



Effect of super-chilled preservation on the water-holding properties of fresh beef during storage

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Abstract

In this study, comparison of the effects of chilled (4 °C), super-chilled (-4 °C), and frozen (-18 °C) storage on the water-holding properties was evaluated by drip loss, surface hydrophobicity, and calpain activity of raw beef. These results indicated that storage temperature can significantly affect the water-holding properties. In contrast to chilled storage, super-chilled storage can maintain a higher thiol group content, which can maintain better myofibrillar protein hydration capacity. Furthermore, super-chilled storage effectively slowed down the increase in the rate of protein hydrophobicity compared with chilled and frozen storage. Additionally, the super-chilled sample exhibited the highest glutathione reductase activity, followed by the frozen sample. These results revealed that super-chilled storage is a good way to preserve the water-holding properties of raw beef.

Keywords: calpain activity; glutathione reductase activity; protein hydrophobicity; water-holding capacity.

Practical Applications: In this study, comparison of the effects of the chilled (4 °C), super-chilled (-4 °C), and frozen (-18 °C) storage on the water-holding properties was evaluated by drip loss, surface hydrophobicity, and calpain activity of raw beef. These results revealed that super-chilled storage can effectively maintain the water-holding capacity of raw beef, resulting in good freshness preservation.

1 INTRODUCTION

Beef is an important worldwide product, and thus the beef industry must ensure the safe delivery of high-quality beef to faraway end-users (Holman et al., 2017). The interaction of water molecules with protein structures is a very important meat quality trait, as it influences the physicochemical properties of meat, particularly in terms of sensory qualities and tenderness (Huff-Lonergan & Lonergan, 2005). For fresh beef, the storage temperature is the most important factor affecting the water-holding capacity, which can determine the shelf life and quality of the meat. Currently, chilled storage (0–4 °C), super-chilled storage (-1 to 4 °C), and frozen storage (-18 to 40 °C) are the most commonly used commercial storage methods (Pan et al., 2019). Compared with freezing, less water is frozen (5–30%) in super-chilled beef, leading to a lower degree of freeze denaturation of the proteins and less mechanical damage to the muscle structure (Melody et al., 2004). Meanwhile, the shelf life of super-chilled beef can be extended by 1.5–4 times compared to conventionally chilled beef. Moreover, super-chilled storage can reduce the mechanical damage, cell collapse, and gas expansion caused by ice crystals generated during freezing, reducing juice loss during thawing and maintaining the original freshness of beef. The application of this technology has solved the safety and freshness problems in the circulation, processing, and marketing of agricultural products. Thus, super-chilled storage got increasing emphasis in the frozen field. To date, many studies focus on microbial quality and meat color of fresh beef under super-chilled storage (Tian et al., 2022; Wen et al., 2022), but the water-holding capacity

under super-chilled storage has rarely been reported, and the exact mechanism responsible for the water-holding capacity is still far from understood and needs to be investigated.

The structure of myofibrillar protein and muscle is crucial for holding water in meat. Thus, in this study, three kinds of storage methods, namely, chilled storage (4 °C), super-chilled storage (-4 °C), and frozen storage (-18 °C), were performed to store the fresh beef. Comparison of the effects of the chilled, super-chilled, and frozen storage on drip loss, surface hydrophobicity, and calpain activity of fresh beef was determined to evaluate the water-holding capacity. These findings can inform the water-holding capacity of fresh beef during super-chilled storage.

2 MATERIALS AND METHODS

2.1 Sample processing

Fresh beef *longissimus lumborum* muscle was obtained from a local abattoir located in Chengdu, Sichuan Province, China, within 12 h after slaughter. Immediately, the fresh beef was divided into samples (10 cm × 10 cm × 1.5 cm) with a weight of about 100 g and equally divided into three groups labeled as Group C, Group S, and Group F. Subsequently, Group C, Group S, and Group F were stored in chilled (4 °C), super-chilled (-4 °C), and frozen (-18 °C) conditions, respectively.

