Food Science of Animal Products

Research Article

Characterization and identification of frozen-thawed beef: the interactions of frozen storage and chilled display time

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Abstract: The quality differences and Raman spectroscopic features of beef, which was frozen for 0–11 months followed by being thawed and displayed for 0–21 days were investigated. Frozen storage did not significantly affect the color blooming ability, in which minimal change in the color parameter redness and yellowness values of thawed beef in the later stage of frozen storage occurred. As such, it was not able to distinguish between frozen-thawed and non-frozen-thawed meat directly in terms of color changes. The total viable count and the total volatile basic nitrogen content increased sharply in the frozen-thawed meat subjected to 11-month frozen storage. The thiobarbituric acid reactive substances value increased dramatically from 0.18 to 0.29 mg/kg during 11-month frozen storage. Raman spectroscopy data showed that the stability of the protein secondary structure of thawed beef became worse after 3 months of frozen storage, of which the relative content of α-helix and β-sheet decreased significantly to 21.46% and 28.94% during 11-month frozen storage, respectively. The findings suggest that quality deterioration occurred for the frozen-thawed beef, and Raman spectroscopy could be a potential method to distinguish unfrozen meat from frozen-thawed meat.

Keywords: frozen-thawed beef; water-holding capacity; quality characteristics; Raman spectroscopy

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1 Introduction

Nowadays, meat consumption is dominated by both fresh and frozen forms, of which frozen storage has become a popular preservation method for muscle foods to extend their shelf life around the world[1–2]. Moreover, freezing is essential for the regulation of seasonal and regional variations of food production, especially meat and meat products[3]. Additionally, frozen meat is widely distributed, and accepted by consumers, owing to its cost-effectiveness, convenience, and inhibitory effect on microorganism and enzymatic activity[4].

Nevertheless, repeated freeze-thaw cycles of frozen meat, occurring as temperature fluctuation during transportation, storage, and retail, can lead to recrystallisation and aggregation of ice crystals, thereby leading to the deterioration of meat quality[3,5]. The formation of ice crystals can destroy the structure of the muscle, and it also increases the concentration of solutes around the protein contributing to protein denaturation, resulting in a reduction in water holding capacity[6–7]. Due to the aforementioned deterioration of muscle physicochemical properties, the water produced during thawing cannot be reabsorbed by muscle fibers, causing more water and nutrient loss. Worse yet, lipid oxidation is accelerated after freezing-thawing[5]. Conversely, chilled meat can retain its intact structure, with good freshness and flavor, and sensory panelists are more likely to accept chilled meat compared with frozen meat even with higher shear force values[8].

Unfortunately, some merchants deliberately confuse the concepts between frozen-thaw and chilled meat and sell frozen-thawed meat as chilled meat, since it is hard for consumers to directly distinguish between frozen-thawed and chilled meat according to the appearance[9]. Selling thawed meat as high-quality fresh meat is considered commercial fraud, which seriously breaches consumers rights and interests, disrupts market order, and may also lead to food safety issues. To date, frozen-thawed meat is known to exhibit higher water loss than the unfrozen meat[10]. Except for the water loss, there are only a few reports on the meat quality differences, between frozen-thawed meat and chilled meat directly[11]. Moreover, there is little information on the quality characteristics of frozen thawed meat simulating unfrozen chilled meat during display time.

Raman spectroscopy can reveal protein secondary structures such as the amide band of protein, which has been widely used in the determination of food protein conformational changes[12–13]. It also possesses the feasibility of identifying fresh meat and frozen-thawed meat by reflecting the changes in protein secondary structure. Herrero et al.[14] reported that Raman spectroscopy revealed the changes in secondary and tertiary structures of fish

protein in frozen storage. However, the spectroscopic changes of the beef after freezing, especially during simulated retail display, has not been reported yet.

The objective of this study was to determine the quality and spectral characteristics of thawed beef following long term frozen storage simulating unfrozen chilled beef during retail display. This paper proposes a theoretical basis for identifying frozen meat and chilled meat, which is of great significance for ensuring consumer rights and food safety.

2 Materials and methods

2.1 Raw materials and sample processing

Both left and right *M. longissimus lumborum* were randomly collected from 5 beef carcasses, sourced from one abattoir at 24 h post-mortem. Both striploins were cut into three portions (see detailed information in Figure 1), all the portions from one carcass were regarded as one group. In this group, one portion were assigned as unfrozen (control), which were cut into four 3 cm thick beef steaks, and directly vacuum skin (VS-410, Dajiang Machine Co., Ltd., Nanjing, China). The packing material was a polyethylene film (Lid 1050, oxygen permeability of 16 654 $cm^3/(m^2 \cdot 24\ h \cdot 0.1\ MPa)$, moisture permeability of 23.5 $g/(m^2 \cdot 24\ h)$, Sealed Air Co., Ltd., Shanghai, China) and trays (D538, Sealed Air Co., Ltd., Shanghai, China). The packages were randomly assigned to 4 display times (0, 7, 14, 21 days). The other five portions were assigned to five frozen periods (at −20 °C) for 1 day, 1, 3, 6, and 11 months, and then thawed under running water at room temperature (24 ± 2) °C and each portion was cut into four 3 cm-thick beef steaks, vacuum skin packaged and randomly assigned to 4 display times (0, 7, 14, 21 days) at 0–4 °C. The following traits were determined at each display time for each steak.

2.2 Determination of quality attributes of beef

2.2.1 pH

The determination of pH was conducted as described by Chen et al.[1]. The pH value of the steaks was determined using a portable pH meter (Senvon2 Go-S2, Mettler-Toledo, Switzerland), and was averaged from three measurements. Before measurement, the pH probe was calibrated using buffers at pH 4.00 and 7.00, both the pH calibration and measurement were conducted at chiller temperature.

