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Changes in the Quality of Chicken Breast Meat due to Superchilling and Temperature Fluctuations during Storage

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The aim of this study was to determine the changes in chicken breast meat quality (water-holding capacity, color, texture, myofibrillar fragmentation index (MFI), total protein solubility, thiobarbituric acid reactive substances (TBARS), total viable count (TVC), and lactic acid bacteria (LAB) count) due to storage under superchilling conditions (−1.3℃) and fluctuating temperatures (ranging from −20℃ to −5℃) as compared to the quality of meat stored at chilled (2–4℃) and frozen (−20℃) temperatures, respectively. Results indicated that the TVC and LAB count of the chilled and superchilled breast meat increased with storage time. TVC of the chilled and superchilled breast meat reached the safety level of 7 log cfu/g at approximately day 8 and 18, respectively. This suggested that the superchilling method extended the storage duration by 10 days. Weight loss and TBARS of the chilled and superchilled samples tended to increase with increasing storage time. The color, texture, protein solubility, and MFI were stable throughout the entire storage period of the chilled (9 days) and superchilled (28 days) samples. Results indicated that while three cycles of storage temperature fluctuation influenced the weight loss and dry matter of the meat, they did not affect the TVC, LAB count, texture, color, pH, MFI, and protein solubility. The superchilling technique (−1.3℃) could extend the shelf-life of meat and maintain the quality of chicken breast meat. Fluctuations in temperature during frozen storage decreased the water-holding capacity of chicken breast meat, indicating that temperature stability should be maintained during frozen storage.

Key words: chicken breast meat, quality, storage, superchilling, temperature fluctuation, traditional chilling

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Introduction

Storage at a chilled temperature extends the shelf-life of chicken meat by slowing down the growth of microorganisms, reducing the rate of chemical reactions, and decreasing the activity of enzymes (Al-jasser, 2012; Stonehouse and Evans, 2015). Traditional chilling temperatures are usually between 0℃ and 7℃ (Xu *et al*., 2012; Latou *et al*., 2014). However, chilled poultry is still perishable, and its quality is dependent on factors such as the initial microbial load and packaging (Patsias *et al*., 2008; Dawson *et al*., 2013).

Freezing is a safe and reliable technique used to preserve chicken meat that is supplied to the global markets. Freezing allows for a longer shelf-life as compared to chilling; frozen poultry meat stored at −18℃ has a shelf-life of 7–18 months (Taub and Singh, 1998), whereas the shelf-life of chilled poultry meat is approximately 1 week (Jiménez *et al*., 1997). However, the freezing process damages the structural integrity of chicken meat and decreases its ability to retain water, which greatly affects consumer acceptance (Leygonie *et al*., 2012; Gambuteanu *et al*., 2013). Temperature fluctuations can occur at several steps before the frozen meat reaches the consumer, such as during storage and transportation under an unstable freezer temperature, loading, unloading, and retail. Temperature fluctuations of frozen food have

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been observed in the range of −30℃ to −6℃ (Giannakourou, 2016). This is a critical factor, as temperature fluctuations may promote ice recrystallization, causing growth of ice crystals and increased damaging to the structural integrity of the meat. Previous studies have found that increasing the number of freeze-thaw cycles led to greater changes in the TBARS, texture, protein oxidation, color, and water-holding capacity of meat (Xia *et al*., 2009; Ali *et al*., 2015). In these cases, the samples reached core temperatures of 0−4℃, resulting in a significant reduction in the meat quality. Freezing temperature fluctuations may lead to even more significant changes in the core temperatures of chicken breast meat, which can range from −20℃ (normal freezing temperature) to −5℃ (minimum freezing temperature); less is known regarding the effect of temperature fluctuations in this range on the quality of meat.

Consumer preference for chilled poultry meat is much higher than the preference for frozen meat, since the former is perceived to be more fresh, less processed, and more convenient for cooking (Stonehouse and Evans, 2015). Consequently, the price of chilled chicken in the market is higher than the price of frozen chicken, despite the higher energy costs and impact on sustainability of frozen poultry meat. However, the shorter shelf-life of chilled chicken meat reduces its compatibility for shipping or long storage (Patsias *et al*., 2008). Therefore, storage techniques that can retain the quality and extend the shelf-life of fresh chicken meat beyond those offered by the refrigeration technique are needed in the poultry industry.

During superchilling, meat is stored at approximately 1−2℃ below its initial freezing point (Kaale *et al*., 2011; Shen *et al*., 2015), which for chicken breast meat, has been reported to be around −0.4℃ (Marini *et al*., 2014). Indeed, Zhou *et al*. (2010) reported that poultry meat in the USA stored at temperatures above −3.3℃ can be marketed as fresh meat. As compared with traditional chilling, superchilling further reduces microbial growth, and helps maintain the freshness of meat for a longer period. For example, the shelf-life of broiler half-carcasses was extended by more than 16 days under superchilling storage (−2℃) as compared to its shelf-life under traditional storage (4℃) (Zhang *et al*., 2015). Moreover, superchilling could reduce the need for freeze-thaw, thereby resulting in increased production yield and reduced energy consumption, labor, and transportation costs (Kaale *et al*., 2011).

