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**Development of temperature control algorithm for supercooling storage of pork loin and its feasibility for improving freshness and extending shelf life**

Abstract (within 250 words)

Supercooling storage refers to lowering the product temperature below its freezing point without phase transition and has the potential to extend shelf life. Nevertheless, supercooled objects are in a thermodynamically unstable state, and nucleation can occur spontaneously. To achieve supercooling storage, slow cooling and insulation are essential. Hence, a stepwise algorithm for the supercooling storage of pork loins was designed and validated in this study. Pork loins were stored at 3°C, -18°C, and -3°C (freezing), and supercooled for 16 days. All samples remained in a supercooled state and were unfrozen at the end of storage. Supercooled pork loins were superior in terms of drip loss, cooking loss, and water-holding capacity compared to frozen samples. Additionally, supercooling treatment prevented discoloration, increase of volatile basic nitrogen, and microbial growth. Thus, supercooling of pork loin was achieved using a stepwise program and was effective at maintaining meat quality.

**Keywords:** pork loin, freshness, stepwise algorithm, supercooling storage

## Introduction

Meat has been recognized as a food source that provides essential human nutrients such as proteins (Hwang and Hong, 2020). However, the meat quality is degraded during distribution and storage, often due to microbial activity (You et al., 2020). Thus, lowering the storage temperature is necessary to extend the acceptable shelf life by reducing the rates of reactions that lead to deterioration, including microbial spoilage, lipid oxidation, and proteolysis (Pomponio and Ruiz-Carrascal, 2017; Stonehouse and Evans, 2015). Refrigeration is an effective method to delay food degradation, but this storage method can only extend the shelf life for a few days (Kang et al., 2020). Freezing is considered as an efficient storage method to preserve the freshness of meat. However, quality degradation of meat occurs due to ice crystals that are generated during the freezing process (Yun et al. 2021), resulting in the collapse of cells in the food leading to drip loss.

Supercooling treatment is a novel technique used to replace the freezing storage method, which is defined as lowering the temperature of a product below its freezing point without phase transition (Stonehouse and Evans, 2015). Although storage at deep freezing temperatures can extend the shelf life of pork loins, quality loss is inevitable due to the phase transition of the freezing process (Hong et al., 2009). Furthermore, the costs of frozen storage and thawing are additional concerns (Kang et al., 2020). Thus, supercooling storage has potential value for extending shelf life without structural damage and denaturation induced by ice crystal formation during the freezing process as well as satisfy the demands of customers, who regard foods stored at the refrigerated condition are fresher than frozen foods (Park et al., 2021a; Stonehouse and Evans, 2015). However, a supercooled product is theoretically unstable, and nucleation can occur spontaneously (Bédécarrats et al., 2010; Fukuma et al., 2012). Fukuma et al. (2012) detailed that vibration and temperature fluctuations can induce nucleation in supercooled foods.

Owing to the limitations in the achievement and maintenance of supercooling, a limited number of studies have been conducted on the supercooling storage of meats. Mok et al. (2017) combined pulsed electric fields and oscillating magnetic fields for supercooling storage of a chicken breast and extended the supercooled state for 12 h at  $-6.5^{\circ}\text{C}$ , despite that conventionally cooled samples are frozen at this temperature. Without specific instruments for supercooling foods, Fukuma et al. (2012) reached supercooled state of fish using only temperature control. The authors employed stepwise cooling to achieve supercooling storage for 10 mm-thick fish slices (from yellow tail, flatfish, and red sea bream) in incubators. The set temperature was reduced by  $0.5^{\circ}\text{C}$  or  $1.0^{\circ}\text{C}$  per day and the fish slices were unfrozen in the range of  $-8.0^{\circ}\text{C}$  to  $-3.5^{\circ}\text{C}$  depending on the fish types and conditions, delaying the loss of texture. Furthermore, Park et al. (2021b) and Kim et al. (2021) reported that mackerel and hair tail preserved in supercooled storage using a stepwise algorithm maintained a 100% supercooled state. That research exploited stepwise algorithms that reduced temperature from  $-1.0^{\circ}\text{C}$  to  $-2.0^{\circ}\text{C}$  by  $0.5^{\circ}\text{C}$  every 18 h and repeated this cycle, considering the freezing point of fish. The researchers speculated that the kinetic energy was insufficient for ice crystallization because of the very slow rate of cooling, and that small molecules in the muscles may be able to depress the freezing point. Nevertheless, research on the supercooling of foods were mainly focused on the achievement and maintenance of supercooled state of foods and few studies have been conducted to for supercooling storage and the effects on the physicochemical properties of supercooled meat.

Therefore, this study was investigated to feasibility of supercooling storage of pork loins. A stepwise algorithm was applied for the temperature control method to accomplish supercooling storage of pork loins without any specific instruments such as electromagnetic fields. This algorithm was designed for reducing the cooling rate, achieving a lower storage temperature, inhibiting ice nucleation by repeating cycle, and consequently extending the

shelf life of pork loins. After cooling treatments, pork loins were analyzed to physicochemical properties and microbial analysis for the investigation on quality of supercooled pork loin, compared to those of conventional refrigerated or frozen pork loins.

## **Materials and Methods**

### **Sample preparation**

Pork loins were purchased at 48 h post-mortem from a local butcher shop, cut into 30 × 30 × 30 (width × length × height) mm cubes. Total 48 samples were covered with kitchen wrap twice. To record the change in temperature in the samples, T-type thermocouples were applied to the surface of the samples, and the surface temperature of the samples was recorded every 1 min during cooling. A data logger (Data Acquisition-MX 100, Yokogawa, Tokyo, Japan) was used to confirm the temperature of the samples was appropriate and ascertain whether the sample was frozen or not. Fresh pork loin was used as the control.

### **Temperature treatments**

Each twelve samples were subjected to four types of cooling treatments. Each 6 samples were placed into an expanded polystyrene box (20 mm thickness) for physicochemical analysis after 8 and 16 days. Refrigeration treatment (3°C) was conducted in a refrigerator (R-F875HBSW, LG Electronics, Seoul, Korea) that was set at 3°C. Supercooling treatment was conducted in a refrigerator (K417SS13, LG Electronics, Seoul, Korea) controlled with a 4-day cycle stepwise algorithm. Initially, the temperature was set to -1°C and reduced by 0.5°C every 0.5 day four times. After reaching -3°C, the temperature was maintained at -3°C for 2 days. This algorithm was repeated every 4 days. Freezing treatments were performed at -3°C and -18°C. Freezing treatment was conducted using refrigerators (K417SS13) set to -3°C and in a refrigerator (R-F875HBSW) set to -18°C to compare the effect of the

temperature between the supercooling and freezing treatments. The boxes were unpacked every eight days for physicochemical analysis. Frozen samples were thawed at 3°C for 0.75 days prior to analysis.

## **Quality characteristics**

### **Drip loss**

Fresh pork loin and treated pork loin cubes with the exudate removed using dry tissues were weighed. Drip loss was determined by calculating the difference in the weight of the fresh and treated samples and expressed with the following formula:

$$\text{Drip loss (\%)} = \frac{\text{Weight of fresh sample (g)} - \text{Weight of thawed sample (g)}}{\text{Weight of fresh sample (g)}} \times 100$$

### **Cooking loss**

The samples were vacuum-sealed in plastic bags and heated in a water bath (BF-30SB, BioFree, Seoul, Korea) at 70°C for 30 min. The heated samples were cooled to ambient temperature for 30 min. Samples were weighed before and after cooking. The cooking loss for the pork loin samples was expressed using the following formula:

$$\text{Cooking loss (\%)} = \frac{\text{Weight of fresh sample (g)} - \text{Weight of sample after cooking (g)}}{\text{Weight of fresh sample (g)}} \times 100$$

### **Water holding capacity**

One gram of each sample was wrapped with absorbent cotton and placed in a centrifuge tube. After centrifugation using a centrifuge separator (1736R, Labogene, Seoul, Korea) at 3,000×g for 10 min at 4°C, the samples were weighed. The water holding capacity (WHC) of

the pork loin samples was expressed as the ratio of the sample weight after centrifugation to the initial sample weight using the following formula:

$$\text{Water holding capacity (\%)} = \frac{\text{sample weight after centrifugation (g)}}{\text{sample weight before centrifugation (g)}} \times 100$$

### **Shear force**

Samples for which the cooking losses were measured were cut into 10 × 20 × 10 (width × length × height) mm cuboid shape. The shear force was measured using a texture analyzer (CT3 texture analyzer, Brookfield, Middlebrow, MA, USA), and the parameters were set as follows: compression type, TA22 probe, TA/SBA fixture, test speed 2.5 mm/s, trigger load 5 g, and target value 10 mm.

### **Color**

Color parameters for each sample were determined using a colorimeter (CR-400 Chroma Meter, Konica Minolta Sensing Inc., Osaka, Japan). The lightness (CIE L\*), redness (CIE a\*), and yellowness (CIE b\*) values of the samples were measured after calibration with a white standard plate (CIE L\* = 96.79, CIE a\* = +0.30, CIE b\* = +1.67). The total color difference (ΔE) was calculated using the following equation for the difference between the fresh and thawed samples.

$$\Delta E = \sqrt{(\Delta \text{CIE } L^*)^2 + (\Delta \text{CIE } a^*)^2 + (\Delta \text{CIE } b^*)^2}$$

### **Freshness characteristics**

#### **pH**

The pH of the samples was measured using a pH meter (Orion 3 Star, Thermo Scientific, Waltham, MA, USA). Five grams of the sample was homogenized with 45 mL of distilled water using a homo-mixer (CNHR 26, Bosch, Ljubljana, Slovenia) for 40 s.