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2.2 Freezing point of beef determination

The freezing point of beef was determined according to the method described by Banerjee and Maheswarappa (2019). Briefly, the thermometer probe was inserted into the center of regular-sized square beef sample (10 cm × 10 cm × 1.5 cm) and then stored in the refrigerator at -18 °C. The decentered temperature was measured and recorded every 1 min. When the temperature reached the plateau period with slight long-term variation, the temperature was the freezing point of beef.

2.3 Water-holding capacity determination

The water-holding capacity of fresh beef was measured with the filter paper press method described by Stadnik et al. (2008). Briefly, a 2 g beef sample was put on a piece of filter paper and then placed between two plexiglass plates and subjected to a mechanical force of 345 kPa for 5 min. The water release was expressed as cm²/g. All measurements were performed in triplicate, and an average was calculated. A low value of cm²/g means that the meat has superior water-holding properties to meat with a high value of cm²/g.

2.4 Texture determination

The texture of the beef sample, including hardness, springiness, and chewiness, was measured by a texture analyzer (TA.XTC-18, Shanghai Baosheng Industrial Development Co., Ltd., Shanghai, China) with a TA/1-SH spherical probe according to the method described by Li et al. (2018). The TPA test conditions were set at 0.07 N pressure, 4 mm/s speed, and 6 s compression deformation.

2.5 Myofibrillar protein extraction

The myofibrillar protein extraction was performed according to the method described by Dava et al. (2021). 1 g of minced beef sample was homogenized with phosphate buffer (20 mmol NaCl, 25 mmol/L KCl, 3 mmol/L MgCl₂, 4 mmol/L EDTA₂Na, and 1 mmol/L protease inhibitor, pH 6.5) for 2 min using a Cuisinart Food Processor (Model CU1 CFP7BC, Cuisinart, East Windsor, NJ), followed by centrifugation at 8,000 r/min for 30 min at 4 °C using a refrigerated centrifuge (Centrifuge 5427 R, Eppendorf, Hamburg, Germany) to separate sarcoplasmic proteins. The resultant pellet was washed two times with phosphate buffer and then centrifuged. The final suspension was myofibrillar protein extraction and was stored at -80 °C. The myofibrillar protein concentration was estimated according to the method described by Lowry et al. (1951) with bovine serum albumin (BSA, Sigma) as the standard.

2.6 Free and total protein thiol group content determination

The free and total protein thiol group content was measured according to the method described by Fu et al. (2015). Briefly, 1 mL of 2 mg/mL myofibrillar protein solution was added to a 9 mL buffer solution (50 mmol/L phosphate buffer, 8 mol/L urea, 0.6 mol/L potassium chloride, 10 mmol/L EDTA, pH 7), and 0.4 mL of 1 g/L DTNB solution was added. Then the mixture solution was incubated for 25 min at 40 °C, and its absorbance

was detected at 412 nm with a molar extinction coefficient of 13,600 M⁻¹/cm⁻¹ to calculate the total sulfhydryl groups. Free thiol group content was determined by incubation of the reaction mixtures without urea for 1 h at 4 °C. Results were expressed in nanomoles of thiol groups per milligram of myofibrillar protein.

2.7 Protein surface hydrophobicity determination

The protein surface hydrophobicity was measured according to the method described by Chelch, Gateller and Sante-Lhoutellier (2006). 40 µL of 1 mg/mL bromophenol blue solution was added into 1 mL of 2 mg/mL myofibrillar protein solution, and the mixture solution was incubated for 15 min at 25 °C, followed by centrifugation at 3,000 r/min for 15 min at 4 °C using a refrigerated centrifuge (Centrifuge 5427 R, Eppendorf, Hamburg, Germany). Then the supernatant was detected at 595 nm. The control group was performed with 1 mL of 20 mmol/L phosphorus acid salt buffer (0.6 mol/L NaCl, pH 6.5) to replace the myofibrillar protein solution. The surface hydrophobicity index is characterized by the binding amount of bromophenol blue (R), which is calculated according to Equation 1.

