Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) *2022 23(7):578-586*
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Research Article

https://doi.org/10.1631/jzus.B2200030

Assessment of quality deviation of pork and salmon due to temperature fluctuations during superchilling

Haoxin CUI[1], Naymul KARIM[1], Feng JIANG[2], Haimei HU[2], Wei CHEN[1,3]✉

[1]*Department of Food Science and Nutrition, College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China*
[2]*Changhong Meiling Co. Ltd., Hefei 230000, China*
[3]*Ningbo Research Institute, Zhejiang University, Ningbo 315100, China*

Abstract: Superchilling is an emerging technology for meat preservation; however, the temperature changes during the process have been commonly ignored. Thus, the effects of temperature fluctuations on meat quality during superchilling are yet to be evaluated. In our study, pork loins and salmon fillets were stored for several days (0, 8, 15, 23, and 30 d) under different temperature fluctuations based on −3.5 ℃ as the target temperature. The results showed that after 15 d of superchilling storage, the values of total volatile basic nitrogen, total viable count, and lipid oxidation were significantly ($P<0.05$) altered in the ±2.0 ℃ fluctuation group compared with the constant temperature group. On the contrary, there was no significant difference in these parameters between the ±1.0 ℃ fluctuation group and the constant temperature group after 30 d of storage. In addition, irregular temperature changes significantly accelerated the modulation of various indicators. In brief, temperature fluctuations and irregular temperature changes accelerated the destruction of muscle structural integrity, increased the water loss, gradually widened the water loss channels, and thereby reduced the edibility by accelerating the spoilage of meat.

Key words: Pork; Salmon; Superchilling; Temperature fluctuation; Meat quality

1 Introduction

Meat is an important part of the human diet. Pork is representative of red meat and occupies a major position in the global market of meat products (Wood et al., 1999). Salmon has become progressively more popular among fish in recent years because of its rich nutrition and delicious taste (Haghiri, 2016). The various nutrients in meat provide an ideal environment for the growth and propagation of common food spoilage organisms (Yang et al., 2017). If fresh meat is not processed appropriately, it can easily spoil due to microorganisms, resulting in poor meat quality (Fidalgo et al., 2021). Therefore, quality and safety assurance are necessary for the development of the meat industry (Xie et al., 2016; Cheng et al., 2017).

✉ Wei CHEN, zjuchenwei@zju.edu.cn
Wei CHEN, https://orcid.org/0000-0002-2373-2437

Received Jan. 16, 2022; Revision accepted Apr. 10, 2022;
Crosschecked June 2, 2022

Temperature is a crucial factor in the meat supply chain. Processes such as the denaturation and degradation of heat-labile muscle proteins, lipid oxidation, and protease reactions are sensitive to temperature changes, which can further lead to the poor quality of meats (Duun and Rustad, 2008).

"Superchilling" was first introduced in 1920 and was defined as the temperature zone where the storage temperature of food is dropped to 1–2 ℃ below its initial freezing point (Duun and Rustad, 2007). This value is between the two temperature zones of traditional refrigeration and freezing (Zhou et al., 2010). With the continuous improvement of superchilling technology, the related cold storage system is becoming more advanced to improve the shelf-life of meats.

One study controlled the temperatures at −1 ℃ to −3 ℃ using refrigerated chambers for the preservation of salmon fillets and seabass (Chang et al., 1998). Compared with −2 ℃, superchilling at −3 ℃ extended the shelf-life of pork as well as maintained better meat quality, which was later measured by the total viable count, total volatile basic nitrogen (TVB-N),

and thiobarbituric acid reactive substances (TBARSs) (Ding et al., 2020). Superchilling was reported to minimize biochemical degradation, protein denaturation, and structural damage in Atlantic salmon compared with frozen storage (Gallart-Jornet et al., 2007). Superchilling was reported to improve the shelf-life of foods by approximately 1.5–4.0 times compared to traditional refrigeration (Kaale et al., 2011). The temperature control capability of superchilling equipment on the market shows variation. The associated challenges prompt the standardization of the thermal process and determination of the optimal degree of superchilling for different product groups to adequately improve shelf-life (Banerjee and Maheswarappa, 2019).