### **Volatile basic nitrogen**

The volatile basic nitrogen (VBN) content of the pork loin samples was determined according to Conway's micro-diffusion method (Kim et al., 2020). Five grams of the sample was homogenized with 45 mL of distilled water using a slap-type homogenizer (WS-400, Shanghai Zhisun Equipment, Shanghai, China) for 180 s. The homogenate was filtered using Whatman No.1 filter paper (GE Healthcare Life Science, Sheffield, UK). After filtering, 1 mL of the filtrate was placed in the outer section of a Conway dish with a mixture of 1 mL of 0.01 N H<sub>3</sub>BO<sub>3</sub> and 100 µL of Conway solution (mixture of 0.066% methyl red in ethanol and 0.066% bromocresol green in ethanol) was dropped into the inner section of the dish. Then, 1 mL of 50% K<sub>2</sub>CO<sub>3</sub> was added to the outer section of the dish. The Conway dish was closed and incubated at 37°C for 2 h, then titrated using 0.02 N H<sub>2</sub>SO<sub>4</sub> until the Conway reagent changed to a red color. The VBN content was determined following the addition of 0.02 N H<sub>2</sub>SO<sub>4</sub> to the inner section of the Conway dish. The VBN values were calculated using the following formula:

$$VBN (mg/100 g) = \frac{14.007 \times (a - b) \times f \times 100 \times c}{S}$$

where a is the titration volume for the sample (mL), b is the titration volume for the blank (mL), f is the factor for H<sub>2</sub>SO<sub>4</sub>, S is the weight of the sample (g), and c is the dilution amount.

### **Thiobarbituric acid reactive substances**

The thiobarbituric acid reactive substances (TBARS) in each sample were measured according to the procedure described by Park et al. (2021). Five grams of the sample was homogenized with 45 mL of distilled water for 60 s. The homogenate was filtered using Whatman No.1 filter paper (GE Healthcare Life Science, Sheffield, UK). The filtrate (0.5 mL) was transferred to a test tube and 4.5 mL of TBA solution (0.25 N HCl, 15% trichloroacetic acid, and 0.375% TBA reagent) was added. The tube was heated (95°C for 15 min) in a water bath (BF-30SB), cooled to room temperature, and centrifuged at 3,000×g at 4°C for 10 min. Then, 200 µL of the supernatant was placed in a 96-Well plate (SPL Life Science, Pocheon, Korea) and the absorbance was measured using a spectrophotometer (Multiskan™ GO UV/VIS, Thermo Fisher, Waltham, MA, USA) at 535 nm. The TBA values of the samples are presented as mg malondialdehyde/kg (mg MDA/kg).

### **Total aerobic count**

One gram of sample was collected from the surface of the pork loin sample and homogenized with 9 mL of sterilized 0.85% NaCl solution for 1 min using a slap-type homogenizer (WS-400). The supernatant was diluted by serial dilution and 1 mL of diluted solution was placed on ready-to-use Petrifilm aerobic count plates (3M Petrifilm, 3M, St. Paul, MN, USA) and incubated for 2 days at 37°C in an incubator (SI-600R, Jeio Tech, Seoul, Korea). The sample colonies were counted and were expressed as log colony-forming units per 1 g of sample (log CFU/g) to obtain the total aerobic count (TAC).

### **Statistical analysis**

All results are expressed as mean ± standard deviation (SD). Statistical analysis was performed with ANOVA test using SPSS software (version 24.0; SPSS Institute, Chicago, IL,

USA). Differences were considered significant at  $p < 0.05$ .

## **Results and Discussion**

### **Temperature curves**

For stable supercooling storage, a stepwise cooling algorithm was designed considering three main parameters: 1) minimization of temperature deviation during storage, 2) suitable setting of the lowest temperature at the end of one cycle for the type of food, and 3) returning to the initial temperature of one cycle at the end point (Figure 1). From our previous experiment (Lee, 2020), the fluctuation of temperature was more than  $0.3^{\circ}\text{C}$ , which is difficult to supercool during the storage period. The second parameter was set to the lowest suitable temperature. According to Kim (2021), the lowest temperature of chicken breast adopted for the stepwise cooling algorithm was set at  $-2.5^{\circ}\text{C}$  for 0.75 day (18 h), which can preserve the supercooled state without phase transition. Although the samples maintained a supercooled state at the lowest temperature owing to the small temperature fluctuations, the supercooled state was thermodynamically unstable. Therefore, to prevent unexpected cancellation of supercooled conditions, it is necessary to limit the exposure time of food to the lowest temperature and return to the initial temperature at the start of the second cycle.

The temperature curves for the refrigeration, supercooling, and freezing treatments of pork loin samples are shown in Figure 2. Refrigeration treatment was conducted at  $3^{\circ}\text{C}$ , and all pork loin samples reached  $3^{\circ}\text{C}$  after 0.08 day (2 h) of storage (Figure 2A). Supercooling treatment was conducted by employing the stepwise algorithm (Figure 2B), and the mean target temperature during storage was about  $-2.4^{\circ}\text{C}$ , which was below the initial freezing temperature of  $-0.6^{\circ}\text{C}$  for the pork loin samples (Kim and Hong, 2016). Thus, in this study, the stepwise algorithm was regarded as supercooling for pork loin. The temperatures of the pork loin samples were slowly cooled in stages and maintained at  $-3^{\circ}\text{C}$  for 2 days (48 h).

Then, the target temperature was raised to  $-1^{\circ}\text{C}$  and the stepwise algorithm was repeated. While repeating the program, the sample temperatures followed the target temperature below  $0.5^{\circ}\text{C}$ . In 6-8th day, the sample temperatures were maintained in the range of  $-3.5^{\circ}\text{C}$  to  $-3.2^{\circ}\text{C}$  without phase transition. Ten of twelve samples retained the supercooled state for up to 16 days of storage, and no ice was detected on the surface of the supercooled pork loin samples at the end of storage. The center temperatures of the pork loin samples frozen at  $-3^{\circ}\text{C}$  reached the target temperature after 0.10 day (2.5 h) and generally remained within the range of  $-3.5^{\circ}\text{C}$  to  $-3.0^{\circ}\text{C}$  (Figure 2C). The sample temperature reached  $-18^{\circ}\text{C}$  after 0.29 day (7 h) in a refrigerator set to  $-18^{\circ}\text{C}$  (Figure 2D). These samples were stored at  $-18^{\circ}\text{C}$  to  $-19^{\circ}\text{C}$  for 16 days.

## **Quality characteristics**

### **Drip loss**

The drip loss for pork loin samples subjected to the various treatments and storage periods are presented in Table 1. Drip not only affects meat quality, such as weight and nutrition, but also plays a role as a good substrate for microbial growth (den Hertog-Meischke et al., 1997). Refrigerated pork loin samples showed approximately 10.5% drip loss, regardless of the storage period. The drip loss of supercooled pork loin sample increased during storage. After 8 days of storage, the supercooled pork loin sample had the lowest drip loss ( $p<0.05$ ) at 8.24%, which increased to 9.44% after 16 days of storage. The two freezing treatments exhibited different patterns. There was no significant difference between pork loin samples frozen at  $-18^{\circ}\text{C}$  and  $-3^{\circ}\text{C}$  ( $p>0.05$ ) after 8 days of storage. The drip loss on day 16 for samples frozen at  $-3^{\circ}\text{C}$  and  $-18^{\circ}\text{C}$  was 10.49% and 6.74%, respectively.

Extrinsic factors such as freezing and thawing processes induce a larger amount of drip from meat due to ice crystallization, leading to rupture of the fiber membrane (Benjakul and

Visessanguan, 2010; Lambert et al., 2001; Turan et al., 2003). Many studies have analyzed factors affecting thawing loss, such as post-mortem aging, frozen storage, and thawing conditions (Añón and Calvelo, 1980; Kondratowicz et al., 2006; Kondratowicz et al., 2008). Thus, quickly lowering the temperature of meat can avoid various metabolic processes related to drip loss (Huff-Lonergan and Sosnicki 2002), and low-temperature storage of meat is necessary to reduce the overall quality loss after slaughter. Duun and Rustad (2008) observed that pork roasts stored at -2°C had less drip loss than samples stored at 3.5°C and -36°C. Similarly, supercooled chicken breast had lower drip loss compared to refrigerated and partially frozen samples after 12 h of storage (Mok et al., 2017).

In this study, supercooling treatment was regarded as superior for the reduction of drip loss compared to conventional refrigeration and freezing, as the drip loss was no higher after 16-day storage. Consequently, it was concluded that supercooling and freezing at lower temperatures are advantageous for drip loss in meat.

## **Cooking loss**

The cooking losses for pork loin samples subjected to various treatments and storage periods are presented in Table 1. Cooking loss is the weight loss of the released liquid and the hydrophilic content during cooking (Aaslyng et al., 2003). The cooking loss for fresh pork loin samples was approximately 30.9% and significantly increased with storage ( $p < 0.05$ ), ranging from 35.5% to 38.0%. There was no marked difference in cooking losses for pork loin samples after eight days of storage, regardless of the cooling treatment ( $p > 0.05$ ). On the 16th day, the cooking losses for pork loin samples did not increase, regardless of treatment, compared to the losses after eight days ( $p > 0.05$ ). However, the cooking loss of supercooled pork loin sample was 35.5% which was the lowest value among the treatment groups and although the difference was small.