The water present in the product during superchilling storage mainly exists in a super-cooled state, and is only partially frozen (Magnussen *et al*., 2008; Lawrance *et al*., 2010). The size of ice crystals in meat depends on the cooling rate during the superchilling process (Kaale *et al*., 2013). In fact, superchilling may affect the structural integrity of chicken meat via partial ice crystal formation, leading to an increased drip loss during storage. Nevertheless, the extent of changes on the final quality of the chicken meat during superchilling as compared with its quality during traditional chilling or freezing remains unknown.

Several studies have reported the quality of fish under superchilled conditions (Olafsdottir *et al*., 2006; Duun and Rustad, 2007; Shen *et al*., 2015); fewer studies have investigated the quality of chicken meat under superchilled conditions (Zhang *et al*., 2015). To the best of our knowledge, no study has reported the quality of superchilled chicken breast meat, which is the most valuable cut in the chicken and for which the gap between the prices of fresh and frozen meat is likely to be large.

Therefore, this study aimed to establish the extent to which the quality of chicken breast meat (water-holding capacity, color, texture, myofibrillar fragmentation index (MFI), total protein solubility, thiobarbituric acid reactive substances (TBARS), total viable count (TVC), and lactic acid bacteria (LAB) count) is affected by storage under superchilled conditions (−1.3℃) as compared to the quality of chicken breast meat by storage under traditional chilled storage. Additionally, the effect of temperature fluctuation cycles on chicken breast quality during the frozen storage as compared with the quality at constant temperature freezing was evaluated.

Materials and Methods

Experimental Design

Fresh chicken breasts (120) were delivered to the Copenhagen University by HK-Scan Denmark A/S (Vinderup, Denmark) on the same day of slaughtering. Chicken breast meat was immediately vacuum packaged (1 breast per package) and stored according to the experimental design.

Vacuum packaged chicken breast meat (80) were stored either under traditional chilling (TC; 2−4℃) or under superchilling (SC; −1.3±0.1℃) conditions. Control chicken breast meat was used to determine the core temperature of TC and SC groups during the entire storage period via a thermocouple.

Quality attributes of the TC samples were measured on days 1, 4, and 9 of storage; the same attributes were measured on days 4, 9, 15, 21, and 28 in the SC samples (n=10 on each sample day). Weight loss, microbiological parameters (TVC and LAB count), pH, color, texture, and dry matter were analyzed on the sampling day. Samples for TBARS, myofibrillar fragmentation index (MFI), and protein solubility measurements were stored at −80℃ until analysis.

Vacuum packaged chicken breast meat (40) samples were also stored under frozen conditions (−20℃); ten of those were kept under stable frozen conditions (F) during the entire storage period. The rest of the samples were subjected to either 1, 2, or 3 temperature fluctuation cycles (C1, C2, and C3 groups, respectively). For each cycle, frozen chicken breast meat were thawed at 4℃ in a refrigerator until the core temperature reached −5℃. Thereafter, chicken breast meat samples were frozen until their core temperature reached −20℃. The first temperature fluctuation cycle was conducted after 8 days of frozen storage in all the three groups. Only groups C2 and C3 were subjected to the second temperature fluctuation cycle, which was conducted 4 days after the first fluctuation cycle, as shown in Fig. 1. Three vacuum packaged chicken breast meat samples from each storage

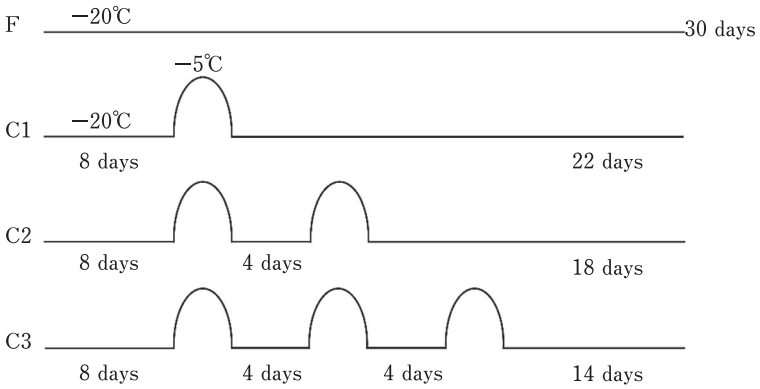

Fig. 1. **Schematic representation of frozen (F) and temperature fluctuation samples for 1 (C1), 2 (C2), and 3 cycles (C3)**

condition were used as controls to determine and record the core temperatures during the freeze-thaw cycles using a thermocouple.