Most of the existing research has focused on the effect of a single temperature (such as −3 ℃) on meat quality (Ye et al., 2020). Nowadays, refrigerators and other low-temperature storage devices sold in the market still feature temperature changes within the storage space, which is usually ignored by merchants and consumers alike (Derens-Bertheau et al., 2015; Khan et al., 2017).

Our study is mainly focused on temperature fluctuations during long-term superchilling and their impact on the quality deterioration of pork and salmon. The results provide a new theoretical basis for reducing the temperature fluctuations in low-temperature storage equipment, in order to improve the meat quality during cold storage.

2 Results and discussion

2.1 Design of temperature fluctuations

During the freezing process, the core temperature of meat dropped rapidly at the initial stage of freezing, followed by a plateau period, after which the temperature dropped continuously. The temperature range of the plateau period is the freezing point of the meat. According to previous studies, the initial freezing points of pork loins and salmon fillets are about −1.0 ℃ and −1.4 ℃, respectively (Duun and Rustad, 2008; Duun et al., 2008; Pomponio and Ruiz-Carrascal, 2017). Superchilling storage usually controls the temperature of food at 1–2 ℃ below the initial freezing point. In our experiment, −3.5 ℃ was selected as the temperature for the superchilling storage of pork and salmon.

The temperature inside a refrigerator shows complex fluctuations. When the superchilling temperature of an ordinary refrigerator was set to −3.5 ℃, the temperature changes in the central area of refrigerator were observed (Fig. 1a). These temperature changes were dominated by fluctuations. In our study, the refrigerators had been remodeled to obtain only the temperature fluctuations during storage. The constant temperature space was assembled in a place where the temperature fluctuations were minimized, such that the internal temperature changes were kept within ±0.3 ℃ (Fig. 1b). Subsequently, the internal temperature of the refrigerator was adjusted so that the temperature fluctuations stabilized, and the integral average temperature value was −3.5 ℃. The temperature fluctuations of (−3.5±1.0) ℃ and (−3.5±2.0) ℃ were shown in Figs. 1c and 1d, respectively. The temperature changes in the center and surface of pork and salmon under different temperature conditions were shown in Fig. S1.

2.2 Effects of temperature fluctuations on meat quality indicators

TVB-N generally refers to the basic nitrogen-containing substances such as volatile ammonia and amines, which are produced by the decomposition of proteins due to the joint interaction of muscle enzymes and bacteria during meat storage (Olafsdóttir et al., 1997). The changes in TVB-N in the different study groups during superchilling were shown in Fig. 2. In general, the acceptable limit of TVB-N value for fresh meat during cold storage is 15 mg/100 g (Zhang et al., 2011; Fidalgo et al., 2021). The average TVB-N of pork increased gradually from Day 0 (8.87 mg/100 g). From Day 8, the average TVB-N values of the constant temperature group and the ±1.0 ℃ fluctuation group were significantly altered compared to the other groups. On Day 30, the mean TVB-N value of the constant temperature group was 11.55 mg/100 g, while the value for the ±2.0 ℃ fluctuation group was 14.23 mg/100 g. The TVB-N of the control group exceeded 15 mg/100 g on Day 25. This parameter did not exceed 15 mg/100 g for the other three groups at Day 30; however, there was a significant difference between the ±2.0 ℃ fluctuation group and the constant temperature group (P<0.05). The average TVB-N of salmon gradually increased from Day 0 (10.03 mg/100 g), and the differences compared to