Cooking meat leads to protein denaturation which deprives the protein structure of entrapped water due to the loss of capillary forces (Aaslyng et al., 2003). Vieira et al. (2009) mentioned that chemically bound water in meat is released by melting fat and denaturing proteins during cooking. Several studies report that the moisture loss caused by cooking is not vastly different between unfrozen meat and frozen and thawed meat (Leygonie et al., 2012; Vieira et al., 2009). In contrast, Kim et al. (2015) reported that the cooking loss of frozen/thawed pork was higher than that of fresh pork, regardless of various freezing and thawing processes, due to ice crystallization and recrystallization. There have been irreconcilable reports regarding cooking loss in meat stored under refrigeration. Straadt et al. (2007), observed an increase in the tendency of cooking loss for pork stored at 4°C for four days, with a constant loss after this period for up to 14 days due to the disintegration of damaged fibers during aging. Furthermore, Lu et al. (2019) found higher cooking loss in *M. longissimus lumborum* muscles from Chinese yellow cattle stored via superchilling at -4°C than in those stored under conventional refrigeration (2°C) and freezing (-18°C). They attributed this result to severely damaged cell membranes during superchilling storage. It was noted that supercooling treatment for unfrozen pork loin samples was advantageous for cooking loss within 16 days of storage.

### **Water holding capacity**

The WHC values of pork loin samples subjected to various treatments and storage periods are presented in Table 1. WHC is the ability of the muscle to retain water from loss to external forces (Huff-Lonergan and Sosnicki, 2002). The loss of WHC is associated with the destruction of muscle fiber structures and protein denaturation and/or modification, which shrinks myofibrils (Savage et al., 1990; Huff-Lonergan and Sosnicki, 2002). The WHC of fresh pork loin sample was 85.1%. On day 8 of storage, the WHC values for all pork loin

samples decreased. However, there were no significant differences compared to that of the 0 day and other treated samples ( $p>0.05$ ), with WHC above 80%. However, unfrozen (refrigerated and supercooled) and frozen pork loin samples had different WHC after 16 days of storage. The WHC of unfrozen pork loin samples was above 83% and was not markedly different from that of fresh pork loin samples ( $p>0.05$ ). This result is consistent with that obtained by Kristensen and Purslow (2001). The authors reported that WHC of *longissimus* muscle was maintained after 10 days during aging process (stored at 4°C), though the WHC initially increased and decreased. Other research also reported about these phenomena, which showed the WHC of non-frozen meat did not decline (Joo et al., 2008; Miller et al., 2007). Kristensen and Purslow (2001) attributed to the proteolysis of cytoskeletal proteins and weight loss of the muscle joints caused by water loss. In contrast, frozen pork loin samples had significantly lower WHC ( $p<0.05$ ), below 80%, although there was no obvious difference based on the freezing temperature ( $p>0.05$ ). This may be ascribed to the structural changes in muscle proteins induced by ice crystals due to freezing and thawing (Huff-Lonergan and Lonergan, 2005; Straadt et al., 2007). Thus, refrigeration and supercooling treatments were regarded as beneficial for sustaining muscle fiber structures within 16 days based on the different WHC trends between frozen and unfrozen pork loin samples.

### **Shear force**

The shear forces of pork loin samples subjected to various treatments and storage periods are presented in Table 1. Meat tenderness is an important property for consumers (Pomponio et al., 2018). The shear force of fresh pork loin sample was 6,412 g, which was reduced by the different treatments and storage periods. In general, unfrozen pork loin samples had lower shear force. After eight days of storage, unfrozen samples had shear forces under 4,500 g, whereas those of frozen samples were approximately 4,800 g. There were no significant

differences among the treatment groups ( $p>0.05$ ). On the 16th day, the shear force of refrigerated pork loin samples was approximately 4,300 g and that of supercooled samples was approximately 3,900 g, which was the lowest value obtained.

A decrease in the shear force of pork under refrigeration is consistently reported due to initial tenderization within the first week after slaughter (Juárez et al., 2009; Juárez et al., 2011; Rees et al. 2002) followed by a steady state (Xiong et al., 2006). This decrease in shear force may have been caused by the degradation of muscle fibers and major myofibrillar proteins by enzymatic proteolysis related to calpains and cathepsins (Huff-Lonergan et al., 2010). The calpain system is a group of several proteins.  $\mu$ - and m-calpains are associated with the proteolysis of myofibrillar proteins, which are calcium dependent and activated during autolysis (Goll et al., 2003; Huff-Lonergan et al., 2010; Pomponio et al., 2008). Cathepsins are peptidases that exist in lysosomes and tenderize meat after leakage through the disintegration of lysosome membranes caused by accumulated lactic acid (Ertbjerg et al., 1999). Thus, temperature is a major factor in physiological proteolysis and tenderization (Huff-Lonergan et al., 2010; Pomponio and Ertbjerg, 2012). Pomponio et al. (2018) also observed a decreasing tendency in the Warner-Blatzer shear force of pork loins under refrigeration and superchilling storage ( $-1^{\circ}\text{C}$ ). Conversely, freezing treatment led to an increase in the shear force, which was above 5,000 g. The pork loin frozen at  $-3^{\circ}\text{C}$  had a shear force of approximately 5,500 g, which was the highest value compared to that of the 0 day. Consequently, it was concluded that supercooling treatment resulted in the tenderization of pork loin samples.

## **Color**

The colors of pork loin samples subjected to various treatments and storage periods are presented in Table 2. The CIE  $L^*$  value of fresh pork loin sample was 43.96 and the storage

temperature significantly affected the CIE  $L^*$  values. The CIE  $L^*$  of refrigerated pork loin sample increased to above 55 after 16 days of storage. However, the supercooling treatment maintained the CIE  $L^*$  value of pork loin sample on the 16th day. The CIE  $a^*$  value of fresh pork loin samples was 6.64 and the refrigerated pork loin samples showed a decrease in the CIE  $a^*$  value to 5.83 after eight days of storage, and eventually to 5.55 ( $p < 0.05$ ). In contrast, supercooling and freezing treatments did not lead to a change in the CIE  $a^*$  of pork loin samples during the storage period ( $p > 0.05$ ). The CIE  $b^*$  values were significantly increased by the different treatments and storage periods ( $p < 0.05$ ). The CIE  $b^*$  of fresh pork loin samples was 2.98 and increased to 4.95-5.78 after eight days of storage. However, the CIE  $b^*$  value did not change substantially within the 16 days of storage in all treatment groups. Similarly, the total color difference ( $\Delta E$ ) of pork loin samples also varied. After eight days of storage, there were no significant differences among the treatment groups ( $p > 0.05$ ). However, after 16 days of storage, the total color difference for refrigerated pork loin samples increased to 11.75, which was the highest value ( $p < 0.05$ ).

Color is considered an index of meat quality, and bright red is regarded as an indicator of freshness by consumers (Mancini and Hunt, 2005; Ye et al., 2020). Martin et al. (2013) indicated that a higher discoloration rate was induced by a higher storage temperature for meat and the color change was alleviated at sub-zero temperatures (Gill and McGinnis, 1995). Hence, lower storage temperatures enhance the color stability of meat during storage (Buys et al. 1993). This was attributed to the higher oxymyoglobin content and lower metmyoglobin content in supercooled steak than in those stored at 5°C, indicating that supercooled meat is redder than those stored under refrigeration conditions (Jeremiah and Gibson, 2001). These findings demonstrate that refrigeration results in discoloration of pork loin samples after 16 days of storage, resulting in the highest CIE  $L^*$  and the lowest CIE  $a^*$  and CIE  $b^*$  values. Lu et al. (2019) also reported that superchilled storage led to less change in the color of beef steak

than in refrigerated samples. Accordingly, supercooling induced stability in the change color of pork loin samples at a similar level of freezing without phase transition. It was concluded that supercooling could maintain an acceptable quality for pork loins with higher storage temperatures compared to freezing for 16 days of storage.

## **Freshness characteristics**

### **pH**

The pH values of pork loin samples subjected to various treatments and storage periods are presented in Table 3. The pH of meat affects physicochemical properties such as WHC and tenderness (Kauffman et al., 1961). The pH of fresh pork loin sample was 5.64 and varied by treatment and storage periods. Overall, unfrozen meat had a lower pH than the frozen samples. After eight days of storage, the pH values for refrigerated and supercooled pork loin samples were approximately 5.6, whereas those for frozen meats were 5.68 and 5.87, depending on the freezing temperature. After 16 days of storage, the pH values of the samples increased by approximately 0.1 units except for pork loin samples frozen at -18°C, which decreased to 5.78.

According to James (1972), the pH of fresh meat ranges from 5.5 to 5.8 and that of putrid meat is above 8.0. At slaughter, the pH of meat is approximately 7.2 and initially decreases due to the depletion of ATP and anaerobic glycolysis (Ko and Yang, 2001; Scherer et al., 2005). Numerous studies have demonstrated an increase in the pH of meat due to the production of basic substances including ammonia and amines by microbes and enzymes (Deymer and Vandekerckhove, 1979; Holly et al., 1994; Kim et al., 2004; Muela et al., 2010). Choi et al. (2002) also reported an increase in the pH of pork loins stored at 4°C, ranging from 5.61 to 5.81. Vacuum packaging prevented a significant pH shift in pork during refrigeration storage (Kim et al., 1998; Seong et al., 2009). Lu et al. (2019) also indicated that

superchilling (storage at  $-4^{\circ}\text{C}$ ) limited the increase in the pH of meat within 12 weeks. Ding et al. (2020) reported slightly different trends in the pH of superchilled pork loins depending on the storage temperature. The pH of pork loins stored at  $-1^{\circ}\text{C}$  gradually increased, whereas those of samples stored at  $-3$  and  $-2^{\circ}\text{C}$  decreased and rebounded during 1 month of superchilling storage, ascribed to the disintegration of buffer proteins and the release of hydrogen ions resulting from formation of ice crystals.

In this study, the pH of supercooled pork loin sample was maintained during the 16 days of storage ( $p>0.05$ ) and was comparable to that of the fresh state, which is consistent with the results obtained by Seong et al. (2009). This result may be attributed to the relatively air-tight packaging method used in this study. In contrast, the pH values of frozen samples significantly increased depending on the storage period ( $p<0.05$ ). The results suggested that the supercooling treatment was helpful for retaining the original pH and related reactions.

