which effectively slowed down the lipid oxidation of meat. While the TBARS increased significantly with storage time ($P < 0.05$). Lipid oxidation was largely dependent on the solubility of oxygen in food, especially at low temperatures [36]. Therefore, the accumulation of lipid oxidative metabolites was the main reason for the increase in TBARS values over time.

3.3. Metabolomics profiles analysis of lamb under different thawing methods

3.3.1. Multivariate statistical analysis

To assess the differences in the metabolic profiles of lamb during refrigerated storage between the two thawing methods, an unsupervised PCA approach was used to obtain an overview of metabolite phenotypic differences. The PCA score plot showed tight clustering among QC samples, confirming the robustness of the method and the reliability of the data (Fig. S1). A comparison of UT1 and FW1 showed that the first two principal components explained 42.22 % of the variation, with 30.48 % for PC1 and 11.74 % for PC2 (Fig. 2A). In the comparison between the UT7 and FW7 groups, the first two principal components explained 39.25 % of the change, with 27.14 % for PC1 and 12.11 % for

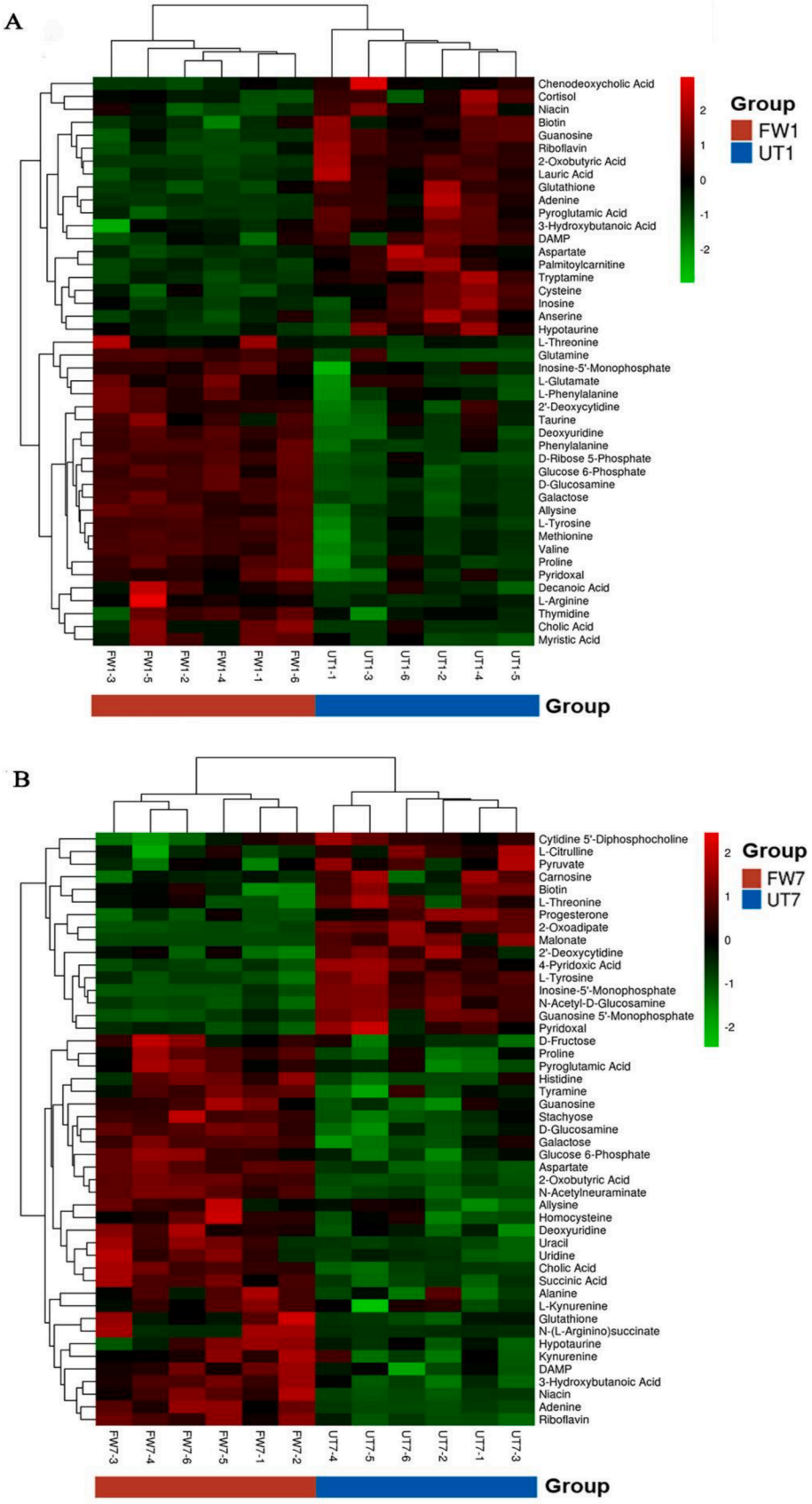

Fig. 3. The heat-map of different metabolites in lamb samples during refrigerated storage. (A) differential metabolites from UT1 and FW1; (B) differential metabolites from UT7 and FW7. Each column indicates the lamb sample and each row indicates the metabolite in the heat-map. UT1, day 1 of refrigerated storage after ultrasound-assisted thawing; UT7, day 7 of refrigerated storage after ultrasound-assisted thawing; FW1, day 1 of refrigerated storage after flow water thawing; FW7, day 7 of refrigerated storage after flow water thawing.