$$R = 40 \times \frac{A_{control} - A_{sample}}{A_{control}} \quad (1)$$

2.8 Calpain activity determination

The calpain activity was determined by fluorescence detection kit (Biovision, USA). Briefly, 100 mg muscle tissue and 100 µL extract buffer solution was mixed and homogenized, and then the mixture was incubated for 20 min at 0 °C followed by centrifugation at 10,000 r/min for 15 min at 4 °C using refrigerated centrifuge (Centrifuge 5427 R, Eppendorf, Hamburg, Germany). After centrifugation, 80 µL supernatant was mixed with 85 µL extract buffer solution, and then 10 µL 10× reaction buffer and 5 µL calcium activating enzyme substrate (Ac-LLY-AFC) was added. Immediately, the mixture was incubated in dark for 60 min at 37 °C. Then absorbance of sample was detected by the microplate reader (SpectraMax M5, Molecular Devices, USA), and the excitation wavelength and emission wavelength were set to 400 nm and 505 nm, respectively. The result was expressed in relative fluorescent units.

2.9 Glutathione reductase activity determination

The glutathione reductase activity was determined by a glutathione reductase activity test kit (Boxbio AKPR010, Beijing Boxbio Technology Co., Ltd., Beijing, China). Briefly, 100 mg of muscle tissue and 1 mL extract buffer solution were mixed and homogenized for 1 min at 0 °C. Then the mixture was centrifuged at 10,000 r/min for 10 min at 4 °C using a refrigerated centrifuge (Centrifuge 5427 R, Eppendorf, Hamburg, Germany). The supernatant was the crude enzyme solution for determination. The reaction solution was added to the supernatant and reacted for 10 s at 37 °C, followed by the detection of absorbance at 340 nm, which was recorded as A_{1T}, and for 190 s at 37 °C, followed by the detection of absorbance at 340 nm, which was recorded as A_{2T}. The control group was performed with distilled

water in place of the reaction solution and recorded as A_{1C} and A_{2C} , respectively. The result recorded as A was calculated according to Equation 2.

$$A = (A_{2T} - A_{1T}) - (A_{2C} - A_{1C}) \quad (2)$$

2.10 Sensory evaluation

Sensory evaluation is a scientific discipline to evaluate the changes in raw meat quality during storage (Paglarini et al., 2020; Vidal et al., 2020). The sensory evaluation was performed according to the method described by Wang et al. (2022). Briefly, the sensory quality was evaluated by their organoleptic characteristics, namely, color, odor, texture, appearance, and viscosity, using a 5-point scale based on attribute degrees by 11 experienced sensory panelists.

2.11 Statistical analysis

Three replicates were performed for all samples, and these results were expressed as the mean \pm standard deviation (SD) unless otherwise mentioned. The Student's *t*-test was used to calculate the significance, accepting $p < 0.05$ as the level of significance using the SPSS 15.0 statistics software (IBM, Chicago, IL, USA).

3 RESULTS AND DISCUSSION

3.1 The result of the freezing point of beef

The freezing curve of beef muscle is shown in Figure 1. The central temperature of beef dropped rapidly at the beginning of freezing and dropped to -1.9 to -2.1 °C, which was maintained for about 40 min. Then the temperature continued to drop. Therefore, the freezing point of beef was determined to be -2 °C. The super-chilled storage is usually controlled by keeping the food temperature 1 – 2 °C below the freezing point. Thus, -4 °C was selected as the temperature for super-chilled storage in this experiment.

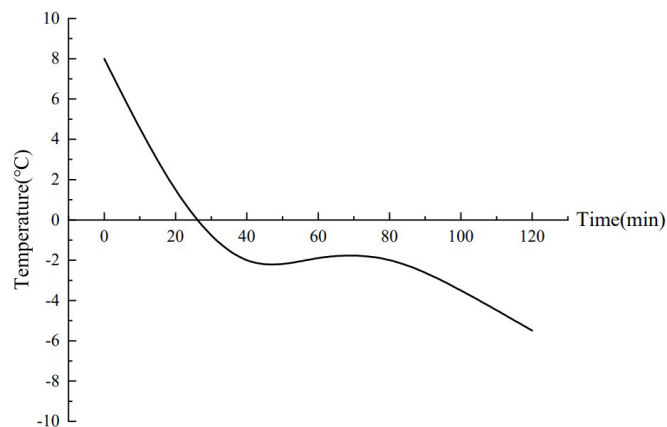


Figure 1. Freezing curve of beef muscle.

