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# The effect of super-chilled preservation on shelf life and quality of beef during storage

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**Abstract**

The effect of super-chilled storage (-2 °C) on shelf life and quality of beef was investigated, compared with chilled (4 °C) and frozen (-18 °C) storage. The pH value, color, TVB-N, water-holding capacity, total viable counts (TVC) and sensory evaluation were used to evaluate the freshness, shelf life and quality of beef. These results indicated that the fresh beef stored in super-chilled condition demonstrated better pH and color stability compared to chilled storage, and exhibited better water-holding capacity and sensory score compared to frozen storage. Based on the TVB-N level and TVC, the super-chilled storage was conducive to prolong the shelf life of beef with good quality. The super-chilled storage is a good way to preserve freshness of fresh beef. In conclusion, this work provides data for the preservation of fresh beef which will benefit the meat industry.

**Keywords:** beef; freshness; super-chilled storage; shelf life.

**Practical Application:** In this study, the effect of super-chilled storage on shelf life and quality of beef was investigated, compared with chilled and frozen storage. The result revealed that the super-chilled storage was a good way to preserve freshness of fresh beef. Our work provides data for the preservation of fresh beef which will benefit the meat industry.

## 1 Introduction

Currently, beef is one of the main red meat resources and continues to be the major contributor to total meat consumption in China (Liu et al., 2012), which satisfies the protein requirement and is a rich source of niacin, vitamin $B_6$, vitamin $B_{12}$, phosphorus, potassium, iron, and zinc. However, during the distribution processes and storage, chemical interactions and microbial contamination would deteriorate meat quality (Ding et al., 2020; Tian et al., 2022). The storage temperature is closely related to the shelf life and quality of meat. Thus, a main challenge in this respect is to maintain a stable and sufficiently low temperature which is often more difficult in fresh foods than in frozen foods. Low temperatures can inhibit the growth and reproduction of microorganisms, reduce enzyme activity and slow down various biochemical reactions in the meat. The chilled storage (0-4 ºC) and frozen storage (-18--40 ºC) are the most commonly commercial storage (Pan et al., 2019). Fresh beef with chilled storage has a limited shelf life, primarily due to the growth of microorganism and the activities of endogenous proteolytic enzymes (Pellissery et al., 2020). Compared to chilled storage, frozen storage could extend significantly the shelf life of fresh beef. However, freezing and thawing can result in the reduction of water holding capacity, the increase of drip loss and the denaturation of protein, which affect the quality of fresh beef (Pomponio et al., 2018).

Super-chilled storage that implies temperatures in the borderline between chilling and freezing is a process by which the temperature of a food product is lowered to 1-2 °C below the initial freezing point of the product. The advantages of temperatures below the freezing point can inhibit the microbial growth as well as endogenous enzyme activity. Therefore, the super-chilled storage not only can prolong the storage period, but also effectively maintain the freshness of meat products (Choe et al., 2016). At present, as a new physical preservation technology, super-chilled storage has been extensively applied in fruits and vegetables.

In this study, super-chilled storage was performed in fresh beef storage and evaluate the effect of different storage temperatures (4 °C, -2 °C, and −18 °C) on beef freshness. Freshness is the main quality parameter in fresh beef either for direct consumption or as raw material for the processing industry. Loss of freshness is a complex process including biochemical, chemical and physical changes and microbiological contamination. Some non-sensory indexes, such as total volatile basic nitrogen (TVB-N) and total viable counts (TVC), can be used to determine product freshness (Liang et al., 2021; Rumape et al., 2022). Due to the wide variety and component complexity of aquatic products, one index or property alone cannot adequately assess the freshness of beef. Generally, two or more indexes are examined simultaneously to give a comprehensive assessment. Thus, the color, TVB-N, water-holding capacity and TVC were used to evaluate the freshness, shelf life and quality of beef. These results may provide a novel approach to extend the shelf life of fresh beef.

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## 2 Materials and methods

### 2.1 Sample processing

Fresh beef *longissimus lumborum* muscle was obtained from a local market of agricultural products located in Chengdu, Sichuan Province, China, within 24 h after slaughter. After purchasing, the fresh beef was immediately kept in an insulation ice box and transported to laboratory within 15 min. Then the fresh beef was divided into samples (10 cm × 10 cm × 1.5 cm) with about weight of 100 g for experiments. Subsequently, all samples were equally divided into three groups. Each group contains 24 samples for experiments. Each sample was packaged in moisture-impermeable polyethylene bags and stored in chilled (4 °C), super-chilled (-2 °C) and frozen (-18 °C) conditions for 0, 1, 3, 5 and 7 d, respectively.