2.2.2 Color

The determination of color was conducted as described by Chen et al.[1]. The steaks were removed from the packaging, and then the steaks were bloomed at 4 °C for 30 min. Color parameters of the meat surface were determined using a colorimeter (SP62, X-Rite, Inc., Grand Rapids, USA) fitted with an aperture of 8 mm diameter and using 10° standard observer and Illuminant A settings. And record the brightness value (L^*), redness value (a^*), and yellowness value (b^*). Six measures were recorded at different locations for each steak.

2.2.3 Lipid oxidation

Lipid oxidation was determined by measurement of thiobarbituric acid reactive substances (TBARS) value, as described by Siu et al.[15], with slight modifications. Samples (1 g) were removed from the meat surface, and subjected to an automatic grinder to be ground with 4 mL of distilled water for 90 s. Subsequently, 4 mL 10 g/100 mL trichloroacetic acid solution was added into the mixture, which was then vortexed, and filtered through Whatman filter paper (#1). Filtrate (4 mL) was mixed with 0.25 mL 0.06 mol/L of thiobarbituric acid (TBA) solution, and incubated in a water bath (80 °C) for 90 min. The absorbance at 532 nm was then measured

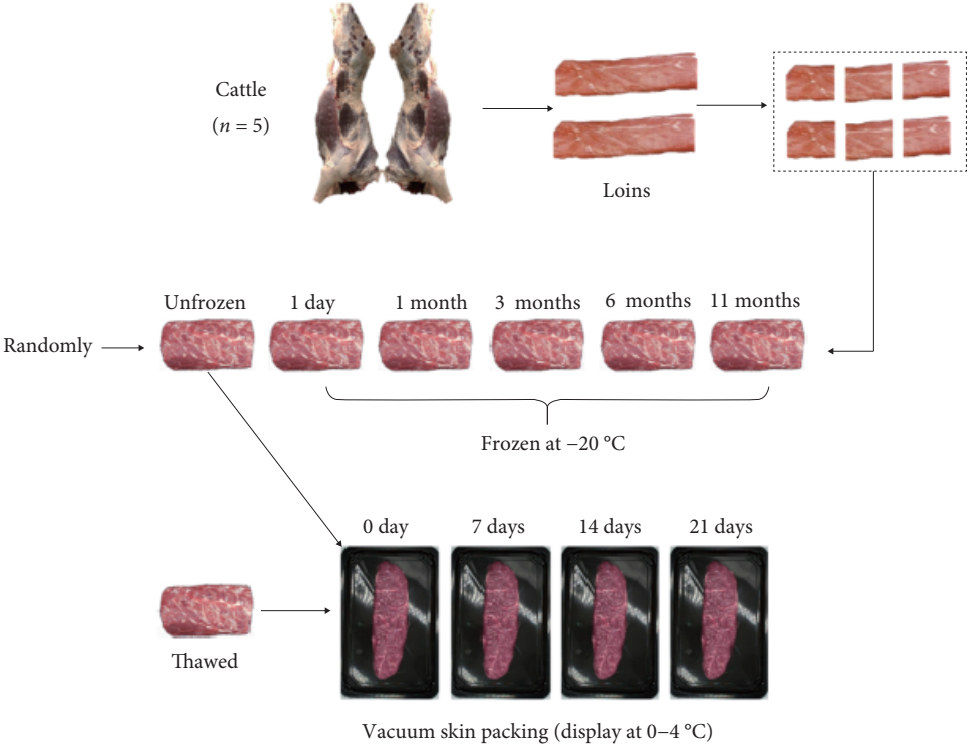

Figure 1 Schematic figures illustrating the experimental design of study and sample allocations.

by an ultraviolet spectrophotometer (TU 1901, Purkinje General, Beijing, China), while the blank was set as the mixture of 1 mL of distilled water + 1 mL of 10 g/100 mL trichloroacetic acid solution + 0.25 mL of 0.06 mol/L TBA solution. A standard curve was plotted with the different concentrations of malondialdehyde standard solutions as the abscissa and the corresponding absorbance values as the ordinate. According to the standard curve, TBARS value was expressed as mg malondialdehyde/kg beef.

2.2.4 Total viable count (TVC)

The TVC was determined as described by Yang et al.[16] with some modifications. Meat surface samples of 10 g were weighed aseptically into sterile stomacher bags (BagPage®, Interscience, St Nom, France) with 90 mL sterile physiological saline, then were mixed in a blender (BagMixer® 400, Interscience, St Nom, France) at room temperature for 1 min. The mixture (1 mL) was serially diluted in 0.1 g/100 mL sterile physiological saline. Each of the three appropriate dilutions of 1 mL were incubated onto plate counting agar (PCA) and stored at 37 °C for 48 h. The results of TVC were expressed as lg (CFU/g).

2.2.5 Total volatile basic nitrogen (TVB-N) content

The TVB-N content was determined using the automatic Kjeldahl method as outlined by Chen et al.[1] with some modifications. Meat samples were chopped and stirred with 70 mL distilled water for 30 s. Homogenates were kept at room temperature for 30 min. The homogenates were distilled for 3 min on a Kjeldahl automated distillation unit (K-355, Buchi Co., Ltd., Switzerland). The volatile components were absorbed with boric acid solution (20 g/L, 30 mL) and titrated with hydrochloric acid standard solution (0.01 mol/L). The TVB-N content was determined according to the consumption of hydrochloric acid and calculated as follows:

$$\text{TVB-N content}\,(\text{mg}/100\ \text{g}) = \frac{(V_1 - V_2) \times c \times 14 \times 100}{m} \quad (1)$$

Where V_1 is the titration volume of hydrochloric acid in the sample (mL), V_2 is the titration volume in the blank (mL), c is the concentration of hydrochloric acid (0.01 mol/L), and m is the weight of the meat sample (g).