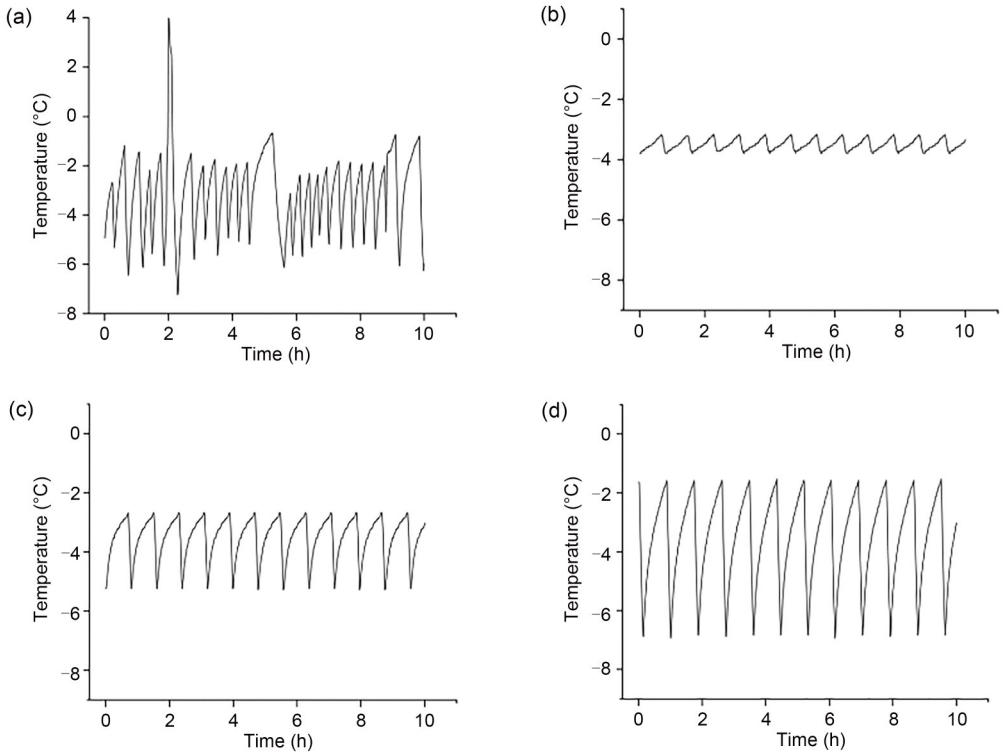

Fig. 1 Study of temperature changes during the superchilling process. (a) The actual temperature changes (control) of the superchilled area in an ordinary refrigerator (−3.5 ℃); (b) Simulation of −3.5 ℃ constant temperature in a refrigerator; (c) Simulation of refrigerator temperature fluctuations of (−3.5±1.0) ℃; (d) Simulation of refrigerator temperature fluctuations of (−3.5±2.0) ℃.

the experimental groups were not clear on Day 8. The TVB-N values of the control group and the ±2.0 ℃ fluctuation group exceeded 15 mg/100 g on Days 16 and 23, respectively, accompanied by the clear smell of ammonia. On Day 30, the average value of the constant temperature group was 13.53 mg/100 g, and there was no significant difference between the constant temperature group and the ±1.0 ℃ fluctuation group (P>0.05). On Day 30, the average TVB-N value of the control group of salmon was 20.53 mg/100 g, which was much higher than that of the pork control group (15.52 mg/100 g). This was because salmon fillet had a lower freezing point than pork loin. The temperature in the superchilling area of the ordinary refrigerator was irregular, and the temperature showed great fluctuation, which could easily be above the freezing point of salmon, greatly reducing the amount of ice crystals in salmon fillets. Pork loins had a higher degree of ice crystallization than salmon fillets. Another reason for the above phenomenon is that salmon has more free nitrogen than pork. Therefore, the TVB-N value increased more slowly in pork loins.

The observed TVB-N values were consistent with the previously reported growth trend (Cordoba et al., 1994). The results showed that the irregular temperature changes had a great influence on the TVB-N value during superchilling storage. With the increase of storage time, ±2.0 ℃ fluctuations induced the significant increase of TVB-N value, while ±1.0 ℃ fluctuations did not significantly change the TVB-N value compared with the constant temperature group.