### 2.2 Physicochemical parameters

*pH measurement*

The pH values of beef samples were measured according to the method described by Wang et al. (2015b) using a pH meter (Testo 205, Testo International Trade Co., Ltd., Shenzhen, China) with automatic temperature compensation (NTC) electrode. All measurements were performed in triplicate and an average was calculated.

*Color measurement*

The color of samples was measured using an auto color chromameter (CS-22, Hangzhou CHNSpec Technology Co. Ltd, Hangzhou, China) according to the method described by Wang et al. (2015a). The color evaluation was made through the CIE L∗(lightness), a∗(redness), and b∗(yellowness) space obtained from 5 different cut areas of each sample and an average was calculated.

*TVB-N measurement*

The TVB-N concentration was measured in accordance to Chinese standard protocols GB/T 5009.228-2016 (National Health and Family Planning Commission of China, 2016) by using an automatic azotometer (KDN-1000, Shanghai Xin Rui instrument and Meter Co. Ltd, Shanghai, China). The TVB-N concentration was expressed as mg/100 g sample. All measurements were performed in triplicate and an average was calculated.

*Drip loss analysis*

Drip loss was measured according to the method described by Wang et al. (2022a). Briefly, the initial weight of sample was recorded before treatment. After storage, the samples were placed at 4 °C overnight and reweighed after wiping drips from their surface. Drip loss was estimated as a percentage of the original weight according to the following equation (Equation 1):

$$Drip\,\mathrm{loss}(\%) = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100\% \quad (1)$$

### 2.3 Total Viable Counts (TVC) determination

Total viable counts (TVC) determination was performed according to the method described by Wang et al. (2021). Specifically, beef sample (5 g) was mixed with 95 mL sterile saline containing 0.9% NaCl and the sample was homogenized for 1 min. Then the mixture was serially diluted with sterile saline and 0.1 mL of diluent was plated onto agar plates (CM101, Beijing Luqiao Technology, Beijing, China). The plates were incubated at 36 ± 1 °C for 48 h for colony forming units (CFU) counting. The results were expressed in lg CFU/g.

### 2.4 Sensory evaluation

The freshness of beef samples was assessed by human sensory analyses according to the method described by Wang et al. (2022b). The freshness of beef samples was evaluated by their organoleptic characteristics, namely color, odor, texture, appearance and viscosity, using a 5-point scale based on attribute degrees. All samples were coded with three digit numbers and evaluated by 11 experienced sensory panelists according to sensory ranking.

### 2.5 Statistical analysis

Three replicates were performed for all samples and these results were expressed as mean ± standard deviation (SD). Duncan's multiple range test (p-value < 0.05) was employed for the independence of error terms using the SPSS 15.0 statistics software (IBM, Chicago, Ill., U.S.A.).

## 3 Results and discussion

### 3.1 The effect of super-chilled storage on the pH value

The pH value plays a critical role in fresh beef quality, which could affect color stability, the water holding capacity and microbial growth (Ramanathan et al., 2013). The changes in the pH values of beef during chilled, super-chilled and frozen storage were shown in Figure 1. The pH values of sample stored

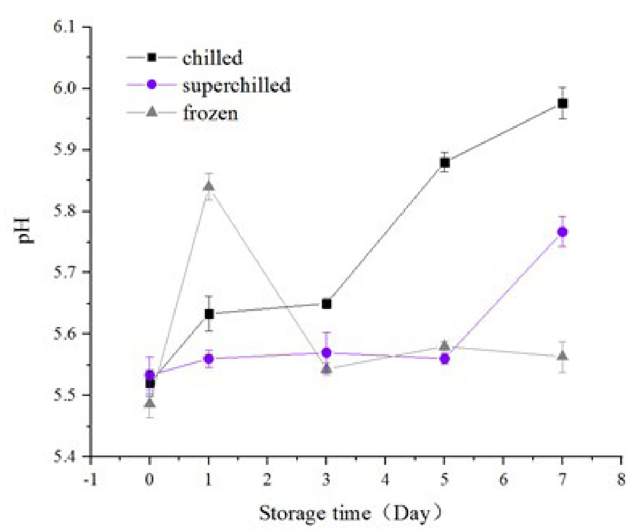


**Figure 1**. The effect of super-chilled storage on the pH of beef during storage.

in chilled condition increased markedly with the storage times extend, and increased from the initial 5.52 to 5.95. In contrast, the pH values of sample stored in super-chilled condition maintained at a stable level (5.52-5.31) within the initial 5 d, and the pH values increased to 5.72. The increase could can mainly be attributed to the production of ammonia, amines, and other basic substances from the degradation of proteins by meat spoilage microorganisms and endogenous enzymes (Ding et al., 2020). There was no significant change ($p > 0.05$) in pH values of sample stored in frozen condition from 0 to 7 d. The results revealed that the super-chilled storage effectively inhibited the increase of pH value compared to chilled storage, which was conducive to freshness maintenance. The results were in agreement with results from Liu et al. (2014).