2.2.6 Centrifugal loss

Centrifugal loss was determined as described by Wang et al.[17], with some modifications. Samples (2 g) were wrapped with filter papers, and centrifugated at 9 000 r/min for 10 min at a temperature of 4 °C in a centrifuge (5840R, Eppendorf, Hamburg, Germany).

Centrifugal loss was obtained as follows:

$$\text{Centrifugal loss}\,(\%) = \frac{m_1 - m_2}{m_1} \times 100 \quad (2)$$

Where m_1 and m_2 correspond to the mass of the meat samples (g) before and after centrifugation, respectively.

2.2.7 Low-field nuclear magnetic resonance (LF-NMR) measurement

Water migration was measured by LF-NMR with reference to the method of Cheng et al.[18]. Transversal relaxation features of the meat samples were determined using a LF-NMR analyzer (NMI20-015V-I, Suzhou Niumag Analytical Instrument Co., Suzhou, China) equipped with a magnet probe. Prior to the measurement, a 30-min warm-up was required to reach the desired test temperature of 32 °C, and the instrument was calibrated using standard oil samples. After removal of the surface water with filter paper the steaks were cut into 1 cm × 1 cm × 2 cm columnar samples parallel to muscle fiber orientation. The samples were then weighed and put into the NMR test tubes. T_2 transversal relaxation curves were determined using the Carr-Purcell-Meiboom-Gill (CPMG) sequence.

2.3 Raman spectroscopy

The determination of Raman spectroscopy was conducted as described by Yang et al.[19]. The scanned surface was the same for the color determination, that was, after removal of the packaging, a fresh surface was cut and allowed to bloom for 30 min at room temperature. A portable Raman instrument (Raman Pro, B&WTek, LLC, USA) equipped with a 785 nm laser diode was used to scan perpendicular to the muscle fibers, with the following parameters: laser power of 100 mW and scan time of 20 s (scan time 10 s, 2 times). Raman spectra were averaged from 8 independently replicated scans for each steak, ranging from –239 to 3 231 cm^{-1}. All Raman spectra in this experiment were obtained by scanning in a closed dark room.

Raman shift was narrowed to the region of 500–3 000 cm^{-1} of the averaged spectra. The amide I band (1 600–1 700 cm^{-1}) in Raman spectra was fitted by a peakfit software (version 4.0, USA), for calculating the content of each component of protein secondary structure (α-helix, 1 650–1 660 cm^{-1}; β-sheet, 1 620–1 640 and 1 670–1 680 cm^{-1}; random coil, 1 660–1 670 cm^{-1}). Through baseline correction, deconvolution, second derivative function was analyzed to determine the peak. Gaussian area function was used to fit the peaks, and then the peak areas of each component were quantified. The relative contents of protein secondary structures were calculated as follows:

$$\text{Relative content}\,(\%) = \frac{A_c}{A_t} \times 100 \quad (3)$$

Where A_c was the peak area of the corresponding component, and A_t was the peak area of amide I band.

2.4 Statistical analysis

The quality and spectral indicators were analyzed for significance using the MIXED procedure in the SAS statistical software (version 9.0, USA), with frozen period, display time and their interaction as fixed terms, and cattle carcasses (n = 5) as a random effect. Least squares means were separated by using the PDIFF option, and $P < 0.05$ indicated that the differences were statistically significant.

3 Results and discussions

3.1 pH change of beef

The pH values of the meat samples are outlined in Table 1. There was a significant interaction between frozen storage time and display time on pH value ($P < 0.05$). After 6 months of freezing, the pH value of the meat samples increased significantly after 21 days of display ($P < 0.05$), and following an 11-month frozen period the pH value increased after 7-day of display and then stabilized. Generally, the generation of ammonia and other alkaline substances resulting from endogenously enzymatic and microbial reactions are considered to contribute the increase in pH value[20]. Conversely, considering the main effect of frozen storage time or

display time, little difference on the pH values occurred, and the pH value of all samples after freezing and thawing were within the normal pH value range of beef. This was consistent with the findings in the study of Lu et al.[21], which showed that pH values of beef remained stable during the frozen storage periods.

3.2 Color change of beef

The color parameters of the beef samples are shown in Table 2. There was no significant interaction between frozen storage time and display time for L^*, whereas frozen storage time had an effect on the L^* ($P < 0.05$). The L^* gradually decreased within the first three months, and then slightly increased at 6 and 11 months ending up being no different to the unfrozen group. Similar results were also reported by Holman et al.[22], where the L^* of beef decreased after 4 or 8 weeks frozen at −18 or −12 °C. The storage time, display time, and their interaction all affected the a^* significantly ($P < 0.05$). The initial a^* of the meat samples (unfrozen and frozen for 1 day) was relatively low. This was likely due to the high mitochondrial activity, which can compete with myoglobin to absorb oxygen, contributing to the formation of deoxymyoglobin. As the extension of storage time, the a^* significant increased ($P < 0.05$), with the values between 16.21 to 17.67 after frozen for 1 to 6 months (display 0 day). This indicates that the mitochondrial activity was getting lower at 1 month frozen storage, resulting less deoxygenated myoglobin thus an increased redness. Moreover, significant increasement of a^* was also observed with increased display time for the early stage of the frozen storage ($P < 0.05$). The unfrozen and 1 day samples reached the highest values on the 21st display, while the 1 and 3 months samples obtained the peak values on the 14th and 7th days of the display, respectively, and the values did not increase significantly for 6 and 11 months frozen samples. This suggests that beef still possessed good color blooming ability after thawing, consistent with previous studies[23], however, the blooming ability was getting weak as the frozen time extended to 11 months. Moreover, frozen storage time and display time showed significant effects on the b^* of beef ($P < 0.05$) individually, while

Table 1 Effect of different display times on the pH value of beef steaks after different frozen storage periods.