The growth and reproduction of microorganisms can lead to the spoilage of meat products. Total viable count (TVC) is a common indicator for evaluating the quality and shelf-life of meat products. The changes of TVC in pork and salmon during superchilling were shown in Fig. 3. In general, the acceptable limit of TVC value for fresh meat during storage is 1×10^6 colony forming units per gram (CFU/g) (Coombs et al., 2017). The average TVC of pork gradually increased from Day 0 (3.87 lgCFU/g). From Day 8, the TVC values of the ±2.0 ℃ fluctuation group and the control group were significantly higher compared to the other two groups (P<0.05). The control group exceeded

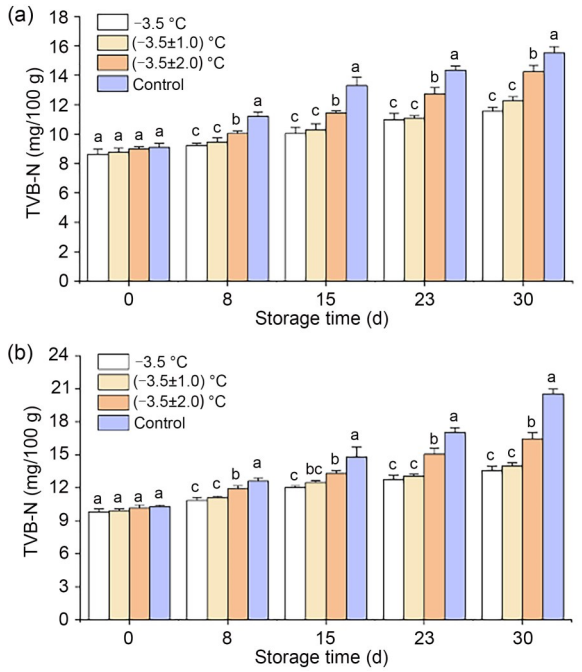

Fig. 2 Changes of total volatile basic nitrogen (TVB-N) during superchilling storage at −3.5 °C, (−3.5±1.0) °C, (−3.5±2.0) °C, and the control. (a) Changes of TVB-N in pork; (b) Changes of TVB-N in salmon. The different lowercases indicate the significant differences among storage conditions for the same storage time (P<0.05). Data are presented as mean±standard deviation (n=3).

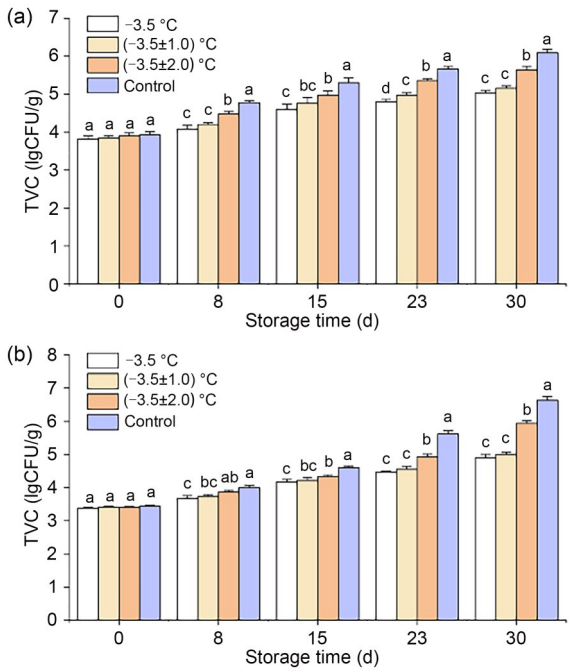

Fig. 3 Changes of total viable count (TVC) during superchilling storage at −3.5 °C, (−3.5±1.0) °C, (−3.5±2.0) °C, and the control. (a) Changes of TVC in pork; (b) Changes of TVC in salmon. The different lowercases indicate the significant differences among storage conditions for the same storage time (P<0.05). Data are presented as mean± standard deviation (n=3). CFU: colony forming units.

6 lgCFU/g on Day 27. The average TVC of salmon increased gradually from Day 0 (3.41 lgCFU/g). The control group and the ±2.0 °C fluctuation group exceeded 6 lgCFU/g on Days 16 and 26, respectively. There was no significant difference between the constant temperature group and the ±1.0 °C fluctuation group (P>0.05).