### **Volatile basic nitrogen**

The VBN values of pork loin samples subjected to various treatments and storage periods are presented in Table 3. The VBN of fresh pork loin samples was 5.84 mg/100 g, and that of pork loin samples stored by refrigeration was the highest among the treatment groups ( $p<0.05$ ). After 8 days of storage, the VBN content of refrigerated pork loin sample was 6.67 mg/100 g. On the 16th day, the VBN level increased to 6.84 mg/100 g, although the difference was not significant ( $p>0.05$ ). The VBN content also increased in the other samples but was not significantly ( $p>0.05$ ) higher than that of fresh meat. Regardless of the cooling treatment and storage period, all VBN values were approximately 6.10 mg/100 g.

VBN comprises ammonia and amines from disintegrated proteins in meat and is regarded as a freshness indicator for meat products. These nitrogen-containing substances are generated by the proteolytic activities of enzymes and bacteria (Ding et al., 2020; He et al.,

2013). Thus, low temperature storage of meat is essential to reduce the amount of VBN by inhibiting the metabolic reactions associated with VBN compound generation (Choi et al., 1998; Lee and Jang, 2013). Ding et al. (2020) reported that superchilled pork loin stored at -3°C and -2°C had lower VBN than that stored at -1°C. Lower VBN content was also reported in superchilled Chinese yellow cattle than in refrigerated samples, though frozen cattle meat had the lowest VBN over 24 weeks (Lu et al., 2019). Superchilled grass carp fillet (Liu et al., 2013) and rabbit meat (Lan et al., 2016) had insignificant results for VBN. Therefore, supercooling treatment was superior in terms of VBN due to its lower value compared to refrigeration and no difference compared to freezing treatment, without undergoing freezing itself.

#### **Thiobarbituric acid reactive substances**

The TBARS values of pork loin samples subjected to various treatments and storage periods are presented in Table 3. The TBARS amount present in the fresh pork loin sample was 0.1927 mg MDA/kg, and all TBARS values were under 0.2500 mg MDA/kg. After 8 days of storage, the amount of TBARS in the refrigerated pork loin sample was 0.2380 mg MDA/kg and that in other samples was approximately 0.2100 mg MDA/kg, with no significant difference among treatments ( $p>0.05$ ). After 16 days of storage, the TBARS values decreased, showing 0.1751-0.1898 mg MDA/kg depending on the treatment. Among the samples, the supercooled pork loin sample had the lowest TBARS content ( $p>0.05$ ).

Lipid oxidation is a time- and temperature-dependent procedure associated with a rancid odor, discoloration, and carcinogenesis (Amaral et al., 2018; Domínguez et al., 2019). Thus, lipid oxidation of meat can be restrained by low temperature storage, similar to VBN. There have been studies on the effectiveness of refrigeration on lipid oxidation in pork and the results indicated that TBARS values increase with the longer storage periods (Choi et al.,

1998; Kim et al., 2004; Seong et al., 2010). Superchilling was also performed to lower the TBARS of meats. Pomponio and Ruiz-Carrascal (2017) reported that superchilled pork loins had approximately 0.2 mg MDA/kg of TBARS after up to 12 weeks of storage. Ding et al. (2020) asserted that ice crystals formed by superchilling reduced TBARS in pork loins, and a lower storage temperature resulted in lower TBARS levels. Lu et al. (2019) observed a higher TBARS value in beefsteak than in frozen samples and ascribed these findings to damage in the cells during superchilling storage and leakage of prooxidants through the damaged regions.

In this study, all pork loin samples had trace amounts of TBARS, and a rancid odor induced by lipid oxidation was not detected after storage. It was concluded that the low-temperature treatment of pork loin samples limited lipid oxidation. Furthermore, the initially low lipid content of the pork loin samples could be the reason for the low TBARS values throughout the storage period. Consequently, low-temperature treatment is effective for restricting lipid oxidation, and supercooling storage results in lipid oxidation stability in pork loin.

#### **Total aerobic count**

The TAC for pork loin samples subjected to various treatments and storage periods is presented in Table 3. The TAC of the fresh pork loin sample was 3.8 log CFU/g, similar to the value obtained by Choi et al. (1998), who reported that fresh pork loin had 2-4 log CFU/g of TAC. After 16 days of storage, the TAC of refrigerated pork loin sample was 6.1 log CFU/g, which was the highest value ( $p>0.05$ ). Refrigeration induced a large amount of microbial growth in pork loin samples due to the higher storage temperature and the TAC exceeded 6 log CFU/g, which meets the criteria for putridity (Egan and Shay, 1982). The TAC for both supercooled and frozen pork loin samples at  $-18^{\circ}\text{C}$  was 3.3 log CFU/g and that

of samples frozen at -3°C was 3.6 log CFU/g, showing a considerable reduction compared to refrigerated samples.

Microbial activity is a primary factor related to the shelf life of meat and is affected by temperature (Hong et al., 2012; Lu et al., 2019). Microorganisms decompose macromolecules such as proteins into smaller substances, including ammonia, amines, mercaptan, and hydrogen sulfide, which are the origins of the off-odor (Choi et al., 2002; Nychas and Arkoudelos, 1990; Ordonez et al., 1991). Microbial spoilage also results in discoloration and/or slime production (Reid et al., 2017). Thus, a low storage temperature is indispensable for controlling microbial counts in meat (Ye et al., 2020). Bellés et al. (2017) also reported that superchilled (-1°C) lamb had a lower TAC than samples refrigerated at 4°C. Ye et al. (2020) recommended storing pork at -3°C to inhibit microbial growth.

In this study, the overall pattern of TAC depending on treatment was similar to the trend for VBN. Refrigeration induced a large amount of microbial growth in pork loins due to the higher storage temperature and the TAC exceeded 6 log CFU/g, which meets the criteria for putridity (Egan and Shay, 1982; Nottingham et al., 1982). Concomitant with this criterion, refrigerated pork loins had strong off-odors at the end point of storage. Freezing was effective to inhibit the microbial spoilage of pork loins during the storage period. Compared to other treatments, supercooling also prevented an increase in the TAC of pork loins, with similar TAC values as control and frozen samples without freezing. Consequently, it was concluded that supercooling can be a profitable storage treatment method for pork loins without phase transition, as it suppresses microbial spoilage during 16 days of storage.

## **Conclusion**

A stepwise algorithm was designed to achieve supercooling storage of pork loins. Using this algorithm, pork loin samples successfully remained in a supercooled state for 16 days,

and the meat quality was maintained. Supercooled pork loin samples showed superior drip loss and similar levels of cooking loss compared to the other treatment groups. Unfrozen pork loin samples had a higher WHC than frozen samples, with no marked change compared to fresh meat. Supercooled samples had pH values similar to those of fresh meat, whereas frozen samples showed a significant increase. The shear force for refrigerated and supercooled meats decreased during the initial storage stage and remained constant until the end of storage. Supercooling treatment also inhibited discoloration during the storage period, resulting in total color difference values similar to those of frozen samples without freezing. The pattern for VBN is analogous to that of the total color. Importantly, supercooling treatment prevented microbial growth for up to 16 days. In contrast, although refrigerated pork loin samples had a higher WHC, they presented discoloration and the highest VBN values. These samples met the criteria for putridity and were regarded as inedible. Freezing generally maintained meat quality and prevented an increase in the TAC. However, freezing and thawing reduced the WHC of pork loin samples. This may be attributed to mechanical damage to the membranes from the ice crystals formed during the phase transition. Consequently, supercooling storage was effective for maintaining the freshness of pork loins for up to 16 days.

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## **References**

Aaslynga MD, Bejerholma C, Ertbjergb P, Bertramc HC. 2003. Cooking loss and juiciness of

523 pork in relation to raw meat quality and cooking procedure. *Food Qual Prefer* 14:277-288.

524 Amaral AB, da Silva MV, da Lannes SCS. 2018. Lipid oxidation in meat: Mechanisms and  
 525 protective factors-A review. *Food Sci Technol* 38:1-15.

526 Añón MC, Calvelo A. 1980. Freezing rate effects on the drip loss of frozen beef. *Meat Sci*  
 527 4(1):1-14.

528 Bédécarrats JP, David T, Castaing-Lasvignottes J. 2010. Ice slurry production using  
 529 supercooling phenomenon. *Int J Refrig* 33:196-204.

530 Bellés M, Alonso V, Roncalés P, Beltrán JA. 2017. The combined effects of superchilling  
 531 and packaging on the shelf life of lamb. *Meat Sci* 133:126-132.

532 Benjakul S, Visessanguan W. 2010. Impacts of freezing and frozen storage on quality  
 533 changes of seafoods. In *Physicochemical aspects of food engineering and processing*. S.  
 534 Devahastin, CRC Press. pp 283-306. Boca Raton, FL, USA.

535 Buys EM, Nortje GL, Steyn PL. 1993. The effect of wholesale vacuum and 100% CO<sub>2</sub>  
 536 storage on the subsequent microbiological, color and acceptability attributes of PVC  
 537 overwrapped pork loin chops. *Food Res Int* 26:421-429.

538 Choi YI, Cho HG, Kim IS. 1998. A study on the physicochemical and storage characteristics  
 539 and freshness of lamb meat. *Korean J Food Sci Anim* 40(1):959-969.

540 Choi YS, Park BY, Lee SK, Kim IS, Kim BC. 2002. Composition and physico-chemical  
 541 properties of vacuum packaged Korean pork loins for export during cold storage. *Korean J*  
 542 *Food Sci Ani Resour* 22:151-157.

543 den Hertog-Meischke MJA, van Laack RJLM, Smulders FJM. 1997. The water-holding  
 544 capacity of fresh meat. *Vet Q* 19(4):175-181.