Storage time	Display time (day)				SE	*P* value		
	0	7	14	21		Storage time	Display time	Storage time × display time
Unfrozen	5.58^{axy}	5.56^{ay}	5.55^{ay}	5.56^{ay}	0.021	< 0.001	0.018	0.011
1 day	5.55^{axy}	5.54^{ay}	5.56^{ay}	5.57^{ay}				
1 month	5.49^{by}	5.56^{axy}	5.58^{axy}	5.56^{ay}				
3 months	5.53^{ay}	5.59^{axy}	5.56^{ay}	5.56^{ay}				
6 months	5.57^{axy}	5.54^{ay}	5.53^{ay}	5.64^{bx}				
11 months	5.59^{bx}	5.62^{abx}	5.63^{abx}	5.66^{ax}				

Notes: The different letters (a–c) in same row indicate significant differences between display time for the same storage time ($P < 0.05$), and the different letters (x–z) in same column indicate significant differences between storage time at the same display time ($P < 0.05$). SE represents standard error.

Table 2 Effect of different display times on the color of beef steaks after different frozen storage periods.

Color parameters	Storage time	Display time (day)				Mean	SE	*P* value		
		0	7	14	21			Storage time	Display time	Storage time × display time
L^*	Unfrozen	36.74	36.54	36.76	36.17	36.55^{xy}	0.401	0.011	0.374	0.918
	1 day	35.75	35.78	35.86	35.18	35.64^{y}				
	1 month	34.81	35.17	35.23	35.93	35.28^{y}				
	3 months	34.32	34.80	36.06	35.71	35.22^{y}				
	6 months	37.28	35.66	37.04	37.10	36.77^{x}				
	11 months	35.32	35.80	36.55	36.60	36.07^{xy}				
b^*	Unfrozen	12.62	13.36	13.55	14.11	13.41^{y}	0.289	< 0.001	0.009	0.795
	1 day	12.26	12.59	12.83	12.94	12.66^{z}				
	1 month	12.24	12.91	13.81	13.64	13.15^{yz}				
	3 months	12.80	13.92	13.66	13.69	13.52^{y}				
	6 months	14.07	13.90	14.77	14.11	14.21^{x}				
	11 months	14.72	14.46	14.49	14.87	14.64^{x}				
	Mean	12.80^{b}	13.34^{ab}	13.72^{a}	13.70^{a}		0.261			
a^*	Unfrozen	14.91^{cz}	16.72^{by}	17.54^{bx}	18.82^{ax}		0.420	< 0.001	< 0.001	< 0.001
	1 day	13.92^{cz}	16.21^{by}	16.21^{by}	17.89^{axy}					
	1 month	16.21^{by}	17.08^{aby}	17.99^{ax}	17.39^{aby}					
	3 months	16.55^{bxy}	18.68^{ax}	17.49^{bx}	17.57^{aby}					
	6 months	17.67^{ax}	17.97^{axy}	18.42^{ax}	17.96^{axy}					
	11 months	16.46^{ay}	17.13^{ay}	17.39^{ax}	17.13^{ay}					

Notes: The different letters (a–c) in same row indicate significant differences between display time for the same storage time, and the different letters (x–z) in same column indicate significant differences between storage time at the same display time ($P < 0.05$). When the interaction effect of the two factors was insignificant, while the effect of the individual factor was significant, the means of the results for different storage times and display times were calculated respectively, and the influence of the two individual factors on the indicator was analyzed. SE represents standard error.

their interaction had no significant effect, the b^* of the meat gradually increased with the extension of the freezing and display time. Xia et al.[24] pointed out that yellow pigment formation in pork could be due to non-enzymatic browning reactions between lipid oxidation products and the amine in the phospholipid head groups or the amine in protein, which may also occur in the frozen beef. Frozen storage did not significantly affect the color blooming ability, as such it was not possible to distinguish between frozen-thawed and non-frozen-thawed meat in terms of color changes.

3.3 Lipid oxidation of beef

Lipid oxidation can occur accompanied by the freeze-thaw process, which is considered to be closely related to the variations on the flavor, color, texture, and nutritive value of meat[25]. As shown in Table 3, it could be seen that freezing and display time significantly affected the TBARS value individually ($P < 0.05$), while their interaction was insignificant. The TBARS value of the meat samples increased during the display period. Additionally, the TBARS value of the samples changed inconspicuously throughout the frozen storage and post-thaw display period, but greatly increased to 0.36 mg/kg at the 21st day of thawed display after 11 months of frozen storage. Results suggested that 11-month frozen storage resulted in an increase in lipid oxidation, and also made higher oxidation degree than the unfrozen group within the display period. This indicated that although low temperature could inhibit the occurrence of lipid oxidation, there was still unfrozen water that caused oxidation reaction[26].