### *3.2 The effect of super-chilled storage on the color*

A bright red color is considered a positive attribute for freshness and superior quality of beef (Holman et al., 2016). The changes in color of beef samples stored in different temperature conditions were shown in Table 1. The degree of *a*∗of chilled beef is associated with consumer-defined beef color acceptability, and when a* < 14.5 the beef color is considered unacceptable (Holman et al., 2017). As shown in Table 1, the initial a∗value of all samples was about 40, suggesting a desirable color acceptability. Subsequently, the *a*∗ value of chilled sample reduced rapidly along with the storage and the *a*∗ value reduced to 13.35 on the 5th day and reached the rejection level (a∗ < 14.5). In contrast, the the a∗value of super- chilled sample and frozen sample maintained a relatively stable a∗value during the storage for 7 days, which was significantly higher than the chilled beef ($p < 0.05$). The *a*∗ value of chilled sample and frozen sample maintained 35.15 and 34.46 on the 7th day, respectively, which much higher than the threshold (a∗ > 14.5). The results revealed that the super-chilled storage effectively maintained the beef color, which was conducive to freshness maintenance.

### *3.3 The effect of super-chilled storage on the TVB-N level*

The TVB-N level is an important index to reflect the freshness of meat (Wang et al., 2022a).The changes in TVB-N concentration of beef samples stored in different temperature conditions were shown in Figure 2. The TVB-N concentration of chilled sample and super-chilled sample increased with storage time extension, and there was no significant changes of frozen samples ($p > 0.05$). The TVB-N concentration of chilled beef sample exhibited the highest rate of growth from 4.82 mg/100 g to 36.28 mg/100 g on the 7th day, and the TVB-N concentration increased up to 19.7 mg/100 g on the 3rd day. Meanwhile, the TVB-N concentration of super-chilled sample and frozen sample maintained 10.45 mg/100 g and 10.63 mg/100 g on the 3rd day, respectively. Moreover, the TVB-N concentrations of super-chilled sample and frozen sample were still lower than 15 mg/100 g on the 7th day. In this study, compared with chilled storage, the super-chilled storage could obviously stable the TVB-N level, as well as the pH value and color. The he upper limit of TVB-N level in fresh meat is 15 mg/100 g and reaches the threshold of acceptability according to the National Food Safety Standard of China (GB 2707-2016) (Wang et al., 2022a). These results indicated that the shelf life of beef stored in chilled condition is less than 3 days, while the the shelf life of beef stored in super-chilled condition is longer than 7 days. The super-chilled storage obviously displayed a longer shelf life than that of chilled storage. Similar results (Zeng et al., 2005) showed that superchilling is great importance for freshness maintenance of fresh meat.

### *3.4 The effect of super-chilled storage on the water-holding capacity*

The water-holding capacity is regarded as an essential meat quality parameter, which is usually assessed by measuring drip