Quality attributes of all the groups (F, C1, C2, and C3) were evaluated at the end of the frozen storage period (30 days). Weight loss, microbiological parameters (TVC and LAB count), pH, color, texture, and dry matter were analyzed. Samples used for TBARS, MFI, and protein solubility measurements were stored at −80℃ until analysis.

Methods

Microbiological Analysis

From each storage condition and time point, 10 g of each sample including the meat surface area was collected under aseptic conditions in triplicates and was mixed with 90 g peptone solution (0.1% (w/v) peptone, 0.9% (w/v) NaCl) in stomacher filter bags. This solution was homogenized for 60 s (Seward Stomacher 400); further decimal dilutions were made in the peptone solution (ISO 6887-1, 1999). TVC was determined using plate count agar (PCA, Thermo Scientific OXOID) (ISO 4833-2, 2013); LAB count was determined using MRS agar (Thermo Scientific OXOID) (ISO 15214, 1998). One set of plates was incubated at 30℃ for 3 days and another set at 4℃ for 14 days.

Texture

Texture of the whole chicken breast was measured using a TA.XT2 Texture Analyser (Stable Micro Systems, Surrey, UK) equipped with a Meullenet-Owens razor shear blade (Meullenet *et al*., 2004) and a 30 kg load cell. Incisions were made on the surface of the chicken breast sample at 5 different locations using a Meullenet-Owens razor shear blade. The crosshead speed, sample shear depth, and trigger force were set at 10 mm/s, 20 mm, and 10 g, respectively. The Meullenet-Owens razor force (MORSF, N) and the maximum shear force were also measured. The Meullenet-Owens razor blade was replaced and recalibrated after every 50 measurements (10 breasts) to prevent dulling of the blade (Meullenet *et al*., 2004; Cavitt *et al*., 2005; Lee *et al*., 2008a).

pH

Sample pH was measured in duplicates using a pH meter (Knick-Portamess, 911 pH, Germany). For measurement, minced chicken breast meat (5 g) was homogenized in 15 mL MilliQ water with a spatula.

Color

The color of chicken breast meat was recorded after 3−5 minutes of blooming at room temperature (25±2℃). Measurements were performed at three locations on the bone side surface of the breast via a Minolta CM600d spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan). Before each series of measurements, the instrument was calibrated using a white ceramic tile. The color of the chicken breast meat was reported based on L* (lightness), a* (redness), b* (yellowness), hue angle (h°), and chroma (C*) values.

Weight Loss

Initial weight of chicken breast samples was recorded. Following storage, samples without being taken out from the package, were blotted with a paper towel and weighed. The percentage of weight loss was calculated using equation 1 (Eq. 1).

Weight loss (%)=(Initial weight−Final weight)/Initial weight×100 (Eq. 1)

Dry Matter

Moisture content was determined by drying the samples (2 g) at 102℃ (AOAC, 2000). Samples were analyzed in duplicates. Dry matter was calculated using equation 2 (Eq. 2).

Dry matter (%)=(Dried sample weight/Raw sample weight)×100 (Eq. 2)

Total Protein Solubility

Protein solubility was determined using the method described by Joo *et al*. (1999). Briefly, 1 g minced meat was homogenized in 20 mL buffer (0.05 M K-phosphate, 0.55 M KI, pH 6.0). Homogenates were stored overnight at 2±2℃ and were then centrifuged at 1,500 *g* for 20 min. Protein concentration in the supernatants was determined by the bicinchoninic acid (BCA) method (Pierce BCA® Protein Assay Kits, Thermo Scientific). Total protein solubility was reported as mg soluble protein per g meat sample.

Myofibrillar Fragmentation Index

Chopped meat (2.5 g in duplicates) was homogenized in 30

mL cold MFI-buffer (100 mM KCl, 20 mM potassium phosphate (pH 7.0), 1 mM EDTA, 1 mM $MgCl_2$) at 20,500 rpm using an Ultra-Turrax homogenizer (Ultra-Turrax T25, IKA, Germany) equipped with a S25N-18 G dispersing element. Myofibril particle size and distribution were measured using a Mastersizer (Malvern, WR14 1AT, Worcestershire, UK), according to the previously described methods of Lametsch *et al*. (2007). The distribution of the myofibrillar fragment sizes was determined as the surface mean diameter D [3,2] (μm).