3.4 Microbial analysis of beef

TVC represented the growth and reproduction of microorganisms, generally considered to be closely associated with the meat spoilage and shelf life. As shown in Figure 2A, significant interaction between storage time and display period was observed ($P < 0.05$), and the TVC of the samples with longer frozen storage time increased faster during the display period. Prolonging the frozen storage time decreased the TVC, indicating that long-term freezing reduced the initial colony number and thereby a lower TVC was obtained in the corresponding display period[27]. For the same frozen storage time, TVC of beef samples showed an upward trend during the display period. The unfrozen sample during the display period increased from 4.41 to 5.42 (lg (CFU/g)), while that with 11-month frozen storage time increased from 3.09 to 6.43 (lg (CFU/g)). This indicated that the microorganisms inhibited during freezing rejuvenated after thawing. It also found that frozen-thawed treatment contributed rapid microbial growth during display period. This was due to the fact that long-term freezing could cause more serious physical damage to muscle tissue than short-term freezing, which led to rapid proliferation of microorganisms in the thawed beef, and on the other hand, the display temperature of 0–4 °C greatly enhanced microbial activity[26]. Kluth et al.[28] also declared similar experimental conclusions, as the effects of freezing temperature and freezing duration on the quality and safety of raw turkey and fresh sausage products were investigated. In general, long-term freezing reduced the initial colony number but contributed significant microbial growth during display period due to serious physical damage of muscle tissue.

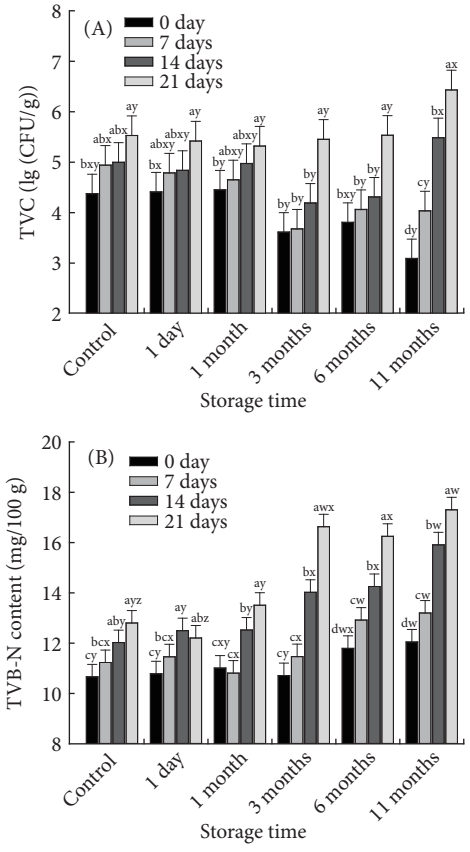

Figure 2 Effects of different display time on the (A) TVC and (B) TVB-N content of beef steaks after different frozen storage periods. The different letters (a–c) indicate significant differences between display time for the same storage time, and the different letters (w–z) indicate significant differences between storage time at the same display time ($P < 0.05$).

Table 3 Effect of different display times on the TBARS value of beef steaks after different frozen storage periods.

Storage time	Display time (day)				Mean	SE	*P* value		
	0	7	14	21			Storage time	Display time	Storage time × display time
Unfrozen	0.18	0.19	0.24	0.22	0.21^{y}	0.009	< 0.001	0.001	0.107 2
1 day	0.23	0.26	0.19	0.24	0.23^{y}				
1 month	0.21	0.22	0.20	0.23	0.22^{y}				
3 months	0.20	0.24	0.21	0.24	0.22^{y}				
6 months	0.21	0.22	0.22	0.26	0.23^{y}				
11 months	0.29	0.31	0.31	0.36	0.32^{x}				
Mean	0.22^{c}	0.24^{ab}	0.23^{bc}	0.26^{a}		0.017			

Notes: The different letters (a–c) in same row indicate significant differences between display time for the same storage time, and the different letters (x–y) in same column indicate significant differences between storage time at the same display time ($P < 0.05$). When the interaction effect of the two factors was insignificant, while the effect of the individual factor was significant, the means of the results for different storage times and display times were calculated respectively, and the influence of the two individual factors on the indicator was analyzed. SE represents standard error.

3.5 TVB-N content change of beef

TVB-N content is one of the important indicators for assessing the freshness of protein-rich foods, and the TVB-N content limit for fresh meat is 15 mg/100 g. As shown in Figure 2B, significant interaction between storage time and display period was observed on the TVB-N content ($P < 0.05$). When the frozen storage period was within 1 month, the TVB-N content of the samples during the 21-day display period were all below the limit, corresponding 12.80, 12.21, and 13.51 mg/100 g for the unfrozen, 1 day, and 1 month sample, respectively. However, after 3 and 6 months of freezing, the TVB-N content increased from 10.71 and 11.80 to 16.63 and 16.25 mg/100 g at the 21st day of display, respectively; the meat sample with 11-month storage time exhibited a TVB-N content of 15.91 mg/100 g for 14-day display period. More specifically, the beef frozen for 3 and 6 months could only be displayed on the shelf for 14 d, and those frozen for 11 months only possessed a 7-day shelf life for displaying. These results further demonstrated that frozen-thawed meat had a higher microbial growth rate during the display period, which also shorted shelf life than unfrozen meat and likely brought certain food safety risks. This was consistent with the experimental results of Qian et al.[29], which proposed that TVB-N could accumulate even when microbial growth is inhibited, but lower storage temperature can slow the growth rate of beef TVB-N. The results reported by Lu et al.[21] also confirmed this point of view. As for single factor, it could be seen that the TVB-N content increased slowly during frozen storage, but increased rapidly during post-thaw display (Figure 2B). This could be attributed to that the higher temperature in display period accelerated the corruption process of meat products, consistent with the results of microbial analysis.