3.2 The effect of super-chilled storage on changes in the water-holding capacity of beef

The results of the water-holding capacity of samples stored in three different conditions are shown in Figure 2. At the beginning of the storage, all samples had good water-holding capacity, while the water-holding capacity of all samples gradually reduced with the extension of storage time. The super-chilled sample exhibited significantly higher water-holding capacity than that of the chilled and frozen samples during the whole period of storage. The result showed that super-chilled storage was a good way to maintain the water-holding capacity of beef compared with chilled and frozen storage.

3.3 The effect of super-chilled storage on changes in the texture of beef

Muscle texture is often evaluated to determine the freshness and quality of fresh beef (Bertram, Purslow & Andersen, 2002; Noman et al., 2018). Among all texture indicators, hardness, springiness, and chewiness were significant among the chilled, super-chilled, and frozen samples, as shown in Table 1. The hardness and springiness of the super-chilled sample were significantly higher ($p < 0.05$) than those of the chilled sample. The hardness and springiness of the chilled sample decreased significantly on the third day. The chewiness of all samples significantly decreased over time, with chilled samples exhibiting the lowest chewiness. The decrease in chewiness indicated that muscle fiber structure was destroyed to a certain extent, which corresponded to decreased hardness and springiness. These results suggest that super-chilled storage could better ensure the quality and integrity of beef compared with chilled and frozen storage.

3.4 The effect of super-chilled storage on changes in free and total protein thiol groups of beef

Myosin and actin are the main components of myofibrillar proteins, which contain many sulfhydryl groups (Li et al., 2012). The sulfhydryl group in myofibrillar proteins is prone to form

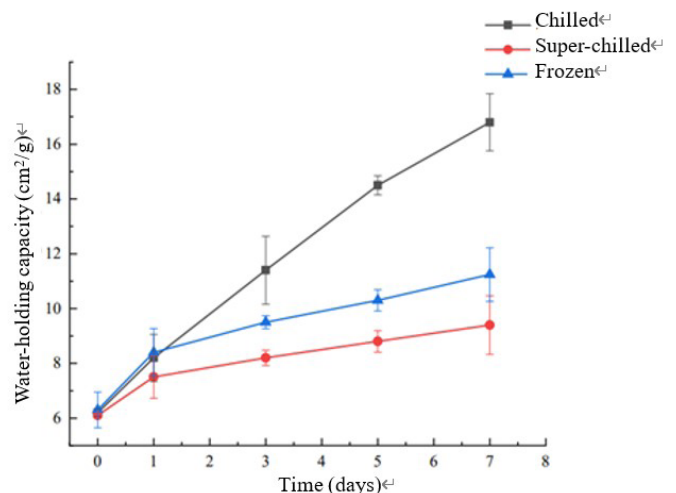


Figure 2. Changes in the water-holding capacity of raw beef during chilled (4 °C), super-chilled (-4 °C), and frozen (-18 °C) storage.

disulfide bond by oxidation resulting in a decrease in sulfhydryl group content. Therefore, thiol group content is regarded as a marker of oxidation (Fu et al., 2015). Both the storage temperature and time had significant effects ($p < 0.05$) on the thiol group content of beef, as shown in Figure 3. The initial total thiol group content of chilled samples, super-chilled samples, and frozen samples was 100.25, 97.26, and 96.39 nmol/mg, respectively. The total thiol group content of the chilled sample decreased to 63.22 nmol/mg on the seventh day and was significantly ($p < 0.05$) lower than those from the super-chilled sample and frozen sample. Few differences were detected in total thiol content of super-chilled sample and frozen sample on the seventh day. As for free thiol group content, the initial content of chilled samples, super-chilled samples, and frozen samples was 83.36, 87.26, and 86.39 nmol/mg, respectively. During storage, the free thiol group content of all samples gradually decreased with storage time and decreased to 52.63, 74.12, and 75.98 nmol/mg of chilled samples, super-chilled samples, and frozen samples on the seventh day, with reductions of 36.85, 15.45, and 12.05%, respectively. The decreased levels of free and total thiol may be involved in the formation of disulfide bonds, which indicate protein oxidation. In this study, the thiol group content of the chilled sample was lowest, indicating that its protein oxidation was the most serious, which will reduce myofibrillar protein hydration capacity. In contrast to chilled storage, super-chilled storage can maintain a higher thiol group content, which can maintain better myofibrillar protein hydration capacity. The result was in agreement with Fu et al. (2015), who showed that thiol group content significantly decreased at 4 °C.