The results showed that temperature changes had a great influence on the variation in TVC values. As the time passed, the ordinary refrigerator and ±2.0 °C fluctuations significantly promoted the increase of TVC, while ±1.0 °C fluctuations did not alter the TVC significantly compared with the constant temperature group. Besides, the TVC of salmon was initially lower than that of pork, but it eventually exceeded it. It was speculated that the high fat and protein contents in salmon were more conducive to the growth and reproduction of microorganisms (Fernández et al., 2009). The degree of ice crystallization in the control salmon fillets was lower than that in the pork loins, and therefore the TVC value increased more rapidly in salmon fillets. At the same time, the growth trend of TVC in each group was consistent with the TVB-N level.

Considering the results of TVC and TVB-N, the shelf-life of pork of the constant temperature group and the ±1.0 °C fluctuation group was approximately 36–39 d, whereas the shelf-life of salmon was approximately 33–35 d. On the other hand, the shelf-life of pork of the ±2.0 °C fluctuation group was approximately 29–32 d, while the shelf-life of salmon was approximately 27–30 d.

TBARS is widely used to determine the degree of oxidative rancidity of fats in foods, especially fats in meat and aquatic products (Campo et al., 2006). An increased TBARS value was mainly associated with the auto-oxidation and enzymatic hydrolysis of lipids (Domínguez et al., 2019). The changes of TBARS in pork and salmon during superchilling were shown in Fig. 4. The TBARSs of pork and salmon gradually increased, starting from 0.17 and 0.43 mg/kg on Day 0, respectively. From Day 15, the difference between the ±2.0 °C fluctuation group and the constant temperature group remained significant (P<0.05). The constant

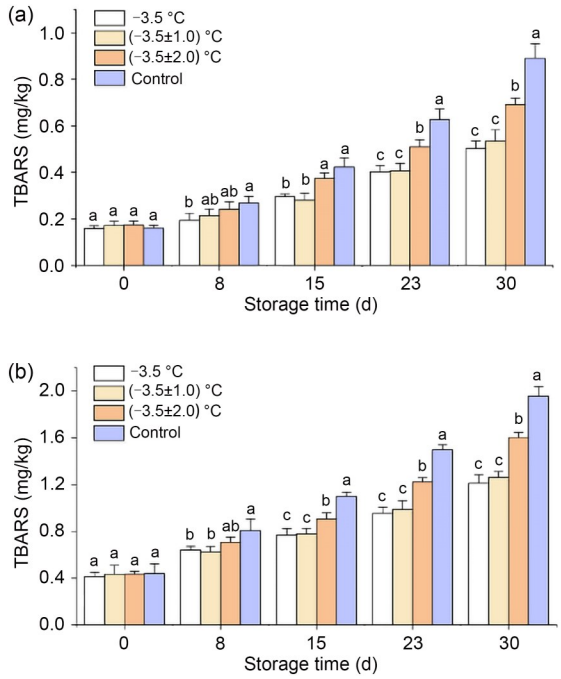

Fig. 4 Changes of thiobarbituric acid reactive substances (TBARSs) during superchilling storage at −3.5 ℃, (−3.5±1.0) ℃, (−3.5±2.0) ℃, and the control. (a) Changes of TBARS in pork; (b) Changes of TBARS in salmon. The different lowercases indicate the significant differences among storage conditions for the same storage time (P< 0.05). Data are presented as mean±standard deviation (n=3).

temperature groups of pork and salmon had no significant difference compared to both ±1.0 ℃ fluctuation groups. Salmon consistently had higher TBARS values than pork. This is because salmon has higher fat content and is more prone to oxidation under superchilling conditions (Thanonkaew et al., 2006). The results showed that irregular temperature changes in the ordinary refrigerator and that with ±2.0 ℃ temperature fluctuations accelerated the lipid oxidation in pork and salmon during storage, which was consistent with the results of TVB-N and TVC.