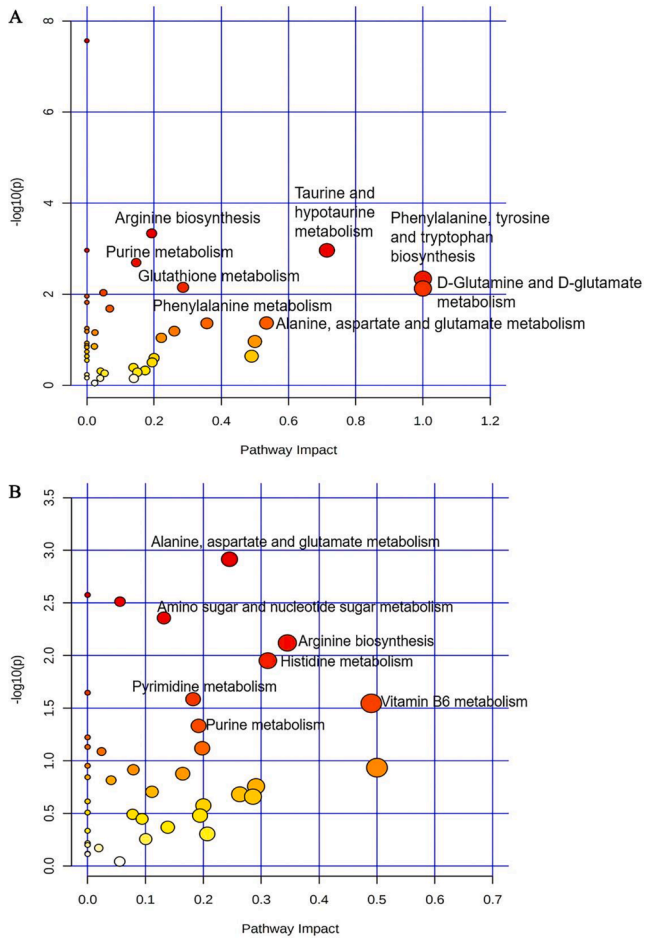

Fig. 4. Topological pathway view map of metabolite enrichment in lamb samples from different thaw treatments (UT and FW) on day 1 (A) and day 7 (B) of refrigerated storage. Larger circle indicates higher pathway enrichment, and darker color represents higher pathway impact values in the metabolome view.

PC2 (Fig. 2B). These results reflected the differences between ultrasound-assisted thawing and flow water thawing treatments, indicating that both thawing methods significantly affected the accumulation of metabolites in lamb.

Supervised modeling based on multivariate statistics, cross-validation with 200 permutation tests confirmed that the OPLS-DA model was reliable (UT1 versus FW1, R2Y = 0.9177, Q2Y = −0.7 < 0; UT7 versus FW7, R2Y = 0.9602, Q2Y = −0.26 < 0) (Fig. 2C and 2D), indicating that no obvious overfitting occurred, and the validity of the model in further screening different metabolites. Based on all PCA and OPLS-DA score plots (Fig. 2), we could separate the metabolites in lamb samples from UT and FW groups at different storage periods. This indicated the effect of the thawing method on the changes of lamb metabolites.

3.3.2. *Identification of metabolites and metabolic pathways*

In the current study, 44 (UT1 vs FW1) and 47 (UT7 vs FW7) compounds were selected and preliminarily identified as potential markers according to the criteria of parameters VIP > 1 and $P < 0.05$ (Table S1 and Table S2). Changes in the content of compounds in different samples could be visualized as heat-map [37]. Metabolites were clustered with heat maps to better understand chemical differences between thaw patterns (Fig. 3A and 3B). The red square in the figure represented higher levels of metabolite, and the green square represented lower metabolite levels. The difference in color between the UT and FW groups showed significant changes in metabolites of the lamb after ultrasound-assisted thawing.

As shown in Fig. 4, according to the pathways reported in the KEGG database, the main metabolic pathways for differential metabolite enrichment in lamb were obtained. The results showed that there were 8 enrichment pathways between UT1 and FW1 and 7 enrichment pathways between UT7 and FW7, respectively. The impact values of these metabolic pathways were higher than 0.1, and the *P* value was less than 0.05. The main pathways involved between UT and FW were amino acid metabolism, including phenylalanine, tyrosine and tryptophan biosynthesis, D-glutamine and D-glutamate metabolism, alanine, aspartate and glutamate metabolism, phenylalanine metabolism, arginine biosynthesis, and histidine metabolism. In addition, 3 metabolic pathways (purine metabolism, alanine, aspartate and glutamate metabolism, and arginine biosynthesis) were observed in highly enriched metabolic pathways in both comparison groups. The results demonstrated a new insight into the effect of ultrasound-assisted thawing on the changes of lamb metabolites during refrigerated storage, which in turn affected the quality of lamb.