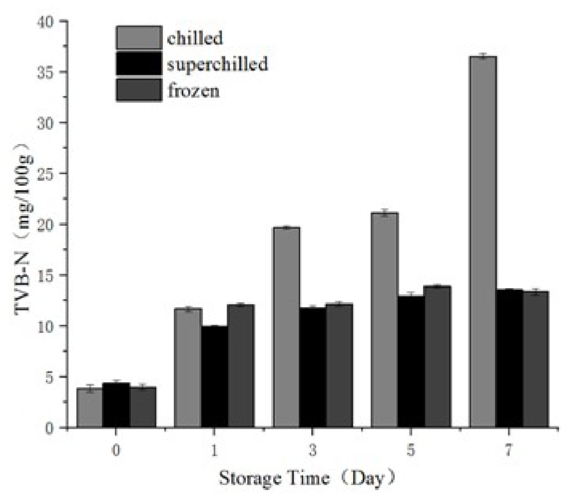


**Figure 2**. The effect of super-chilled storage on the TVB-N level of beef during storage.

**Table 1**. The effect of super-chilled storage on the color of beef during storage.

| | | 0 d | 1 d | 3 d | 5 d | 7 d |
|---|---|---|---|---|---|---|
| L | Chilling | 20.38 ± 1.50 | 21.83 ± 0.76 | 26.99 ± 1.29 | 27.75 ± 1.29 | 33.91 ± 0.31 |
| | Superchilling | 20.86 ± 0.93 | 23.85 ± 0.50 | 23.12 ± 1.30 | 25.59 ± 0.31 | 27.26 ± 1.29 |
| | Frozen | 20.52 ± 0.68 | 26.12 ± 0.32 | 25.86 ± 1.30 | 26.13 ± 0.83 | 29.68 ± 1.29 |
| a* | Chilling | 40.94 ± 1.21 | 33.68 ± 0.75 | 25.19 ± 1.78 | 13.33 ± 1.52 | 8.41 ± 1.20 |
| | Superchilling | 40.5 ± 1.59 | 46.26 ± 0.93 | 43.79 ± 1.52 | 38.27 ± 0.67 | 35.15 ± 1.58 |
| | Frozen | 41.68 ± 1.33 | 45.39 ± 1.10 | 39.51 ± 1.55 | 35.67 ± 0.75 | 34.46 ± 0.69 |
| b* | Chilling | -2.51 ± 0.20 | 0.38 ± 0.25 | 1.76 ± 0.44 | 2.06 ± 0.45 | 3.36 ± 0.36 |
| | Superchilling | -2.55 ± 0.30 | -0.71 ± 0.22 | 0.33 ± 0.17 | 0.49 ± 0.22 | 1.08 ± 0.21 |
| | Frozen | -2.63 ± 1.37 | -0.75 ± 0.41 | 0.42 ± 0.69 | 0.66 ± 0.38 | 1.39 ± 0.25 |

loss which reflects a phenomenon that the structure of aquatic product muscle tissue is damaged by ice crystals and/or endogenous enzymes during storage (Liang et al., 2021).The changes in water-holding capacity of beef samples stored in different temperature conditions were shown in Figure 3. The drip loss in all samples significantly increased ($p < 0.05$) with storage time extension, in which the drip loss of sample stored in super-chilled condition was lower than that of sample stored in chilled condition and frozen condition at each time point of storage. More specifically, the drip loss values measured from sample in super-chilled storage on the 1st, 3rd, 5th and 7th day were around 1.2%, 0.86%, 2.19% and 2.47%, respectively. The water-holding capacity reduction was mainly attributed to the microstructure of muscle fibers damage. Under frozen storage, the water in muscle tissue of beef turns into ice crystals, which may damage muscle tissue structure, resulting in the expansion of muscle fiber space and the change of intramuscular water migration (Li et al., 2020). Under chilled storage, endogenous enzymes and exogenous microorganisms still maintains an active level, resulting in oxidative denaturation of protein in the muscle, which reduced the ability to bind water (Li et al., 2017). In contrast to chilled storage and frozen storage, the super-chilled storage can not only inhibit activity of endogenous enzymes and exogenous microorganisms, but also reduce ice crystal formation during storage. Thus, the beef sample stored in super-chilled condition can maintain better water-holding capacity.

### *3.5 The effect of super-chilled storage on microbial quality*

Microbial quality is one of the main factors affecting the shelf life of fresh meat (Hong et al., 2012), which is usually assessed by total viable counts (TVC). The changes in TVC of all beef samples stored in different temperature conditions were shown in Figure 4. The initial count for TVC was about 3.2 lg CFU/g for three group samples. The TVC values of chilled sample increased remarkably with the prolonging of storage time, and reached up to 6.2 l g CFU/g on the 5th day. In contrast to chilled storage, the TVC values of super-chilled sample were lower and reached up to 4.6 l g CFU/g on the 7th day. Moreover, there was no significant changes in TVC value of frozen samples ($p > 0.05$). Generally, for raw meat, the microbial acceptable limit for TVC value during storage is 6 l g CFU/g (Fernández et al., 2009). At the end of storage, the TVC values of the super-chilled sample and frozen sample were still lower below 5 l g CFU/g, while the TVC value of chilled sample was up to 7.6 l g CFU/g which exceeded the upper tolerable limit for fresh meat. These results indicated that super-chilled storage could inhibit the microbiological activity, which is conducive to the shelf life extension. The temperature and free water are two key factors affecting microbial growth. The advantages of super-chilled storage for inhibiting microbial growth are mainly reflected with respect to the low temperature and free water amount. Specifically, ice crystal formation under super-chilled storage will correspondingly reduce the free water amount, hereby limiting the availability for microbial use. From these result, the the beneficial effect of super-chilled storage will be widely employed in the fresh beef supply chain, compared with traditional refrigeration approach.