3.6 Centrifugal loss change of beef

As shown in Table 4, the interaction of display time and storage time significantly affected the centrifugal loss ($P < 0.05$). Without display, the centrifugal loss of frozen samples decreased with increased storage time, while all frozen-thawed samples showed significant higher ($P < 0.05$) centrifugal loss than the unfrozen. However, the unfrozen beef possessed significantly increased centrifugal loss as the extension of display time ($P < 0.05$), while the values for frozen beef were generally getting lower as the display time extended, expect for the 11-month samples, which maintained relatively stable values with an already low initial centrifugal loss of 23.52%. The high centrifugal loss for the unfrozen samples at the late stage of the display was mainly caused by the aging effect, and calpains and other endogenous proteases contributed to the degradation of myofibrillar proteins to a large extent, and the destroyed networks of myofibrils made water migrate from inside to outside the muscle fiber[30], resulting in obvious centrifugal water loss. On the other hand, the ice crystals formed during freezing could disturb myofibril structures, resulting in increased centrifugal water loss in frozen-thawing samples compared with the unfrozen ones; while as the frozen time extended, the water sublimation occurred, so there was more water dissipation for long-freezing samples, which in turn decreased their centrifugal water loss. Additionally, the freezing denaturation of proteins may also contribute the decreased water-holding capacity for the long term frozen mear[4]. Thus, at the early stage of storage, the destruction of myofibrils increased the centrifugal loss of beef, but after 6-month storage, the centrifugal loss decreased due to more water loss of samples.

3.7 LF-NMR analysis of beef

The moisture state and distribution can be analyzed by measuring the relaxation properties of hydrogen atoms in the meat in a magnetic field[31–32], and the corresponding LF-NMR data are shown in Table 5. According to the difference of the transverse relaxation time T_2, the water in different states in the meat can be distinguished. In this study, three characteristic peaks in the spectra of transverse relaxation time T_2 occurred. The proton pool T_{21} with shortest relaxation time (0.1–10 ms) corresponded to the bound water closely attached on the polar groups of macromolecules; the predominant proton pool T_{22} (10–100 ms) was attributed to immobilized water entrapped within the myofibrillar network; the proton pool T_{23} with longest relaxation time (100–1 000 ms) was assinged to the free water in the myofibril lattice[18]. As shown in Table 5, frozen storage time, display time and their interaction had significant effects on T_{21} ($P < 0.05$), while frozen storage time had a significant effect on T_{22}, display time had a significant effect on T_{23} ($P < 0.05$). At 0th day of display (except for the unfrozen group), significantly lower T_{21} occurred in the samples with 3 or over 3 months of storage. While, for other display times, only the sample with 11-month frozen storage showed significantly lower T_{21} than the un-frozen group. The reduction of T_{21} could be attributed to the denaturation and aggregation of myofibrillar proteins that affected the chemical exchange between water and protons[33]. Generally speaking, the higher T_2 stand for more mobile water with higher mobility, while the lower values represent less mobile water molecules. On the other hand, the immobilized water content accounted for 90% of the total water content, which played an important influence on water holding capacity. Frozen-thawed samples showed a lower T_{22} than the unfrozen, and with the prolongation of storage time, the T_{22} for frozen-thawed samples showed a downward trend. This indicated that the mobility of immobilized water decreased with the extension of frozen storage

Table 4 Effect of different display times on the centrifugal loss of beef steaks after different frozen storage periods.

Storage time	Display time (day)				SE	*P* value		
	0	7	14	21		Storage time	Display time	Storage time × display time
Unfrozen	22.89^{by}	30.58^{ax}	27.85^{ax}	29.35^{ax}	1.265	< 0.001	0.039	0.007
1 day	29.41^{ax}	29.15^{ax}	24.85^{bxy}	26.85^{abxy}				
1 month	27.78^{axy}	27.96^{ax}	27.00^{axy}	27.33^{axy}				
3 months	27.76^{axy}	28.06^{ax}	25.10^{axy}	25.08^{ay}				
6 months	25.71^{ay}	24.29^{ay}	23.94^{aby}	20.74^{bz}				
11 months	23.52^{ay}	23.16^{ay}	22.91^{ay}	23.24^{ayz}				

Notes: The different letters (a–c) in same row indicate significant differences between display time for the same storage time, and the different letters (x–z) in same column indicate significant differences between storage time at the same display time ($P < 0.05$). SE represents standard error.

Table 5 Effect of different display times on the LF-NMR data of beef steaks after different frozen storage periods.