3.5 The effect of super-chilled storage on changes in protein surface hydrophobicity of beef

Protein surface hydrophobicity is an effective index to evaluate the degree of protein denaturation (Xiao et al., 2011). The higher the surface hydrophobicity, the higher the degree of protein denaturation. Both the storage temperature and time had significant effects ($p < 0.05$) on the protein surface hydrophobicity of beef, as shown in Figure 4. The protein surface hydrophobicity in all samples significantly increased ($p < 0.05$) with storage time extension, and the protein surface hydrophobicity of super-chilled storage was the lowest among the three kinds

of samples. The increase in protein surface hydrophobicity will weaken the hydration ability of myofibrillar proteins, resulting in a decline in the water-holding capacity. In this study, the results

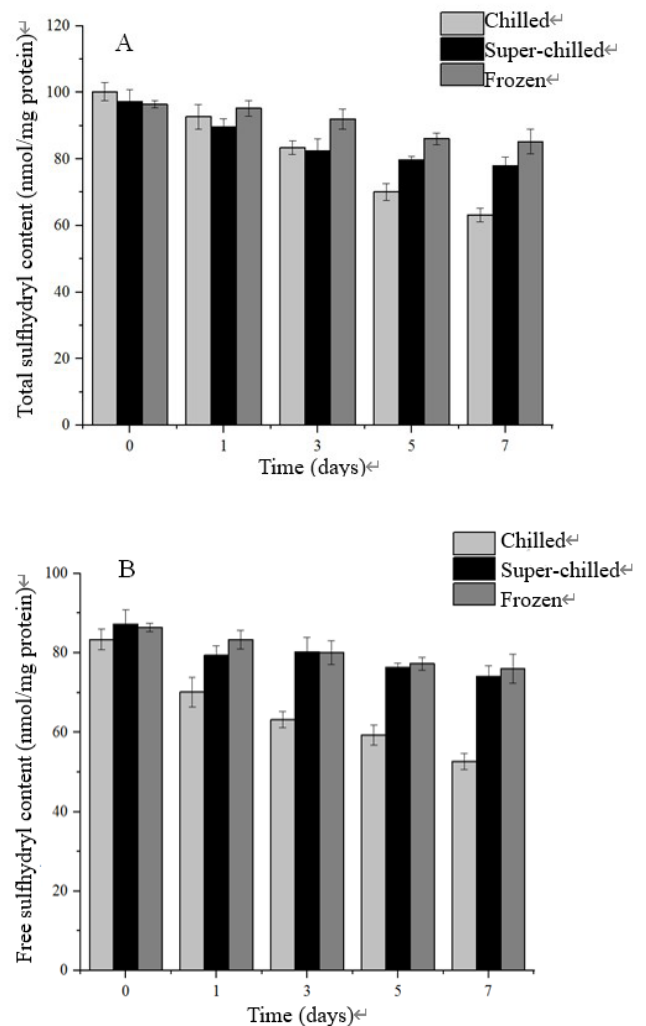


Figure 3. (A) Changes in total sulfhydryl content and (B) free sulfhydryl content of raw beef during chilled (4 °C), super-chilled (-4 °C), and frozen (-18 °C) storage.