Thiobarbituric Acid Reactive Substances

Secondary lipid oxidation products were quantified by TBARS analysis, according to Jongberg *et al*. (2013), and were expressed as malondialdehyde (MDA) equivalents. Aliquots of 5.0 g meat were homogenized in 15 mL 7.5% (v/v) trichloro acetic acid with 0.10% (w/v) propylgallate and 0.10% (w/v) ethylenediaminetetraacetic acid (EDTA) with an Ultra Turrax homogenizer for 45 s at 13,500 rpm. The solution was then filtered, mixed with 20 mM thiobarbituric acid (ratio 1:1), and heat treated (40 min, 100℃). Absorbance was measured at 532 and 600 nm at room temperature. Results were represented as the mean of two replicates from the same sample and were expressed as 2-TBARS in mg $MDAkg^{-1}$ sample using a standard curve prepared from malonic dialdehyde-bis (diethylacetal).

Statistical Analysis

Statistical analysis of the experimental results was performed using the Statistical Package for Social Science (SPSS for windows version 17.0, SPSS Inc., Chicago, IL, USA). Two-way ANOVA was used to determine the statistical significance of changes in measured parameters of chicken breast meat due to storage conditions and time; one-way ANOVA was used to determine the effect of fluctuating frozen storage temperature on chicken breast meat. Significant differences among means within each experiment were evaluated using Duncan's multiple range test at a significance level of $\alpha=0.05$.

Results and Discussion

Effect of Superchilling on the Quality of Chicken Breast Meat as Compared with that of Traditional Chilling

Microbiological Parameters

The most important factor that limits the shelf-life of fresh chicken meat is microbial growth during storage (Zhang *et al*., 2015). The microbial counts (TVC and LAB incubated at either 4℃ or 30℃) of chilled and superchilled chicken breast meat were evaluated. The initial number of TVC in raw chicken breast meat at 30℃ was 5.18 log cfu/g, which was higher than the previously reported number (Meredith *et al*., 2014; Pavelková *et al*., 2014); this was likely due to sample transportation, which resulted in delayed sampling. During the 9 days of storage, the TVC and LAB count were affected by both the storage condition (TC and SC) and storage time. In addition, interaction between storage condition and time was observed in TVC and LAB count, with the exception of LAB count under incubation at 30℃. Growth rate of bacteria (both TVC and LAB) was higher in chilled chicken breast meat than in superchilled chicken breast meat and increased with increasing storage time (Figs. 2 and 3). No significant differences in TVC for superchilled samples were noted on days 0, 4, and 9, whereas the growth rate of TVC in the chilled sample rapidly increased after 4 days of storage. These results demonstrated that superchilling induced a marked increase in the lag phase and a decrease in the growth rate of bacteria as compared to the growth rate using the chilling method. LABs are often involved in the spoilage of vacuum-packaged meat (Adams and Moss, 2008), and our results indicated that the superchilling technique also prolongs the lag phase of LAB, similar to the results obtained by Zhang *et al*. (2015).

It has been reported that the TVC limit for edible fresh chicken meat is 7 log cfu/g (ICMSF, 2011). In our study, such levels were reached after 8 days of storage for chilled samples, and after 18 days of storage for superchilled samples. Strong spoilage odors due to the bacterial growth were observed on days 9 and 28 for chilled and superchilled samples, respectively. These observations were consistent with those in the study by Zhang *et al*. (2015), who reported that superchilling prolongs the time required to reach unacceptable odor levels in packaged chicken half-carcasses (day 28 as compared with day 12 using traditional chilling).

The initial counts of both TVC and LAB were lower in the meat when incubated at 4℃ as compared with the counts observed at 30℃, which reflected a primarily mesophilic population at the outset. Interestingly, LAB numbers of superchilled samples incubated at 4℃ showed counts that were lower than 5 log cfu/g throughout the entire storage period. This indicated that true psychrophilic LAB was not a part of the dominant microbiota.

Color and Texture

Texture and color are important quality factors that affect the consumer preference for poultry meat (Fletcher, 2002; Shen *et al*., 2015). During the 9 days of storage, no effect of storage condition and time was observed on the texture of chicken breast meat. The results revealed that the superchilling technique yielded similar meat texture as compared to the texture obtained by the traditional chilling technique. Moreover, shear force values of superchilled chicken breast showed no significant change during storage, except for superchilled samples on day 28, which exhibited significantly higher values, indicating a tougher meat (Table 1). This result was similar to that of a previous study conducted on superchilled fish meat (Bahuaud *et al*., 2008). Overall, our results indicated that superchilling storage for 21 days has no effect on the texture of chicken breast meat. The increase in shear force observed on day 28 of superchilled samples may be associated with high water loss (determined as weight loss in Table 1). High water loss from the meat results in shrunken protein in the meat, which leads to increased packing and decreased meat tenderness (Lee *et al*., 2008b).