Drip loss, as one of the indicators to evaluate the water retention capacity of meat, is an important parameter for evaluating meat quality (Bueno et al., 2013). The changes in drip loss in pork and salmon during superchilling were shown in Table S1. Drip loss was more severe in pork compared to salmon because of its higher free water content. On Day 30, drip loss in the control group was significantly higher than those in the constant temperature group and the ±1.0 ℃ fluctuation group (P<0.05). According to our results, it was speculated that irregular temperature changes and ±2.0 ℃ temperature fluctuations caused the repeated freeze and thaw cycles of ice crystals in the meat and reduced the ratio of ice formed in pork and salmon. At the same time, the repeated formation of intracellular ice crystals could pierce the cell membrane, reduce the water retention capacity of meat, and subsequently aggravate the drip loss (Kaale et al., 2014).

The changes in pH of pork and salmon during superchilling were shown in Fig. S2. In our study, the average pH of pork changed from 5.89 (on Day 0) to 5.38–5.55 (on Day 15), and then gradually increased. The average pH of salmon changed from 6.57 (on Day 0) to 6.31 (on Day 15), and then gradually increased. This is because in the initial storage period, the glycogen and adenosine triphosphate (ATP) in the meat are decomposed to produce acidic substances (such as lactic acid), which lowers the pH value. With the increase of storage time, the protein in meat is decomposed by the action of microorganisms and produces alkaline substances, which leads to the rise of pH value (Scheffler and Gerrard, 2007). After Day 23, there was a significant difference in pH between the control group and the constant temperature group (P<0.05). It was implied that irregular temperature changes accelerated the spoilage of meat, which was manifested as an accelerated pH value. In addition, it was speculated that pH value of the salmon control group first increased and then decreased from Day 23 to Day 30. This was due to more pronounced spoilage, the denaturation of myofibril proteins, and the release of hydrogen ions, which led to a pH drop in the salmon control group towards the end of storage (Zhang and Ertbjerg, 2019).

Color is an important quality indicator of fresh meat, which directly affects the consumer acceptance. Lightness value (L^*), redness value (a^*), and yellowness value (b^*) are three important parameters for evaluating color (Holman et al., 2016). Sarcoplasmic protein solubility declines with decreasing pH, which contributes to a paler pork color. In addition, a slight water loss can enhance the lightness of pork. The proper coloration of salmon is dependent on the contents of astaxanthin and cantaxanthin. The flesh color should be deep red/orange and can be evenly distributed along the salmon fillet. The altered physical state of salmon myofibrils can affect the light scattering properties and lead to color changes (Erikson and

Misimi, 2008). The changes of meat color in pork and salmon during superchilling were shown in Fig. S3. The L^* value of pork fluctuated from 36.89 to 47.03, the a^* value changed from 4.20 to 6.89, and the b^* value increased from 12.13 to 17.85. In the case of salmon, the L^* value fluctuated from 33.28 to 45.24, while the a^* value fluctuated from 13.45 to 18.62, and the b^* value rose from 16.51 to 22.64. With the passing of storage time, the color changes of pork and salmon were not clear, and the differences among the groups were large. It was speculated that temperature changes under superchilling storage had no significant effect on the color of fresh meat, which is in agreement with previous research (Rosenvold and Wiklund, 2011).

2.3 Effects of temperature fluctuations on the myofibril protein changes

During meat storage, proteins in the muscle become denatured, expose the hydrophobic inner core, and increase the surface hydrophobicity. The binding efficiency of bromophenol blue (BPB) and protein hydrophobic residues reflects the hydrophobicity of meat surface protein (Li et al., 2019). The changes of BPB bound in pork and salmon during superchilling were shown in Fig. S4. The average surface hydrophobicity of pork and salmon in the constant temperature group gradually increased from Day 0 (32.26 and 44.44 μg, respectively) to Day 30 (79.79 and 94.57 μg, respectively). The constant temperature group was significantly different from the control group on Day 15 (P< 0.05), and it was also significantly different from the ±2.0 ℃ fluctuation group on Day 23 (P<0.05). Greater surface hydrophobicity means a higher degree of protein denaturation. The results confirmed that irregular temperature changes and ±2.0 ℃ temperature fluctuations accelerated the protein degradation in pork and salmon.