545 Deymer DI, Vandekerckhove P. 1979. Compounds determining pH in dry sausage. *Meat Sci*  
 546 34:351-362.

547 Ding D, Zhou C, Ge X, Ye K, Wang P, Bai Y, Zhou G. 2020. The effect of different degrees

548 of superchilling on shelf life and quality of pork during storage. J Food Process Preserv  
549 44(4):e14394.

550 Domínguez R, Pateiro M, Gagaoua M, Barba FJ, Zhang W, Lorenzo JM. 2019. A  
551 comprehensive review on lipid oxidation in meat and meat products. Antioxidants 8:429.

552 Duun AS, Rustad T. 2008. Quality of superchilled vacuum packed Atlantic salmon (*Salmo*  
553 *salar*) fillets stored at -1.4°C and -3.6°C. Food Chem 106:122-131.

554 Egan AF, Shay BJ. 1982. Significance of Lactobacilli and film permeability in the spoilage of  
555 vacuum-packaged beef. J Food Sci 47(4):1119-1122.

556 Erthjerg P, Henckel P, Karlsson A, Larsen LM, Møller AJ. 1999. Combined effect of  
557 epinephrine and exercise on calpain/calpastatin and cathepsin B and L activity in porcine  
558 *longissimus* muscle. J Anim Sci 77(9):2428-2436.

559 Fukuma Y, Yamane A, Itoh T, Tsukamasa Y, Ando M. 2012. Application of supercooling to  
560 long-term storage of fish meat. Fish Sci 78:451-461.

561 Gill C, McGinnis J. 1995. The effects of residual oxygen concentration and temperature on  
562 the degradation of the colour of beef packaged under oxygen depleted atmospheres. Meat Sci  
563 39(3):387-394.

564 Goll DE, Thompson VF, Li H, Wei W, Cong J. 2003. The calpain system. Physiol Rev  
565 83(3):731-801.

566 He X, Liu R, Nirasawa S, Zheng D, Liu H. 2013. Effect of high voltage electrostatic field  
567 treatment on thawing characteristics and post-thawing quality of frozen pork tenderloin meat.  
568 J Food Eng 115:245-250.

569 Holly RA, Gariepy C, Delaquis P, Doyon G, Gagnon. 1994. Static controlled (CO<sub>2</sub>)  
570 atmosphere packaging retail pork. J Food Sci 59:1296-1301.

571 Hong GP, Shim KB, Choi MJ, Min SG. 2009. Effects of air blast thawing combined with  
572 infrared radiation on physical properties of pork. Food Sci Ani Resour 29(3):302-309.

573 Hong H, Luo Y, Zhu S, Shen H. 2012. Application of the general stability index method to  
 574 predict quality deterioration in bighead carp (*Aristichthys nobilis*) heads during storage at  
 575 different temperatures. *J Food Eng* 113:554-558.

576 Huff-Lonergan E, Lonergan SM. 2005. Mechanisms of water-holding capacity of meat: The  
 577 role of postmortem biochemical and structural changes. *Meat Sci* 71:194-204.

578 Huff-Lonergan E, Sosnicki A. 2002. Water holding capacity of fresh meat. Fact Sheet, 4669.

579 Huff-Lonergan E, Zhang W, Lonergan SM. 2010. Biochemistry of postmortem muscle-  
 580 Lessons on mechanisms of meat tenderization. *Meat Sci* 86:184-195.

581 Hwang SI, Hong GP. 2020 Effect of high pressure in combination with the type of aging on  
 582 the eating quality and biochemical changes in pork loin. *Meat Sci* 162:10828.

583 James MJ. 1972. Mechanical and detection of microbial spoilage in meats at low temperature.  
 584 *J Milk Food Technol* 35:467-471.

585 Jeremiah LE, Gibson LL. 2001. The influence of storage temperature and storage time on  
 586 colour stability, retail properties and case-life of retail-ready beef. *Food Res Int* 34(9):815-  
 587 826.

588 Joo ST, Kauffman RG, van Laack RLJM, Lee S, Kim BC. 2008. Variations in rate of water  
 589 loss as related to different types of post-rigor porcine musculature during storage. *J Food Sci*  
 590 64(5):865–868.

591 Juárez M, Caine WR, Dugan MER, Hidirolou N, Larsen IL, Uttaro B, Aalhus JL. 2011.  
 592 Effects of dry-ageing on pork quality characteristics in different genotypes. *Meat Sci*  
 593 88(1):117-121.

594 Juárez M, Caine WR, Larsen IL, Robertson WM, Dugan MER, Aalhus JL, 2009. Enhancing  
 595 pork loin quality attributes through genotype, chilling method and ageing time. *Meat Sci*  
 596 83(3):447-453.

597 Kang T, You Y, Jun S. 2020. Supercooling preservation technology in food and biological  
 598 samples: a review focused on electric and magnetic field applications. Food Sci Biotechnol  
 599 29(3):303-321.

600 Kauffman RG, Carpenter ZI, Bray RW, Hockstra WG. 1961. pH of chilled aged and cooked  
 601 pork as related to quality. J Anim Sci 20:918-918.

602 Kim EJ. 2021. Development of novel low temperature preservation technology to extend  
 603 freshness and wholesomeness of foods. Ph. D. thesis, Konkuk Univ. Seoul, Korea.

604 Kim EJ, Lee SY, Park DH, Kim H, Choi MJ. 2020. Physicochemical properties of pork neck  
 605 and chicken leg meat under various freezing temperatures in a deep freezer. Food Sci Anim  
 606 Resour 40(3):444-460.

607 Kim IS, Min JS, Lee SO, Shin DK, Lee JI, Lee M. 1998. Physicochemical and sensory  
 608 characteristics of domestic vacuum packaged pork loins for export during chilled storage.  
 609 Korean J Ani Sci 40:401-412.

610 Kim JJ, Park DH, Kim EJ, Choi MJ. 2021. Effect of supercooling controlled by step-cooling  
 611 algorithm on freshness of hairtail (*Trichiurus lepturus*). Food Eng Prog 25(3):255-263.

612 Kim KI, Shim JB, Yoo SM, Min SG, Lee SY, Jo YJ, Choi MJ. 2015. Effects of various  
 613 freezing and thawing techniques on pork quality in ready-to-eat meals. Afr J Food Sci  
 614 9(11):525-533.

615 Kim SK, Lee MS, Lee KT, Park SK, Song KB. 2004. Changes in quality of pork and beef  
 616 during storage and electronic nose analysis. Korean J Food Preserv 11:441-447.

617 Kim Y, Hong GP. 2016. Effect of artificial supercooling followed by slow freezing on the  
 618 microstructure and qualities of pork loin. Food Sci Ani Resour 36:650-655.

619 Ko MS, Yang JB. 2001. Effects of wrap and vacuum packaging on shelf life of chilled pork.  
 620 Korean J Food Nutr 14:255-262.

621 Kondratowicz J, Chwastowska-Siwiecka I, Burczyk E. 2008. Technological properties of

pork thawed in the atmospheric air or in the microwave oven as determined during a six-month deep-freeze storage. Anim Sci Papers Rep 26(3):175-181.

Kondratowicz J, Chwastowska-Siwiecka I, Matusevicius P. 2006. Sensory quality of pork and total microbial count depending on deep-freeze storage time and thawing method. Vet Zootech 33(55):43-46.

Kristensen L, Purslow PP. 2001. The effect of ageing on the water-holding capacity of pork: role of cytoskeletal proteins. Meat Sci 58(1):17-23.

Lambert IH, Nielsen JH, Andersen HJ, Ørtenblad N 2001. Cellular model for induction of drip loss in meat. J Agri Food Chem 49:4876-4883.

Lan Y, Shang YB, Song Y, Dong Q. 2016. Changes in the quality of superchilled rabbit meat stored at different temperatures. Meat Sci 117:173-181.

Lee KT, Jang MJ. 2013. Quality changes of pork in relation to packaging conditions during chilled storage in households. Food Sci Anim Resour 33:448-455.

Lee SY. 2020. Feasible strategies to improve freshness and wholesomeness of pork loin during extended storage. Ph. D. thesis, Konkuk Univ. Seoul, Korea.

Leygonie C, Britz TJ, Hoffman LC. 2012. Impact of freezing and thawing on the quality of meat: review. Meat Sci 91:93-98.

Liu D, Liang L, Xia W, Regenstein JM, Zhou P. 2013. Biochemical and physical changes of grass carp (*Ctenopharyngodon idella*) fillets stored at -3 and 0°C. Food Chem 140:105-114.

Lu X, Zhang Y, Zhu L, Luo X, Hopkins DL. 2019. Effect of superchilled storage on shelf life and quality characteristics of *M. longissimus lumborum* from Chinese yellow cattle. Meat Sci 149:79-84.

Mancini RA, Hunt M. 2005. Current research in meat color. Meat Sci 71:100-121.

Martin JN, Brooks JC, Brooks TA, Legako JF, Starkey JD, Jackson SP, Miller MF. 2013. Storage length, storage temperature, and lean formulation influence the shelf-life and stability

647 of traditionally packaged ground beef. *Meat Sci* 95(3):495-502.

648 Miller KD, Ellis M, Bidner B, McKeith FK, Wilson ER. 2007. Porcine Longissimus  
 649 glycolytic potential level effects on growth performance, carcass, and meat quality  
 650 characteristics. *J Muscle Foods* 11:169–181.

651 Mok JH, Her JY, Kang T, Hoptowit R, Jun S. 2017. Effect of pulsed electric field (PEF) and  
 652 oscillating magnetic field (OMF) combination technology on the extension of supercooling  
 653 for chicken breasts. *J Food Eng* 196, 27-35.

654 Muela E, Sañudo C, Campo MM, Medel I, Beltrán JA. 2010. Effect of freezing method and  
 655 frozen storage duration on instrumental quality of lamb throughout display. *Meat Sci* 84: 662-  
 656 669.