No interaction between the storage condition (TC and SC) and time (9 days storage) was found to impact on the color of chicken breast meat. The color of chicken breast meat was

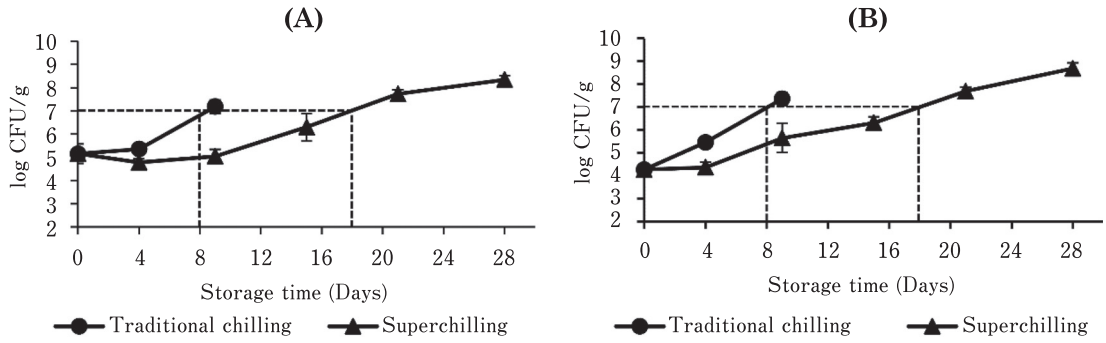

Fig. 2. **Total viable count (log cfu/g) of the chicken breast meat stored under chilling and superchilling conditions upon incubation at 30℃ (A) and 4℃ (B) (*n*＝24 breast meat pieces)**

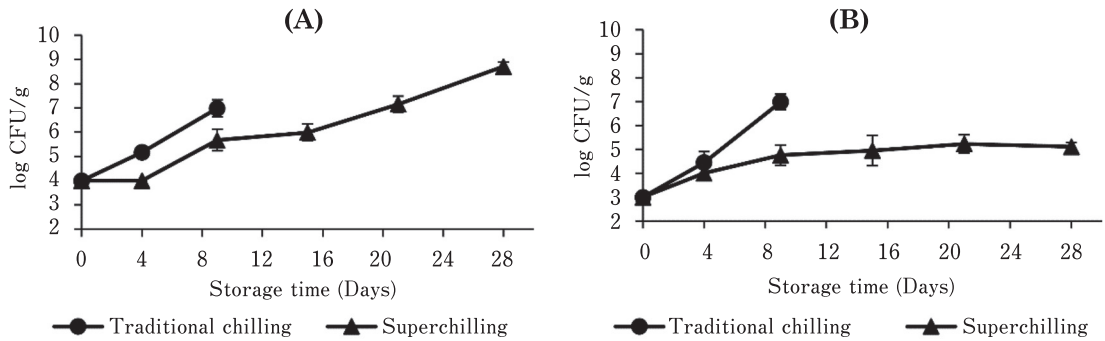

Fig. 3. **Lactic acid bacteria count (log cfu/g) of the chicken breast meat stored under storage under chilling and superchilling conditions upon incubation at 30℃ (A) and 4℃ (B) (*n*＝24 breast meat pieces)**

not affected by storage conditions. We found that the superchilling and traditional chilling techniques maintained the color of the chicken breast meat to a similar degree. Storage times had no influence on the L*, a*, and hue of chicken breast meat, whereas changes in b* and chroma of chicken breast meat were affected by storage time. b* and chroma of superchilled and chilled samples were slightly increased from day 0 to day 4. After day 4, no change was detected in b* and chroma of superchilled samples. Changes in the color of fresh meat under cold storage may be attributed to water loss and lipid oxidation (Shen *et al*., 2015).

Based on the color and texture results in the present study, we have clearly demonstrated that superchilled chicken breast meat exhibits stable color and texture during storage.

pH

The pH of chicken breast meat was not affected by either treatment (TC and SC) or storage time during the 9-day storage. The pH of superchilled chicken breast meat was slightly increased with storage time for 28 days. This could be due to an increase in ammonia and amino acid products following utilization of amino acids by microorganisms (Zhang *et al*., 2016). This result was concomitant with the increased growth rate of TVC in superchilled samples, as shown in Fig. 2.