	Storage time	Display time (day)				Mean	SE	*P* value		
		0	7	14	21			Storage time	Display time	Storage time × display time
T_{21} (ms)	Unfrozen	0.87^{by}	1.04^{ax}	0.94^{abx}	0.94^{abx}		0.042	< 0.001	< 0.001	0.013
	1 day	1.01^{ax}	1.03^{ax}	0.97^{ax}	0.96^{ax}					
	1 month	1.07^{ax}	1.01^{abx}	0.95^{bx}	0.95^{bx}					
	3 months	0.87^{by}	0.94^{abx}	1.02^{ax}	0.84^{bx}					
	6 months	0.88^{ay}	0.92^{ax}	0.86^{ax}	0.70^{by}					
	11 months	0.73^{abz}	0.78^{ay}	0.62^{bcy}	0.59^{cy}					
T_{22} (ms)	Unfrozen	47.73	44.51	43.32	42.71	44.57^{x}	0.491	< 0.001	0.155	0.164
	1 day	44.56	43.72	43.31	43.30	43.72^{xy}				
	1 month	43.63	43.01	41.93	42.75	42.83^{yz}				
	3 months	40.96	42.50	43.29	42.53	42.32^{z}				
	6 months	42.33	41.36	42.37	41.62	41.92^{z}				
	11 months	42.03	41.06	42.07	41.32	41.62^{z}				
T_{23} (ms)	Unfrozen	698.01	524.24	506.13	684.59			0.149	< 0.001	0.241
	1 day	549.43	504.65	561.76	739.37					
	1 month	511.65	505.95	573.21	601.14					
	3 months	455.24	444.97	518.08	578.16					
	6 months	445.05	503.06	649.78	648.63					
	11 months	460.05	518.06	664.78	663.63					
	Means	519.91^{bc}	500.16^{c}	578.96^{b}	652.59^{a}		26.686			
P_{21} (%)	Unfrozen	2.79^{awx}	2.35^{bxy}	2.32^{by}	2.38^{bxy}		0.072	< 0.001	< 0.001	0.001
	1 day	2.27^{ay}	2.16^{ay}	2.12^{ayz}	2.18^{ay}					
	1 month	2.24^{ay}	1.96^{by}	1.97^{bz}	1.96^{bz}					
	3 months	2.25^{ay}	2.31^{axy}	2.09^{bz}	2.19^{ay}					
	6 months	2.88^{aw}	2.46^{bwx}	2.87^{aw}	2.90^{aw}					
	11 months	2.67^{ax}	2.56^{aw}	2.52^{ax}	2.58^{ax}					
P_{22} (%)	Unfrozen	96.66	96.36	96.23	96.94	96.55^{x}	0.123	< 0.001	< 0.001	0.073
	1 day	96.31	96.65	96.20	97.41	96.64^{x}				
	1 month	96.13	96.04	97.01	97.18	96.59^{x}				
	3 months	95.95	95.92	96.75	97.03	96.41^{x}				
	6 months	95.89	96.44	96.46	96.63	96.36^{x}				
	11 months	95.35	95.32	96.15	96.43	95.81^{y}				
	Means	96.05^{c}	96.12^{c}	96.47^{b}	96.94^{a}		0.100			
P_{23} (%)	Unfrozen	0.55^{cy}	1.29^{abxy}	1.45^{awx}	0.81^{bcw}		0.222	< 0.001	< 0.001	0.006
	1 day	1.42^{awx}	1.192^{ay}	1.668^{aw}	0.41^{bw}					
	1 month	1.63^{aw}	2.00^{aw}	1.02^{bxyz}	0.87^{bw}					
	3 months	1.80^{aw}	1.77^{awx}	1.16^{bwxy}	0.78^{bw}					
	6 months	1.22^{awx}	1.10^{ay}	0.67^{abyz}	0.47^{bw}					
	11 months	1.02^{axy}	0.90^{ay}	0.47^{abz}	0.27^{bw}					

Notes: The different letters (a–c) in same row indicate significant differences between display time for the same storage time, and the different letters (x–z) in same column indicate significant differences between storage time at the same display time ($P < 0.05$). When the interaction effect of the two factors was insignificant, while the effect of the individual factor was significant, the means of the results for different storage times and display times were calculated respectively, and the influence of the two individual factors on the indicator was analyzed. SE represents standard error.

time. Cheng et al.[18] reported that the relaxation time of T_{22} decreased significantly with the increase of the number of freeze-thaw cycles, and the shorter relaxation time and lower population of immobilized water was accompanied by weaker water holding capacity. Ali et al.[34] also noticed that in multiple freeze-thaw cycles, the relaxation time of chicken breast fixed water reduced, and the water holding capacity decreased. Generally, the freezing-thawing process causes a large amount of water loss, and the decreased water content in the samples results in a weakened water escape ability. During the display period, while T_{23} showed an overall upward trend. This suggested that after thawed, the freedom degree of immobilized water gradually increased with the extension of display time, and the free water escape capacity slightly increased in the later stage of display. This might be due to freezing and thawing increasing the content of immobilized water in the muscle or reducing the degree of water binding to protein.

P_2 reflects the difference in the relative percentage of water in different states, where P_{21}, P_{22}, and P_{23} represent the proportion of bound, immobilized, and free water, respectively. The interaction between storage time and display time had a significant effect on P_{21}

and P_{23}, and storage time and display time both significantly affected P_{22} ($P < 0.05$). It found that most of the water in the sample presented in the form of immobilized water, as the proportion of P_{22} in all treatment groups was more than 95%, P_{21} was about 2%, and P_{23} was slightly less than 2%.

With the extension of storage time, P_{21} decreased and then increased, P_{22} only had a minor decrease in samples with 11-month frozen storage, while P_{23} showed a trend of increasing and then decreased. On the other hand, P_{21} decreased with 7-day display, but the subsequent changes were insignificant, P_{22} increased slightly during the display period, and P_{23} decreased significantly after 21 days of display.

Results showed that the proportion of free water increased in the early stage of storage due to the conversion of bound and immobilized water to free water, but the proportion of free water decreased in the later stage owing to increased water loss[34]. During the display period, the myofibrillar protein networks were destroyed due to the degradation, leading to excessive water loss and further decrease in the proportion of free water.