657 Nottingham PM. 1982. Microbiology of carcass meat. In *Meat microbiology*. Applied  
 658 Science Publishers Ltd., London. pp. 13.

659 Nychas GJ, Arkoudelos JS. 1990. Microbial and physicochemical changes in minced meats  
 660 under carbon dioxide, nitrogen or air at 3°C. *Int J Food Sci Technol* 25:389.

661 Ordonez JA, de Pablo B, de Castro BP, Senio MA, Sanz B. 1991. Selected chemical and  
 662 microbiological changes in refrigerated pork stored in carbon dioxide and oxygen enriched  
 663 atmosphere. *J Agric Food Chem* 39:668-672.

664 Park DH, Lee SY, Kim EJ, Ji YR, Wi GH, Choi MJ. 2021a. Freshness of deep-frozen  
 665 mackerel and croaker during long-term storage. *Int J Food Prop* 24(1):89-104.

666 Park DH, Lee SY, Lee J, Kim EJ, Jo YJ, Kim H, Choi MJ, Hong GP. 2021b. Stepwise  
 667 cooling mediated feasible supercooling preservation to extend freshness of mackerel fillets.  
 668 *LWT* 152:112389.

669 Pomponio L, Bukh C, Ruiz-Carrascal J. 2018. Proteolysis in pork loins during superchilling  
 670 and regular chilling storage. *Meat Sci* 141:57-65.

671 Pomponio L, Ertbjerg P. 2012. The effect of temperature on the activity of  $\mu$ - and m-calpain  
 672 and calpastatin during post-mortem storage of porcine longissimus muscle. Meat Sci  
 673 91(1):50-55.

674 Pomponio L, Ruiz-Carrascal J. 2017. Oxidative deterioration of pork during superchilling  
 675 storage. J Sci Food Agric 97:5211-5215.

676 Rees MP, Trout GR, Warner RD. 2002. Tenderness, ageing rate and meat quality of pork *M.*  
 677 *longissimus thoracis et lumborum* after accelerated boning. Meat Sci 60(2):113-124.

678 Reid R, Fanning S, Whyte P, Joe K, Lindqvist R, Yu Z, Bolton C. 2017. The microbiology of  
 679 beef carcasses and primals during chilling and commercial storage. Food Microbiol 61:50-57.

680 Savage AW, Warris PD, Jolley PD. 1990. The amount and composition of the proteins in drip  
 681 from stored pig meat. Meat Sci 27:289-303.

682 Scherer R, Augusti PR, Steffens C, Bochi VC, Hecktheuer LH, Lazzari R, Radünz-neto J,  
 683 Pomblum SCG, Emanuelli T. 2005. Effect of slaughter method on postmortem changes of  
 684 grass carp (*Ctenopharyngodon idella*) stored in ice. J Food Sci 70(5):C348-C353.

685 Seong PN, Cho SH, Kim JH, Kang GH, Park BY, Lee JM, Kim DH. 2010. Changes in haem  
 686 pigments, peroxide value, TBARS, free fatty acid contents and fatty acid composition of  
 687 muscles from low fat pork cuts during chilled storage. Food Sci Anim Resour 30(3):427-434.

688 Seong PN, Cho SH, Kim JH, Kim YT, Park BY, Lee JM, Kim DH. 2009. Changes in the  
 689 physicochemical properties of the muscles from low-fat pork cuts during chilled storage.  
 690 Food Sci Anim Resour 29(2):213-219.

691 Stonehouse GG, Evans JA. 2015. The use of supercooling for fresh foods: A review. J Food  
 692 Eng 148:74-79.

693 Straadt IK, Rasmunssen M, Andersen HJ, Bertram HC. 2007. Aging-induced changes in  
 694 microstructure and water distribution in fresh and cooked pork in relation to water-holding  
 695 capacity and cooking loss - A combined confocal laser scanning microscopy (CLSM) and

low-field nuclear magnetic resonance relaxation study. Meat Sci 75:687-695.

Turan H, Kaya Y, Erkoyuncu I. 2003. Effects of glazing, packaging and phosphate treatments on drip loss in rainbow trout (*Oncorhynchus mykiss* W., 1792) during frozen storage. Turk J Fish Aquat Sci 3:105-109.

Vieira C, Diaz MT, Martínez B, García-Cachán MD. 2009. Effect of frozen storage conditions (temperature and length of storage) on microbiological and sensory quality of rustic crossbred beef at different states of ageing. Meat Sci 83(3):398-404.

Xiong YL, Gower MJ, Li C, Elmore CA, Cromwell GL, Lindemann MD. 2006. Effect of dietary ractopamine on tenderness and postmortem protein degradation of pork muscle. Meat Sci 73(4):600-604

Ye K, Ding D, Zhu X, Wu Z, Hu Q, Li R. 2020. Modified atmosphere packaging with a small change in gas ratio could maintain pork quality during -3°C storage. Food Control 109:106943.

You Y, Her J, Shafel T, Kang T, Jun S. 2020. Supercooling preservation on quality of beef steak. J Food Eng 274:109840.

Yun YC, Ramachandraiah K, Hong GP. 2021 Effect of precooling conditions on the ice nucleation temperatures and freezing characteristics of semisolid matrices. J Food Eng 291:110232.

**Table 1.** Changes in the drip loss, cooking loss, WHC, and shear force of pork loins with various treatments and storage periods

Parameters	Treatment	Storage period (days)		
		0 day	8	16
Drip loss (%)	Refrigeration (+3°C)		10.46 ± 1.60 <sup>Aa</sup>	10.54 ± 0.58 <sup>Ax</sup>
	Supercooling		8.24 ± 2.43 <sup>BCb</sup>	9.44 ± 1.16 <sup>ABx</sup>
	Freezing (-3°C)		9.60 ± 1.92 <sup>ABab</sup>	10.49 ± 2.99 <sup>Ax</sup>
	Freezing (-18°C)		9.20 ± 3.12 <sup>ABab</sup>	6.74 ± 0.69 <sup>Cy</sup>
Cooking loss (%)	Refrigeration (+3°C)	30.9 ± 1.9 <sup>C</sup>	36.3 ± 0.7 <sup>AB</sup>	36.6 ± 1.3 <sup>AB</sup>
	Supercooling	30.9 ± 1.9 <sup>C</sup>	36.2 ± 1.6 <sup>AB</sup>	35.5 ± 2.1 <sup>B</sup>
	Freezing (-3°C)	30.9 ± 1.9 <sup>C</sup>	36.8 ± 1.1 <sup>AB</sup>	37.0 ± 2.8 <sup>AB</sup>
	Freezing (-18°C)	30.9 ± 1.9 <sup>C</sup>	37.0 ± 2.1 <sup>AB</sup>	38.0 ± 1.1 <sup>A</sup>
WHC (%)	Refrigeration (+3°C)	85.1 ± 0.9 <sup>A</sup>	82.7 ± 2.7 <sup>AB</sup>	84.2 ± 1.8 <sup>Ax</sup>
	Supercooling	85.1 ± 0.9 <sup>A</sup>	83.7 ± 1.6 <sup>A</sup>	83.1 ± 3.3 <sup>Axy</sup>
	Freezing (-3°C)	85.1 ± 0.9 <sup>A</sup>	80.7 ± 1.4 <sup>AB</sup>	75.7 ± 3.7 <sup>Cz</sup>
	Freezing (-18°C)	85.1 ± 0.9 <sup>A</sup>	81.6 ± 1.9 <sup>AB</sup>	78.3 ± 2.7 <sup>BCyz</sup>
Shear force (g)	Refrigeration (+3°C)	6,412 ± 440 <sup>A</sup>	4,447 ± 816 <sup>BC</sup>	4,291 ± 770 <sup>BCxy</sup>
	Supercooling	6,412 ± 440 <sup>A</sup>	4,237 ± 789 <sup>BC</sup>	3,881 ± 794 <sup>Cy</sup>
	Freezing (-3°C)	6,412 ± 440 <sup>A</sup>	4,829 ± 1,214 <sup>BC</sup>	5,509 ± 1,569 <sup>ABx</sup>
	Freezing (-18°C)	6,412 ± 440 <sup>A</sup>	4,835 ± 1,230 <sup>BC</sup>	5,098 ± 2,132 <sup>ABCxy</sup>

<sup>A-C</sup>Means with different letters within the entire data are significantly different ( $p < 0.05$ ).

<sup>a-b</sup>Means with different letters within the 8-day data are significantly different ( $p < 0.05$ ).

<sup>x-z</sup>Means with different letters within the 16-day data are significantly different ( $p < 0.05$ ).