Weight Loss and Dry Matter

Our study showed that storage condition and time had no effect on dry matter, while weight loss was influenced by storage time. Weight loss of superchilled and chilled samples increased with storage time (Table 1), which was in agreement with the findings of Zhang *et al*. (2015). Weight loss during storage is associated with water loss, which affects both the quality and yield of fresh and cooked meat. Storage temperature and time, and microbiological growth are the main factors that influence the water retention ability of myofibrils in meat during storage under cold conditions (Cheng and Sun, 2008; Zhang *et al*., 2015). Bahuaud *et al*. (2008) reported that myofiber detachment and breakage in superchilled fish fillet (at －1.5℃) due to ice crystal formation were increased with storage time, leading to increased water loss during storage. Interestingly, no difference in weight loss between the chilled and superchilled samples was detected on day 9. Moreover, weight loss of superchilled chicken breast was unchanged from day 9 to day 21. This result indicated that chicken breast meat could be stored

Table 1. **Effects of traditional chilling and superchilling storage conditions on the quality of chicken breast meat**

Parameters	Storage conditions	Storage time (Days)					
		0	4	9	15	21	28
Shear force	TC	8.35±0.36^{b}	8.55±0.70^{b}	8.30±0.41^{b}			
(MORSF, N)	SC	8.35±0.36^{b}	8.83±0.27^{b}	8.39±0.25^{b}	8.87±0.52^{b}	9.68±0.67^{ab}	10.75±0.56^{a}
Color L*	TC	49.85±0.46^{a}	49.87±0.72^{a}	49.78±0.84^{a}			
	SC	49.85±0.46^{a}	50.86±0.91^{a}	49.92±0.39^{a}	50.49±0.75^{a}	50.92±0.89^{a}	51.93±0.70^{a}
a*	TC	−0.26±0.22^{ab}	−0.78±0.18^{b}	−0.16±0.14^{ab}			
	SC	−0.26±0.22^{ab}	−0.22±0.17^{ab}	−0.32±0.26^{ab}	−0.17±0.15^{ab}	0.18±0.22^{a}	−0.06±0.14^{a}
b*	TC	6.13±0.47^{c}	8.73±0.62^{a}	7.93±0.60^{ab}			
	SC	6.13±0.47^{c}	7.30±0.34^{abc}	8.07±0.60^{ab}	7.39±0.52^{abc}	6.61±0.35^{bc}	7.43±0.37^{abc}
Chroma	TC	6.18±0.46^{c}	8.78±0.62^{a}	7.94±0.59^{ab}			
	SC	6.18±0.46^{c}	7.32±0.34^{abc}	8.12±0.59^{ab}	7.41±0.52^{abc}	6.65±0.36^{bc}	7.44±0.38^{abc}
Hue	TC	90.96±1.36^{ab}	95.29±1.27^{a}	91.21±1.03^{ab}			
	SC	90.96±1.36^{ab}	91.38±1.48^{ab}	91.63±1.51^{ab}	91.10±1.48^{ab}	87.35±1.51^{b}	90.64±1.04^{b}
pH	TC	5.96±0.05^{b}	5.97±0.06^{b}	5.99±0.06^{ab}			
	SC	5.96±0.05^{b}	6.09±0.05^{ab}	6.09±0.03^{ab}	6.12±0.02^{a}	6.13±0.02^{a}	6.03±0.03^{ab}
Weight loss (%)	TC	—	2.64±0.23^{c}	3.30±0.38^{bc}			
	SC	—	2.50±0.24^{c}	3.22±0.14^{bc}	3.57±0.30^{b}	3.83±0.15^{b}	6.01±0.47^{a}
Dry matter (%)	TC	24.66±0.31^{b}	24.55±0.36^{b}	25.42±0.33^{ab}			
	SC	24.66±0.31^{b}	24.78±0.22^{ab}	24.98±0.16^{ab}	25.59±0.21^{a}	25.18±0.25^{ab}	25.42±0.18^{ab}
Total protein solu-	TC	160.65±3.26^{bc}	154.05±5.79^{c}	161.63±4.06^{bc}			
bility (mg/g)	SC	160.65±3.26^{bc}	153.25±4.41^{c}	160.67±2.94^{bc}	166.43±3.57^{ab}	159.33±3.03^{bc}	173.11±2.71^{a}
Myofibril particle	TC	20.91±0.95^{a}	22.15±1.14^{a}	22.72±1.22^{a}			
size D [3,2] (μm)	SC	20.91±0.95^{a}	22.92±1.30^{a}	25.36±1.17^{a}	22.78±0.99^{a}	23.35±1.74^{a}	23.40±1.48^{a}

$^{a-c}$ Mean±SE for each parameter under superchilling (SC) and traditional chilling (TC) conditions superscripted with different letters are significantly different ($P<0.05$), $n=80$ breast meat pieces. Classification of color of the meat: L*=lightness, a*=redness, b*=yellowness; MORSF, N=Meullenet-Owens razor force

A two-way ANOVA was applied to study the effects on various meat quality parameters under superchilling (SC) and traditional chilling (TC) conditions for 0–9 days storage ($P<0.05$).

under superchilled condition for a longer duration (21 days) with no significant effect on weight loss. Moreover, James and James (2002) reported that the shelf-life of meat stored at −1.5±0.5℃ could be extended without any surface freezing. We found that dry matter of superchilled samples was slightly increased, which was associated with the increase in water loss with storage time.