We found that these differential metabolites belonged to a variety of compound classes, including amino acids, carbohydrates and their conjugates, nucleic acids, lipids, organic acids, vitamins and cofactors, and carbonyl compounds, among them. Fig. 3A showed that 24 down-regulated metabolites and 20 up-regulated metabolites were found between the UT1 group and the FW1 group. Compared with the FW7 group, 31 metabolites were down-regulated and 16 metabolites were up-regulated in the UT7 group.

Most of the free amino acids showed higher content in the FW group, indicating extensive protein degradation. This could be attributed to the fact that flow water thawing disrupted the structural integrity of the muscle, resulting in the release of proteases that activate protein hydrolysis [38]. However, in a study of the microstructure of muscle by Kong et al. [39], it was noted that the ultrasound-assisted thawing group exhibited minimal changes in muscle fiber structure. The L-glutamate in the UT1 group was significantly lower than that in the FW1 group. Pathway analysis also clarified that the most abundant pathways in the UT1 group include arginine biosynthesis, glutathione metabolism, D-glutamine and D-glutamate metabolism, and alanine, aspartate and glutamate metabolism. These four metabolic pathways were related to L-glutamate, therefore, L-glutamate could be used as a biomarker to identify the lamb from ultrasound-assisted thawing and flow water thawing on day 1 of refrigerated storage. The metabolic pathways involved in the lowest content of aspartate in the UT7 group included alanine, aspartate and glutamate metabolism, arginine biosynthesis, histidine metabolism, and pyrimidine metabolism, indicating the important role of aspartate in the metabolic pathway of ultrasound-assisted thawing. Therefore, aspartate may be a potential biomarker for lamb on day 7 of refrigerated storage after thawing.

Carbohydrates had been reported as the main characteristic metabolite predicting final pH, and the glycolytic pathway was promoted by high carbohydrate storage [40] In addition, the glucose in muscle was mainly composed of glucose 6-phosphate. The contents of carbohydrates and their conjugates, especially glucose 6-phosphate, D-glucosamine and galactose, were significantly reduced in the UT group, which were mainly involved in amino sugar and nucleotide sugar metabolism pathways. The above study demonstrated a strong correlation between glycolytic potential and amino sugar and nucleotide sugar metabolism [41].

Purine metabolism pathways were associated with ATP degradation products, including inosine 5′-monophosphate (IMP), deoxyadenosine monophosphate (DAMP), adenine, guanosine, and guanosine 5′-monophosphate (GMP). Among them, IMP and GMP were important flavor components related to umami in meat [42]. On day 1 of refrigerated storage, the content of IMP in the UT group was lower than that in the FW group, however, on day 7 of refrigerated storage, IMP and GMP were significantly increased in the UT group. This indicated that the flavor of lamb meat would be enhanced with prolonged storage time after

ultrasound-assisted thawing.

Bioactive peptides are specific and have positive effects on health [43]. Anserine, carnosine, and glutathione are important small peptides in animal tissues, all of which have antioxidant properties that reduce oxidative stress and prevent the deterioration associated with meat oxidation during postmortem aging [44–45]. In the present study, we found that ultrasound-assisted thawing increased the content of anserine (UT1), glutathione (UT1) and carnosine (UT7) in lamb, which may ensure the stability of protein and lipid oxidation in lamb.

It was worth noting that the content of carbonyl compounds (kynurenine and L-kynurenine) was significantly reduced in the UT7 group. The carbonyl compound is an important indicator of protein oxidation in meat and meat products. The carbonyl derivative in the UT7 group was less than that in the FW7 group, indicating that there were fewer oxidized products generated in the meat and lower protein oxidation. This also proved that ultrasound-assisted thawing increased the oxidation stability of meat.

4. Conclusion

This study showed that ultrasound-assisted thawing improved the water-holding capacity and increased the color of lamb during refrigerated storage. Furthermore, ultrasound-assisted thawing could also reduce the sulfhydryl content in lamb and inhibit protein oxidation. Moreover, potential metabolites associated with amino acids, carbohydrates and their conjugates, nucleic acids, carbonyl compounds, and peptides could be identified after ultrasound-assisted thawing treatment. Ultrasound-assisted thawing could lead to differences in various amino acid metabolism and purine metabolism pathways. The identified metabolic markers could be used in the future to differentiate the meat between ultrasound-assisted thawing and flow water thawing, which ensured product quality to protect consumers' rights and interests. In addition, this study suggests the huge potential of ultrasound in the field of meat preservation, which can provide a solid theoretical reference for the development and industrial application.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2022.106211.

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