During the superchilling storage of muscle, the structural integrity of myofibrils can be destroyed and myofibrils can break into small fragments (consisting of several sarcomeres), which is measured by the myofibril fragmentation index (MFI). The changes of MFI in pork and salmon during superchilling were shown in Fig. S5. The average MFI of pork and salmon in the constant temperature group gradually increased from Day 0 (50.73 and 67.97, respectively) to Day 30 (83.81 and 101.63, respectively). The constant temperature group was significantly different from the control group on Day 15 (P<0.05), and it was also significantly different from the ±2.0 ℃ fluctuation group on Day 23 (P<0.05). During the post-mortem storage of meat, myosin and actin degradations are important reasons for the increase of MFI (Qian et al., 2020; Xiong et al., 2020). Our results confirmed that irregular temperature changes and ±2.0 ℃ temperature fluctuations accelerated the myofibrillar protein degradation towards the end of storage, which was consistent with the results of BPB bound.

2.4 Evaluation of the structural variation of muscle

The microstructure of cross-section of pork and salmon muscles was visualized by light microscopy (LM) and scanning electron microscopy (SEM) during superchilling storage (Figs. S6 and 5). The images of fresh samples on Day 0 showed that the muscle cells were uniform in size, rigid in structure, and the intercellular gaps were small and uniform. After 15 d of storage, the intercellular gaps slightly increased in different groups. After 30 d of storage, the intercellular gaps in the pork and salmon increased significantly, while they were still relatively tight in the constant temperature group. In comparison, the irregular temperature changes and ±2.0 ℃ temperature fluctuations had a greater degree of compression deformation, such as scattered, twisted, and even partially broken myofibrils, which further accelerated the spoilage of meat.

During superchilling storage, ice crystals firstly form between the muscle cells. With the increase of storage time, the gaps between myofibrils gradually enlarge, and the muscle fibers break down, which makes the tissue structure of muscle sparser. The formation of intracellular and extracellular ice crystals damages the integrity of muscle tissue and promotes microstructural deterioration (Wu et al., 2014). At the same time, the degradation of myofibril protein (including cytoskeletal proteins) during the enzymatic reaction may lead to the destruction of cytoskeleton and the removal of structural connections between muscle fibers (Syamaladevi et al., 2012). This phenomenon further leads to the formation of wider water loss channels, which could be seen from the muscle microstructure. This result was consistent with the results of myofibril protein changes discussed in Section 2.3.