Protein Solubility and Myofibrillar Fragmentation Index

Protein solubility property refers to the protein denaturation in meat (Van Laack *et al*., 2000). Protein denaturation of chilled and superchilled chicken breast meat was determined by increase in MFI during storage. Fragmentation of myofibrils is associated with the degree of proteolysis during storage of meat (Lametsch *et al*., 2007). There was no interaction between storage conditions (TC and SC) and time during 9 days of storage when total protein solubility and MFI were examined. The highest protein solubility of superchilled sample was observed on day 28 (Table 1). Moreover, MFI of superchilled chicken breast meat showed no significant change during the entire storage period. The result of protein solubility and MFI demonstrated that traditional chilling at 4℃ for 9 days and superchilling at −1.3℃ for 21 days have minimal effect on proteins present in the chicken breast meat. Previous research reported that protein solubility of superchilled fish decreased with increasing storage time, which indicated an increased degree of protein denaturation in superchilled fish (Duun and Rustad, 2008). In contrast, protein solubility of superchilled chicken breast was constant, which was likely due to lower sensitivity of meat protein to denaturation as compared to the proteins of fish (Mackie, 1993; Zayas, 1997).

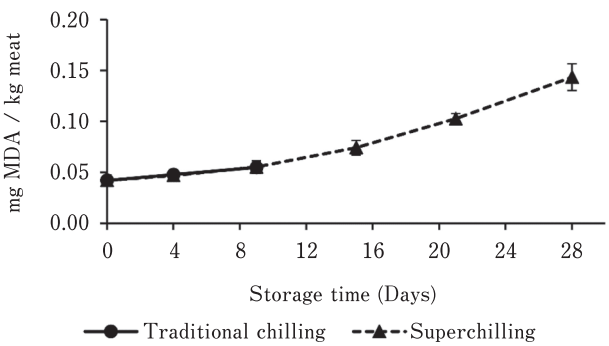

Fig. 4. **Changes in TBARS (mg MDA / kg meat) of the chicken breast meat stored under chilling and superchilling conditions ($n=80$ breast meat pieces)**

Thiobarbituric Acid Reactive Substances

No interaction between storage condition and time was observed in TBARS of chicken breast meat. Increase in TBARS of superchilled chicken breast meat was observed

Table 2. **Effect of the number of bacteria (cfu/g) in frozen chicken breast meat and in chicken breast meat subjected to temperature fluctuation cycles**

Incubation Conditions	Treatments		The number of bacteria (cfu/g)	
			TVC	LAB count
30℃, 3 days	Freeze		5.0×10^{4b}	5.0×10^{4a}
	Temperature fluctuation cycle	C1	5.0×10^{4b}	2.0×10^{4a}
		C2	2.0×10^{5a}	2.4×10^{5a}
		C3	8.6×10^{4ab}	4.0×10^{4a}
4℃, 14 days	Freeze		3.7×10^{4a}	1.0×10^{4a}
	Temperature fluctuation cycle	C1	4.7×10^{4a}	1.0×10^{4a}
		C2	1.6×10^{5a}	2.0×10^{4a}
		C3	1.2×10^{5a}	1.5×10^{4a}

[a–b] Means in a single column representing an incubation condition, with different superscripted letters, are significantly different ($P<0.05$), $n=40$ pieces. TVC=Total viable count; LAB=Lactic acid bacteria; C1, C2, C3=Temperature fluctuation cycles

after 9 days of storage (Fig. 4). The TBARS values of chilled and superchilled samples in the present study were lower than 0.15 mg MDAkg^{-1} sample, which was due to the low fat content in the chicken muscles (Wattanachant *et al*., 2004; Iman Rahayu *et al*., 2008). Additionally, meat samples were vacuum packed, which could slow down lipid oxidation under cold storage conditions (Jouki and Khazaei, 2012).

Effect of Temperature Fluctuation Cycles on the Quality of Chicken Breast Meat

Microbiological Parameters

The effect of temperature fluctuation cycle on the number of TVC and LAB incubated at 30℃ and 4℃ is shown in Table 2. The initial TVC and LAB count of frozen chicken breast meat were 5.0×10^4 cfu/g. TVCs of frozen chicken breast meat samples with and without fluctuating temperature treatment, incubated at 30℃, were slightly altered, whereas TVCs of samples incubated at 4℃ did not change significantly. The TVC in chicken breast meat under fluctuating cycles was less than 7 log cfu/g which did not exceed the limitation of TVC in fresh chicken meat (ICMSF, 2011). Furthermore, temperature fluctuations did not have any significant effect on the LAB count of chicken breast meat. Our results indicated that the three cycles of temperature fluctuations had no effect on the microbial growth of frozen chicken meat. This was likely due to the fact that samples were thawed until −5℃. At this temperature, almost all of the water in the meat should still be in a frozen state (Hsieh *et al*., 2010). Therefore, the states of free water, nutrients, and temperature of breast meat under this condition were not suitable for rapid microbial growth.