3.8 Raman spectroscopy of beef

Variations in the secondary structure of proteins can be reflected by the relative contents of α-helix, β-sheet, β-turn and random coil, which were determined by analyzing the specific sensitive amide I region (1 600–1 700 cm^{-1})[13]. The band seen at 1 650–1 660 cm^{-1} corresponded to intramolecular α-helix structure, while those with predominantly β-sheet structure show the band at 1 620–1 640 and 1 670–1 680 cm^{-1}, and random coil are attributable to proteins with an amide I band centred at 1 660–1 670 cm^{-1}[35]. Generally, α-helix and β-sheet represent ordered structures, while random coil indicates disordered structures. It could be seen that compared with unfrozen beef, the relative contents of α-helix and β-sheet in frozen-thawed beef decreased significantly within 3 months freezing storage time (Table 6, $P < 0.05$). Furthermore, the α-helix relative content reduced significantly with increased display time. After experiencing freeze-thaw cycles, the forces such as hydrogen bonds that maintained the stability of the protein structure were destroyed[7]. This could transform the stable ordered structure (such as α-helix, β-sheet, etc.) of the original protein structure into disordered random coil. Additionally, the hydrogen bonds between the carbonyl group and the amino group are the main force to maintain the stability of the α-helix structure, and the reduction of the α-helix indicated that the hydrophobic group originally in the beef myofibrilla molecule was exposed, and the protein surface hydrophobicity increased[36–37]. As listed, storage time significantly affected the random coil, and more precisely, increased coil content occurred in the sample with 1-day frozen treatment compared with that unfrozen. This was likely due to freezing destroyed the muscle structure, making the protein secondary structure from ordered structure to disordered structure. Furthermore, from 1 to 11 months of storage, the relative content of random coil showed a trend of decreasing and then increased. The disrupted muscle structure and increased protein oxidation could lead to the transformation of ordered structure into disordered structure,

Table 6 Effect of different display times on the proteins secondary structure relative content of beef steaks after different frozen storage periods.

Secondary structure	Storage time	Display time (day)				Mean	SE	*P* value		
		0	7	14	21			Storage time	Display time	Storage time × display time
α-Helix (%)	Unfrozen	31.53	31.05	30.95	23.96	29.37^{x}	0.007	< 0.001	0.001	0.051
	1 day	31.72	28.41	27.68	27.47	28.07^{x}				
	1 month	22.47	22.63	24.80	23.23	23.29^{y}				
	3 months	23.27	23.42	22.69	22.13	22.88^{yz}				
	6 months	20.87	20.02	20.79	20.61	20.77^{z}				
	11 months	21.46	21.91	21.34	20.08	21.20^{yz}				
	Mean	25.22^{a}	24.57^{a}	24.84^{a}	22.42^{a}		0.005			
β-Sheet (%)	Unfrozen	33.92	38.63	36.29	33.27	35.52^{x}	0.008	< 0.001	0.330	0.927
	1 day	32.77	31.27	32.98	32.16	32.30^{y}				
	1 month	32.62	32.06	33.57	30.27	32.13^{y}				
	3 months	28.64	29.81	28.69	28.61	28.94^{y}				
	6 months	28.72	29.20	30.69	28.61	28.85^{z}				
	11 months	28.94	27.15	28.26	27.10	27.86^{z}				
Random coil (%)	Unfrozen	21.90	22.01	22.21	23.56	22.42^{ab}	0.007	0.014	0.341	0.830
	1 day	23.74	23.30	23.25	23.32	23.42^{a}				
	1 month	20.38	21.61	22.25	24.20	20.96^{bc}				
	3 months	19.23	21.25	19.29	20.81	20.15^{c}				
	6 months	20.28	20.79	23.09	23.72	21.80^{abc}				
	11 months	21.36	20.96	23.86	23.70	22.47^{ab}				

Notes: The different letters (a–c) in same row indicate significant differences between display time for the same storage time, and the different letters (x–z) in same column indicate significant differences between storage time at the same display time ($P < 0.05$). When the interaction effect of the two factors was insignificant, while the effect of the individual factor was significant, the means of the results for different storage times and display times were calculated respectively, and the influence of the two individual factors on the indicator was analyzed. SE represents standard error.

increasing random coil content[38]. Results suggested that freezing caused a breakdown of the protein's secondary structural stability and an increase in the hydrophobicity of the protein surface. Findings supported that Raman spectroscopy can provide information on protein secondary structure, which was feasible for rapid and nondestructive discrimination between fresh and frozen-thawed meat.

4 Conclusion

In this work, interactions between frozen storage time and display time on the quality and Raman spectroscopic characteristics of beef were investigated for clarifying the difference between unfrozen meat and frozen-thawed beef. Color analysis indicated that frozen-thawed meat be unable to identify only by the visual color, and blooming ability was also insignificantly affected by frozen-thawed treatment. Lipid oxidation increased with storage time and increased sharply at 11 months frozen storage time; the meat that experienced long-term frozen storage could quickly spoil and quality deterioration during display, reaching higher TBARS value, TVC, TVB-N content, and centrifugal loss. LF-NMR data showed that the bound water and immobilized water in meat could be transferred to free water after freezing and thawing, thereby to easily lose during the display period. Frozen-thawed meat exhibited low protein secondary structure stability with reduced α-helix relative content. Findings supported the characterization of frozen-thawed beef, indicating that the beef with extended storage time showed faster quality deterioration during display, and also proposed a feasible identification approach via Raman spectroscopic features. The following study may focus on the non-destructive detection or identification of frozen-thawed meat by the combination of a variety of spectroscopy methods.

Conflict of interest

The authors declare that they have no conflict of interest in the publication of this article.

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Authors contribution

Yufan An: Writing-original draft; writing-review & editing. **Qiantong Wang:** Formal analysis. **Ping Lu:** Investigation. **Yunge Liu:** Data curation. **Shujuan Gao:** Validation. **Stephanie Fowler:** Methodology. **Rongrong Liang:** Visualization. **Lixian Zhu:** Resources. **Wei Wang:** Validation. **Yimin Zhang:** Conceptualization; funding acquisition; supervision; writing-review & editing.

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