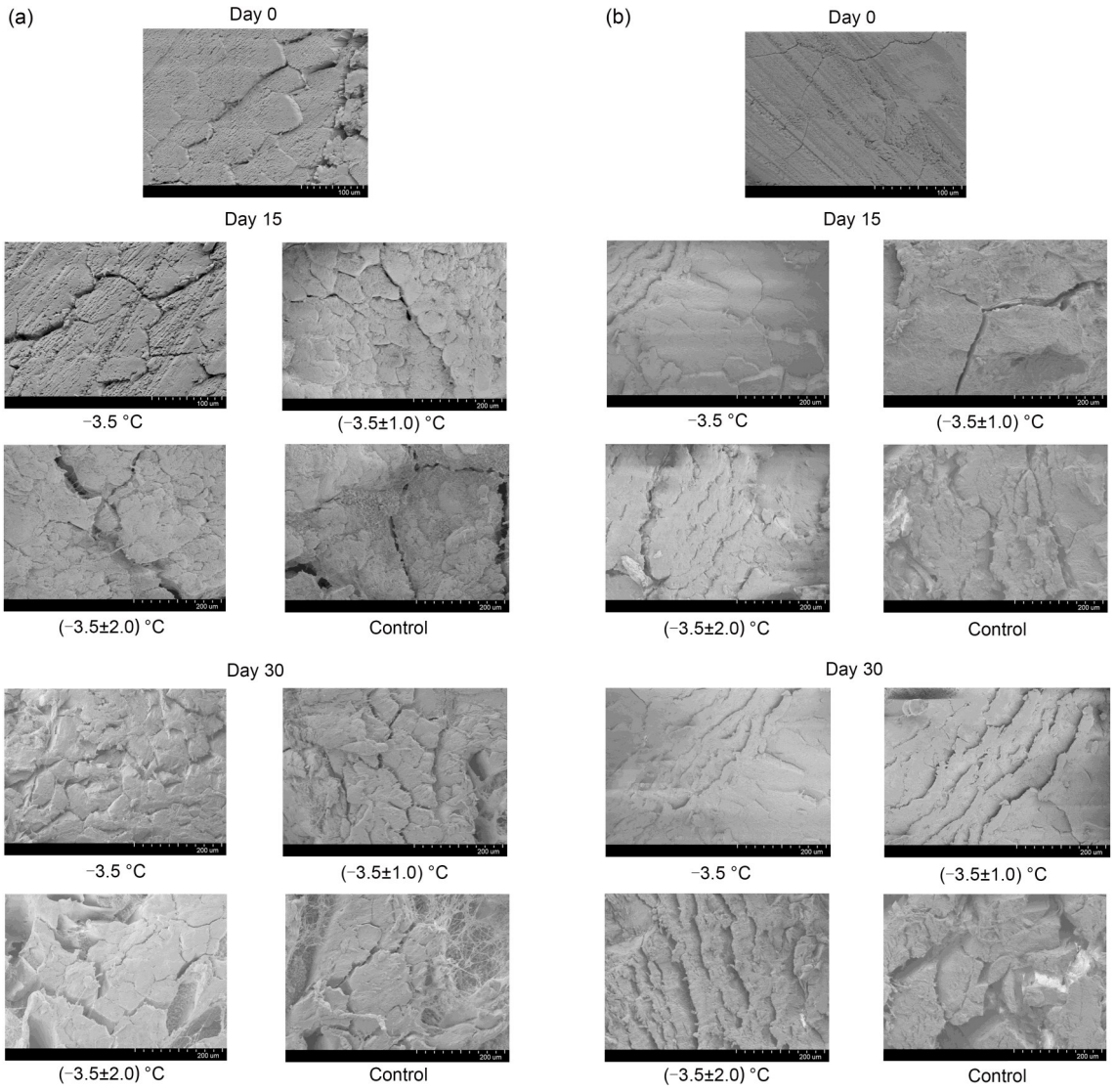

Fig. 5 Microstructures of meat samples stored at −3.5 ℃, (−3.5±1.0) ℃, (−3.5±2.0) ℃, and the control after 0, 15, and 30 d observed using scanning electron microscopy (SEM). (a) Microstructural changes in pork; (b) Microstructural changes in salmon.

3 Conclusions

In summary, this study uncovered the impact of temperature fluctuations and irregular temperature changes on the quality deviation of pork and salmon during superchilling storage. Among the studied groups, the irregular temperature change and ±2.0 ℃ temperature fluctuation groups exhibited significant increases of TVB-N, TVC, and TBARS than the other indicators after 30 d of superchilling storage. Further investigation showed that temperature changes around the freezing point of meat affected the formation of ice crystals, which accelerated the degradation and denaturation of myofibrillar proteins and thereby impaired the structural integrity of the muscle. Furthermore, the number of water loss channels of muscles also increased and they gradually widened with the increase of storage time. These deformations accelerated the spoilage of meat. Overall, the results highlighted the importance of knowledge on the "proper uses of superchilling temperature for storing meat products, especially pork and salmon."

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Acknowledgments

This work was supported by the Starry Night Science Fund of Zhejiang University Shanghai Institute for Advanced Study (No. SN-ZJU-SIAS-0013) and the National Key Technology R&D Program of China (No. 2016YFD0401201).

Author contributions

Haoxin CUI: conceptualization, investigation, methodology, formal analysis, writing-original draft, writing-review & editing. Naymul KARIM: methodology, writing-review & editing. Feng JIANG: methodology, investigation. Haimei HU: methodology, investigation. Wei CHEN: conceptualization, supervision, resources, funding acquisition, writing-review & editing. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Haoxin CUI, Naymul KARIM, Feng JIANG, Haimei HU, and Wei CHEN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Supplementary information

Table S1; Figs. S1–S6; Materials and methods