**WHC, Water holding capacity**

721 **Table 2.** Changes in the color of pork loins with various treatments and storage periods

	Treatment	Color		
		Storage period (days)		
		0 day	8	16
CIE L *	Refrigeration (+3°C)	43.96 ± 0.84 <sup>D</sup>	47.66 ± 2.39 <sup>Ba</sup>	55.43 ± 3.49 <sup>Ax</sup>
	Supercooling	43.96 ± 0.84 <sup>D</sup>	47.78 ± 0.68 <sup>Ba</sup>	46.26 ± 1.12 <sup>BCy</sup>
	Freezing (-3°C)	43.96 ± 0.84 <sup>D</sup>	42.40 ± 2.28 <sup>Db</sup>	44.47 ± 2.08 <sup>CDyz</sup>
	Freezing (-18°C)	43.96 ± 0.84 <sup>D</sup>	43.73 ± 3.48 <sup>Db</sup>	42.87 ± 1.14 <sup>Dz</sup>
CIE a *	Refrigeration (+3°C)	6.64 ± 0.16 <sup>ABC</sup>	5.83 ± 0.35 <sup>CDb</sup>	5.55 ± 1.18 <sup>Dy</sup>
	Supercooling	6.64 ± 0.16 <sup>ABC</sup>	7.49 ± 0.58 <sup>Aa</sup>	7.21 ± 0.26 <sup>ABx</sup>
	Freezing (-3°C)	6.64 ± 0.16 <sup>ABC</sup>	6.63 ± 1.46 <sup>ABCa</sup>	6.40 ± 1.06 <sup>BCDxy</sup>
	Freezing (-18°C)	6.64 ± 0.16 <sup>ABC</sup>	5.93 ± 0.75 <sup>CDb</sup>	6.58 ± 0.48 <sup>ABCx</sup>
CIE b *	Refrigeration (+3°C)	2.98 ± 0.65 <sup>D</sup>	4.95 ± 0.34 <sup>BCb</sup>	4.83 ± 1.12 <sup>Cy</sup>
	Supercooling	2.98 ± 0.65 <sup>D</sup>	5.78 ± 0.25 <sup>Aa</sup>	5.60 ± 0.23 <sup>ABxy</sup>
	Freezing (-3°C)	2.98 ± 0.65 <sup>D</sup>	5.59 ± 0.86 <sup>ABa</sup>	5.76 ± 0.96 <sup>Ax</sup>
	Freezing (-18°C)	2.98 ± 0.65 <sup>D</sup>	5.00 ± 0.36 <sup>BCb</sup>	4.89 ± 0.09 <sup>BCy</sup>
ΔE	Refrigeration (+3°C)		4.43 ± 2.68 <sup>B</sup>	11.75 ± 3.54 <sup>Ax</sup>
	Supercooling		4.86 ± 0.51 <sup>B</sup>	3.62 ± 0.81 <sup>BCy</sup>
	Freezing (-3°C)		4.00 ± 1.00 <sup>BC</sup>	3.60 ± 1.12 <sup>BCy</sup>
	Freezing (-18°C)		3.91 ± 1.01 <sup>BC</sup>	2.42 ± 0.57 <sup>Cy</sup>

722 <sup>A-D</sup>Means with different letters within the same column are significantly different ( $p<0.05$ ).

723 <sup>a-b</sup>Means with different letters within the 8-day data are significantly different ( $p<0.05$ ).

724 <sup>x-z</sup>Means with different letters within the 16-day data are significantly different ( $p<0.05$ ).

**Table 3.** Changes in the pH, VBN, TBARS, and TAC of pork loins with various treatments and storage periods

Parameters	Treatment	Storage period (days)		
		0 day	8	16
pH	Refrigeration (+3°C)	5.64 ± 0.07 <sup>CD</sup>	5.58 ± 0.05 <sup>Dc</sup>	5.66 ± 0.04 <sup>Cy</sup>
	Supercooling	5.64 ± 0.07 <sup>CD</sup>	5.63 ± 0.04 <sup>CDbc</sup>	5.71 ± 0.04 <sup>BCxy</sup>
	Freezing (-3°C)	5.64 ± 0.07 <sup>CD</sup>	5.68 ± 0.05 <sup>Cb</sup>	5.77 ± 0.01 <sup>Bx</sup>
	Freezing (-18°C)	5.64 ± 0.0 <sup>CD</sup>	5.87 ± 0.04 <sup>Aa</sup>	5.78 ± 0.07 <sup>Bx</sup>
VBN (mg/100 g)	Refrigeration (+3°C)	5.85 ± 0.17 <sup>B</sup>	6.67 ± 0.17 <sup>Aa</sup>	6.84 ± 0.17 <sup>Ax</sup>
	Supercooling	5.85 ± 0.17 <sup>B</sup>	6.16 ± 0.20 <sup>Bb</sup>	6.16 ± 0.15 <sup>By</sup>
	Freezing (-3°C)	5.85 ± 0.17 <sup>B</sup>	6.14 ± 0.20 <sup>Bb</sup>	6.15 ± 0.20 <sup>By</sup>
	Freezing (-18°C)	5.85 ± 0.17 <sup>B</sup>	6.11 ± 0.26 <sup>Bb</sup>	6.11 ± 0.26 <sup>By</sup>
TBARS (mg/MDA kg)	Refrigeration (+3°C)	0.193 ± 0.006 <sup>BC</sup>	0.238 ± 0.059 <sup>A</sup>	0.187 ± 0.010 <sup>BCxy</sup>
	Supercooling	0.193 ± 0.006 <sup>BC</sup>	0.222 ± 0.041 <sup>AB</sup>	0.175 ± 0.004 <sup>Cy</sup>
	Freezing (-3°C)	0.193 ± 0.006 <sup>BC</sup>	0.218 ± 0.030 <sup>ABC</sup>	0.183 ± 0.005 <sup>BCxy</sup>
	Freezing (-18°C)	0.193 ± 0.006 <sup>BC</sup>	0.209 ± 0.015 <sup>ABC</sup>	0.190 ± 0.015 <sup>BCx</sup>
TAC (log CFU/g)	Refrigeration (+3°C)	3.80 ± 0.20 <sup>B</sup>		6.08 ± 0.07 <sup>Ax</sup>
	Supercooling	3.80 ± 0.20 <sup>A</sup>		3.28 ± 0.16 <sup>By</sup>
	Freezing (-3°C)	3.80 ± 0.20 <sup>A</sup>		3.59 ± 0.42 <sup>Ay</sup>
	Freezing (-18°C)	3.80 ± 0.20 <sup>A</sup>		3.26 ± 0.10 <sup>By</sup>

<sup>A-D</sup>Means with different letters within the same column are significantly different ( $p < 0.05$ ).

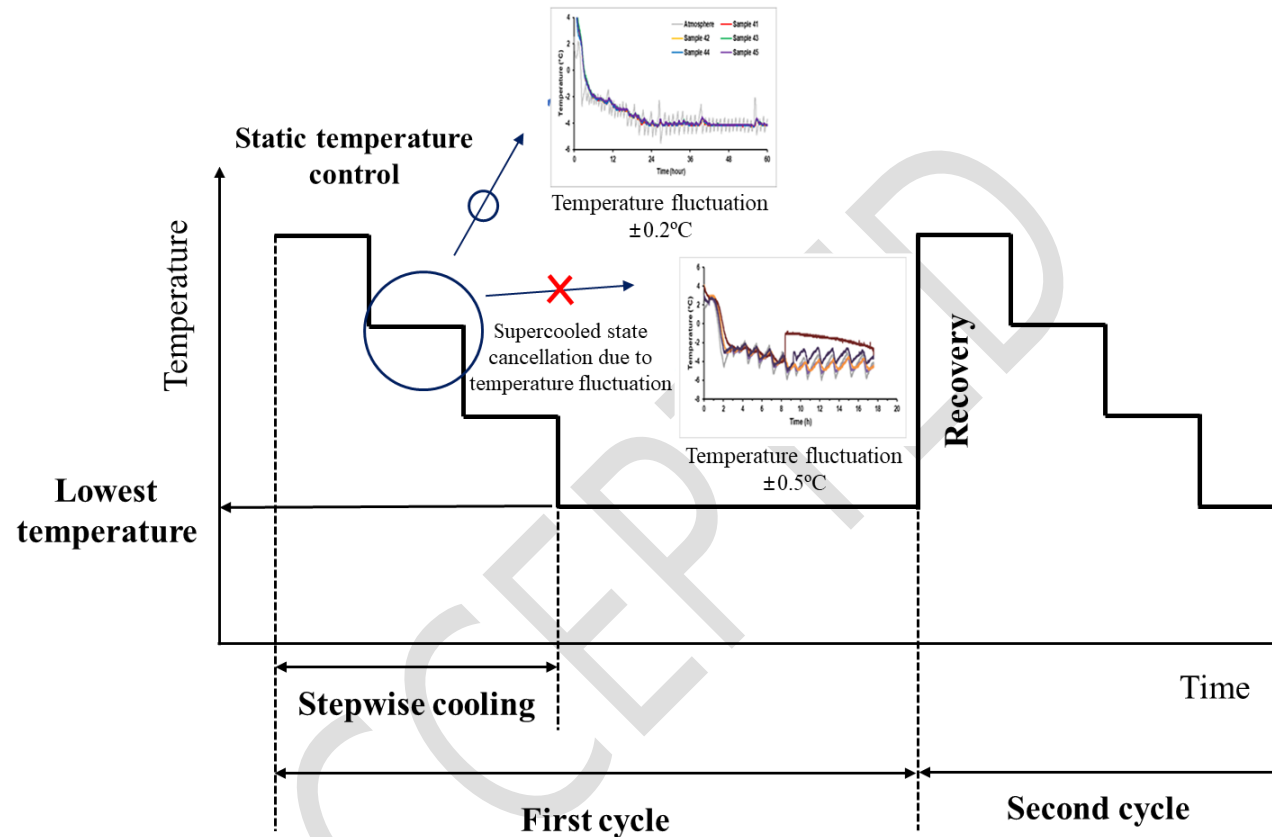
<sup>a-c</sup>Means with different letters within the 8-day data are significantly different ( $p < 0.05$ ).

<sup>x-y</sup>Means with different letters within the 16-day data are significantly different ( $p < 0.05$ ).