Table 1. The changes in texture of raw beef during chilled (4 °C), super-chilled (-4 °C), and frozen (-18 °C) storage

Storage Time	Sample	Hardness/(N)	Springiness/(mm)	Chewiness/(mj)
1 day	Chilled	62.45±3.35	1.92±0.45	19.34±0.87
	Super-chilled	65.24±2.75	2.12±0.32	21.32±0.67
	Frozen	63.32±2.63	1.87±0.49	18.45±0.37
3 days	Chilled	58.82±2.32	1.75±0.14	13.47±0.54
	Super-chilled	62.63±2.04	1.91±0.32	20.32±0.43
	Frozen	57.34±1.89	1.76±0.43	17.56±0.41
5 days	Chilled	38.65±1.75	1.43±0.21	10.34±0.56
	Super-chilled	50.78±2.56	1.76±0.13	17.34±0.43
	Frozen	47.32±1.89	1.69±0.21	16.67±0.56
7 days	Chilled	31.93±1.75	1.03±0.09	8.57±0.46
	Super-chilled	45.57±1.89	1.56±0.12	15.45±0.34
	Frozen	46.76±1.56	1.65±0.32	16.29±0.32

revealed that super-chilled storage effectively slowed down the increase rate of protein hydrophobicity compared with chilled and frozen storage, which was conducive to maintain the water-holding capacity.

3.6 The effect of super-chilled storage on changes in calpain activity of the beef

Calpain, which belongs to a family of intracellular calcium-dependent cysteine proteases, has been regarded as a key factor for the degradation of myofibrillar protein and the improvement of beef quality during postmortem aging (Lu et al., 2015). There was significant difference ($p < 0.05$) in calpain activity under three storage conditions, as shown in Figure 5. The calpain activity in all samples significantly reduced ($p < 0.05$) with storage time extension, with chilled storage decreased fastest, followed by super-chilled storage and frozen storage decreased slowest. The decrease of calpain

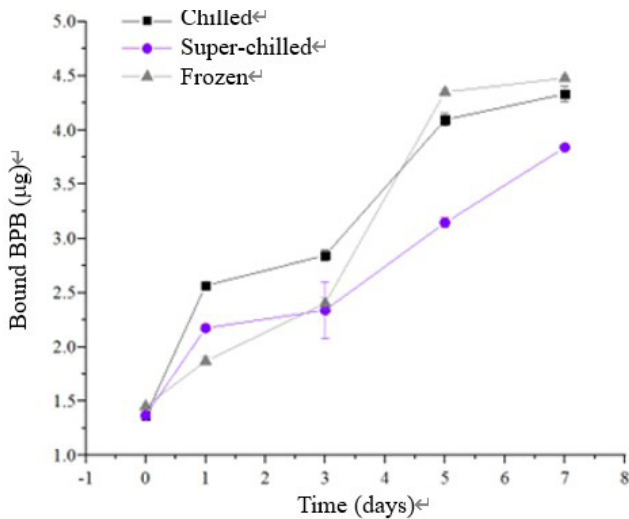


Figure 4. Changes in protein surface hydrophobicity of raw beef during chilled (4 °C), super-chilled (-4 °C), and frozen (-18 °C) storage.

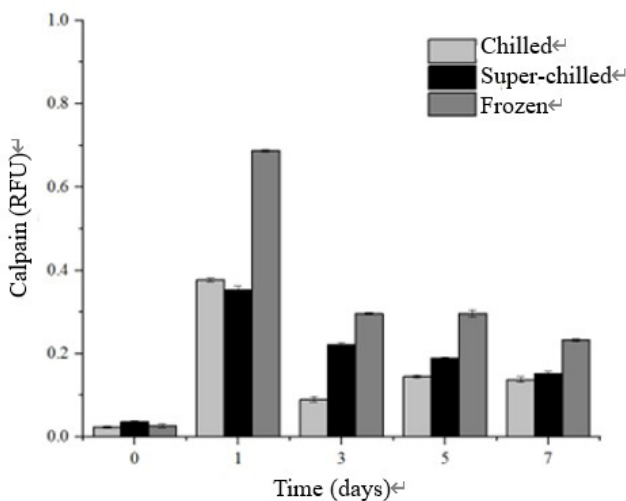


Figure 5. Changes in calpain activity of raw beef during chilled (4 °C), super-chilled (-4 °C), and frozen (-18 °C) storage.

activity in chilled and super-chilled storage is probably due to the autolysis of calpain after activation. While calpain activity is usually inhibited by freezing conditions, resulting in a slow decrease in calpain activity. The result was in accordance with the report of Al-Dalali et al. (2022), who showed that frozen samples obviously had more calpain activity compared with chilled and super-chilled samples.