### *3.6 The effect of super-chilled storage on sensory quality*

The sensory evaluation is a scientific discipline for the purposes of evaluating consumer products (Hyldig, 2012; Vidal et al., 2020). Nowadays, sensory analysis has been a useful tool to evaluate the changes of raw meat quality during storage (Paglarini et al., 2020). The sensory assessment of three group samples was shown in Table 2. Sensory scores decreased with storage time in all the three group samples. The chilled sample had the highest reducing rate among three group sample ($p < 0.05$), while the super-chilled sample bad the best sensory score. The sensory score of chilled sample was only 12.10 on the 5th day, indicating that had bad color, odor, texture and unacceptable appearance, while the sensory score of super-chilled sample still had high degrees (17.30) of quality. On the 7th day, the super-chilled sample still had acceptable quality. These results are in agreement with

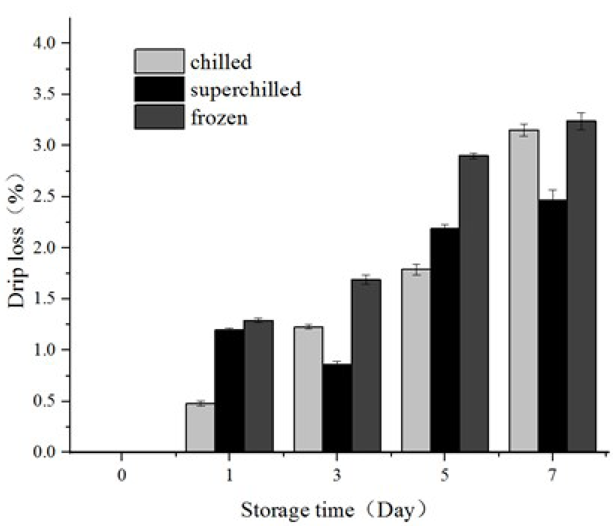


**Figure 3**. The effect of super-chilled storage on the drip loss of beef during storage.

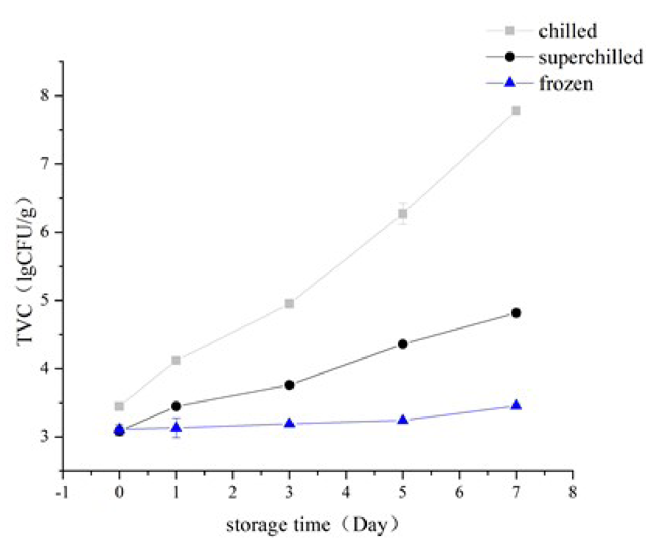


**Figure 4**. The effect of super-chilled storage on the TVC value of beef during storage.

**Table 2**. Sensory score of beef during storage in different low-temperature storage.

| | 0 d | 1 d | 3 d | 5 d | 7 d |
|---|---|---|---|---|---|
| Chilling | 19.30 ± 0.50 | 16.30 ± 0.40 | 14.80 ± 0.16 | 12.10 ± 0.20 | 7.60 ± 0.37 |
| Superchilling | 19.30 ± 0.50 | 18.80 ± 0.36 | 18.10 ± 0.27 | 17.30 ± 0.33 | 16.10 ± 0.29 |
| Frozen | 19.30 ± 0.50 | 18.60 ± 0.33 | 17.60 ± 0.41 | 16.80 ± 0.26 | 15.80 ± 0.43 |

the color and water-holding capacity. Therefore, it might be useful to use sensory evaluation together with TVB-N values and microbial results to judge shelf life.

## 4 Conclusion

In this study, the effect of storage temperatures on shelf life and quality of beef was unraveled in detail. The quality assessment factors such as color, drip loss, TVB-N, TVC, and sensory of beef stored in super-chilled condition (-2 °C) were evaluated and compared with those of the chilled sample (4 °C) and frozen sample (-18 °C). The quality factor analysis indicates that the super-chilled storage could extend the shelf life of beef and is a good way to preserve freshness of beef. The developed super-chilled technique is expected to provide an alternative option to preserve high end fresh meat for short and mid term storage to satisfy commercial need.

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