VBN, Volatile basic nitrogen

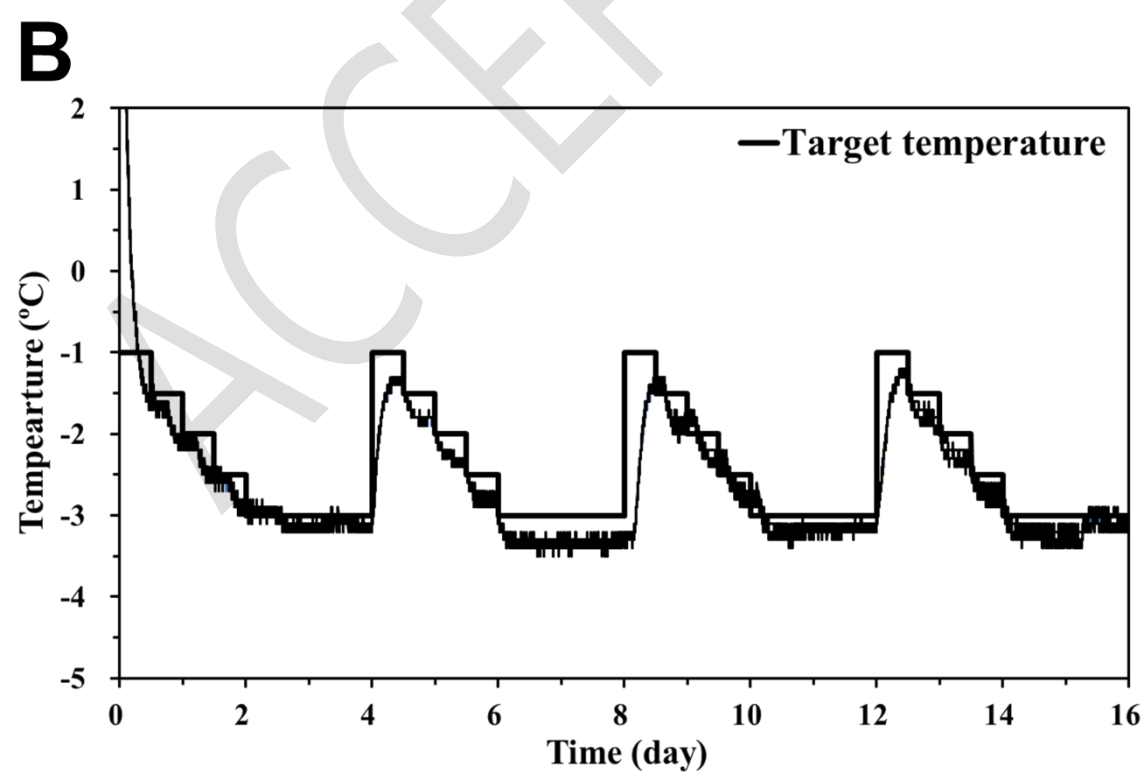
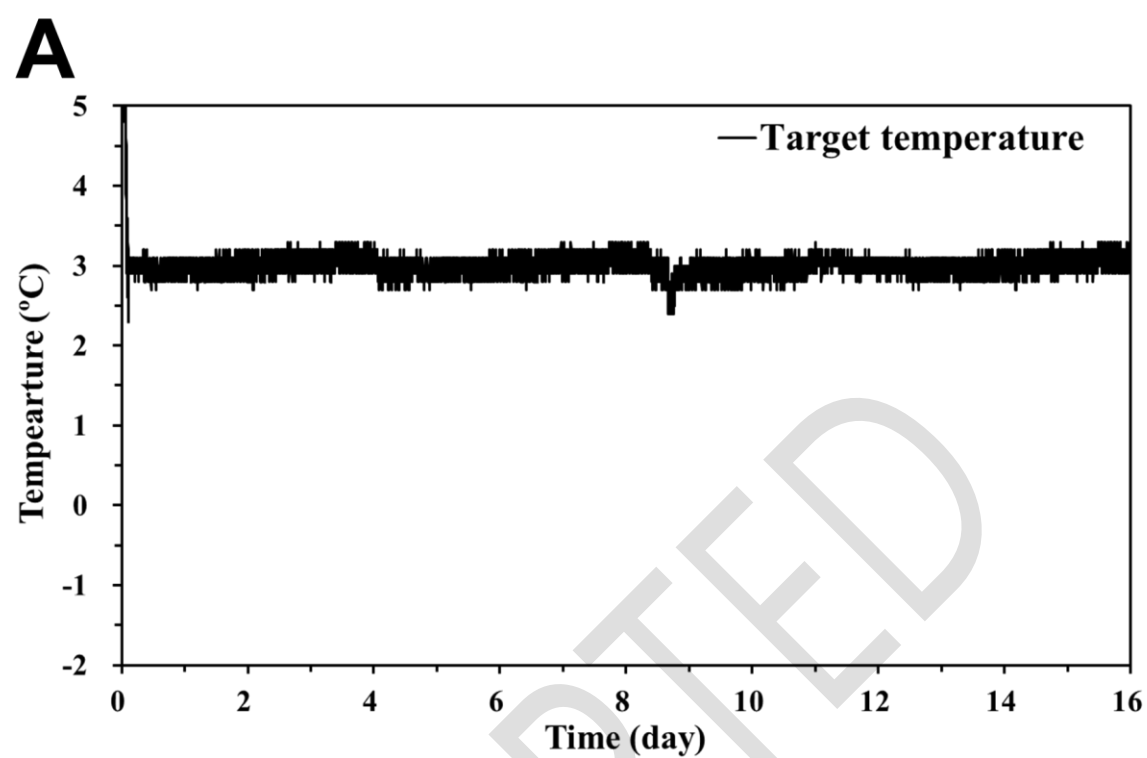
TBARS, Thiobarbituric acid reactive substances

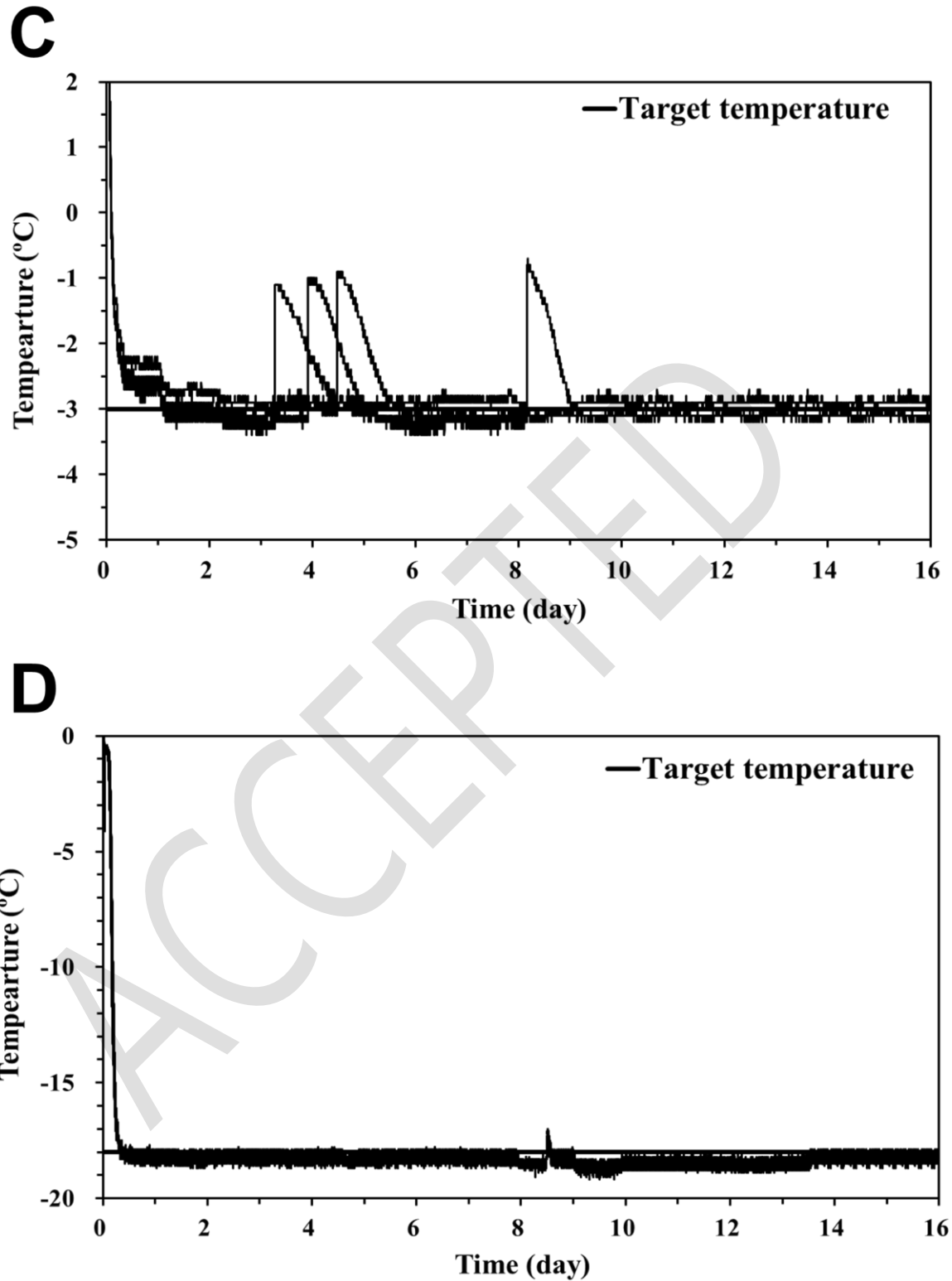
TAC, Total aerobic count



733 **Figure 1.** Concept of supercooling preservation controlled by stepwise cooling process (Kim, 2021).

734 The 3 main factors are consisted of 1) the fluctuation minimization of temperature, 2) setting a suitable the lowest temperature at end of cycle  
 735 according to kind of food, and 3) returning to the initial temperature of one cycle at the end point. The variation of temperature was less than  
 736  $\pm 0.2^\circ\text{C}$ , which caused the stable supercooled state. Temperature fluctuation of more than  $\pm 0.5^\circ\text{C}$ , resulted in the cancellation of the  
 737 supercooled state.





**Figure 2.** Temperature curves of target temperature and pork loins samples under (A) refrigeration, (B) supercooling, (C) freezing (-3°C), and (D) freezing (-18°C) treatments for 16 days.