3.7 The effect of super-chilled storage on changes in glutathione reductase activity in beef

The drip loss of beef is closely related to the integrity and normal function of the cell membrane (Qi et al., 2021). Oxidation of proteins and fatty acids will lead to cell membrane damage. Therefore, oxidation may also be a cause of serious drip loss. Glutathione reductase has the function of scavenging free radicals in cells. Therefore, high glutathione reductase activity is conducive to protecting cell membranes and reducing membrane permeability against drip loss. There was a significant difference ($p < 0.05$) in glutathione reductase activity under three storage conditions, as shown in Figure 6. The glutathione reductase activity in all samples was significantly reduced ($p < 0.05$) with storage time extension. The super-chilled sample exhibited the highest glutathione reductase activity, followed by the frozen sample. The glutathione reductase activity of the super-chilled sample was three times higher than that of the chilled sample on the seventh day.

3.8 The effect of super-chilled storage on changes in the sensory quality of beef

The sensory evaluation of Group C, Group S, and Group F is shown in Table 2. As shown in Table 2, Group S had the highest sensory evaluation score among the three groups, showing good sensory quality. The sensory analysis showed that the super-chilled storage did not have a negative impact on consumer perception.

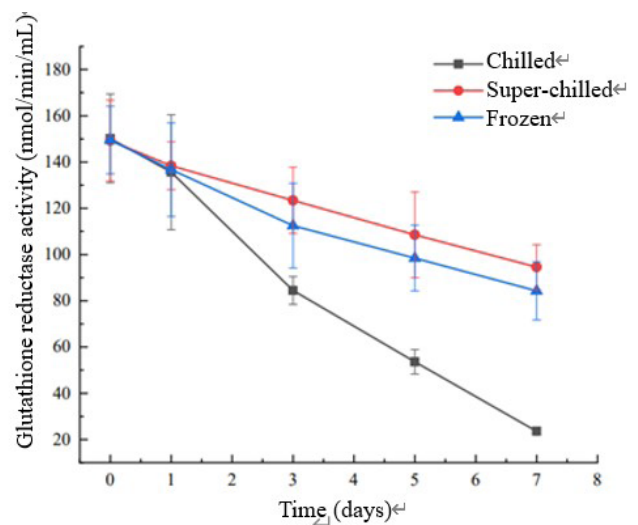


Figure 6. Changes in glutathione reductase activity of raw beef during chilled (4 °C), super-chilled (-4 °C), and frozen (-18 °C) storage.

Table 2. Sensory score of beef during different condition storage.

	0 day	1 day	3 days	5 days	7 days
Group C	24.32±0.40	23.30±0.40	18.80±0.16	14.10±0.20	9.60±0.37
Group S	24.50±0.35	23.80±0.36	22.10±0.27	21.30±0.33	19.10±0.29
Group F	24.36±0.42	21.60±0.33	20.60±0.41	19.80±0.26	19.70±0.43

4 CONCLUSIONS

In this study, the effect of super-chilled preservation on the water-holding properties of fresh beef during storage was unraveled in detail. The holding properties assessment factors such as water-holding capacity, texture, free and total protein thiol groups, protein surface hydrophobicity, calpain activity, and glutathione reductase activity of beef stored in a super-chilled condition (-4 °C) were evaluated and compared with those of the chilled sample (4 °C) and frozen sample (-18 °C). These results indicate that super-chilled storage is a good way to preserve the water-holding properties of fresh beef. The developed super-chilled storage is expected to provide an alternative storage to preserve the high water-holding capacity of fresh beef.

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