Physical and Chemical Characteristics

Based on our results, the texture, pH, and color of chicken breast meat were not affected by temperature fluctuations (Table 3). Furthermore, no change in total protein solubility, MFI, and TBARS of frozen chicken breast meat was detected after three temperature fluctuation cycles. Normally, freeze-thaw cycles of frozen meat, which was thawed until the core temperature of sample reached 0–4℃, affected the texture, color, protein property, lipid oxidation, and water-holding capacity of the meat (Xia *et al*., 2009; Ali *et al*., 2015). The results obtained in this study indicated that temperature fluctuations of breast meat in the range of −20℃ to −5℃ did not affect the structural integrity of the breast muscle until properties such as texture, color, and protein structure changed. However, temperature fluctuations affected the water loss from muscles, resulting in increased weight loss and percentage of dry matter. After two cycles of temperature fluctuation, no effect on weight loss was observed. However, following the third temperature fluctuation, an increased weight loss of frozen chicken breast meat was observed. The increase in water loss could reflect the formation of ice crystals during the conversion of water to ice. Furthermore, ice crystal growth during temperature fluctuations could also have resulted in damage to the muscle tissues (Ngapo *et al*., 1999; James and James, 2002). Since weight loss during storage reduces the total yield of the final product, temperature fluctuations during storage of chicken meat may have direct implications for the poultry industry. The stability of pH was in accordance with the unchanged bacterial count under freezing and fluctuating temperature conditions. Lipid oxidation may have been slowed due to a combination of freezing and vacuum packaging during storage (Śmiecińska *et al*., 2015). This could be attributed to the unchanged TBARS in samples during storage despite temperature fluctuations.

Conclusions

Superchilling markedly decreased bacterial growth, thereby prolonging the period before a TVC of 7 log cfu/g was reached at approximately 18 days which was 10 days longer as compared with traditional chilling technique. This delay was also observed for other measured quality parameters of chicken breast meat. Up to three cycles of temperature fluctuation in the range of −20℃ to −5℃ during the freezing storage of chicken breast meat had no effect on all the quality attributes, with the exception of weight loss. Weight loss of frozen chicken breast meat was increased after samples were

Table 3. **Effects of freezing and number of temperature fluctuation cycles on the quality of chicken breast meat**

Parameters		Freeze	Temperature fluctuation cycles		
			C1	C2	C3
Shear force (MORSF, N)		9.58±0.80[a]	9.08±0.59[a]	9.08±0.56[a]	9.11±0.58[a]
Color	L*	48.62±1.01[a]	48.73±0.78[a]	47.66±0.66[a]	49.35±0.59[a]
	a*	−0.97±0.30[a]	−0.60±0.33[a]	−0.50±0.29[a]	−0.92±0.13[a]
	b*	6.60±0.42[a]	7.28±0.48[a]	6.16±0.37[a]	7.33±0.21[a]
	Chroma	6.71±0.40[a]	7.35±0.49[a]	6.23±0.34[a]	7.40±0.21[a]
	Hue	98.66±2.85[a]	94.23±2.52[a]	92.96±2.49[a]	97.21±1.03[a]
pH		6.13±0.04[a]	6.03±0.04[a]	6.03±0.04[a]	6.03±0.02[a]
Total protein solubility (mg/g)		161.67±5.08[a]	167.15±3.65[a]	171.03±3.26[a]	170.43±5.04[a]
Myofibril particle size D [3,2] (μm)		22.71±1.31[a]	23.38±1.02[a]	21.59±0.85[a]	24.36±0.92[a]
TBARS (mg MDAkg^{-1} meat)		0.044±0.003[a]	0.051±0.006[a]	0.051±0.004[a]	0.055±0.006[a]
Weight loss (%)		3.25±0.37[b]	4.18±0.55[ab]	4.34±0.40[ab]	5.21±0.36[a]
Dry matter (%)		24.15±0.57[b]	24.95±0.30[ab]	25.72±0.23[a]	25.15±0.23[ab]

[a–b] Mean±SE in a single row with different superscripted letters are significantly different ($P<0.05$), $n=40$ breast meat pieces. Classification of color of the meat: L*=lightness, a*=redness, b*=yellowness; MORSF, N=Meullenet-Owens razor force; C1, C2, C3=Temperature fluctuation cycles; TBARS=thiobarbituric acid reactive substances; MDA=Malondialdehyde

subjected to three temperature fluctuation cycles. Therefore, temperature stability during cold storage must be controlled to reduce productivity loss. In general, superchilling prolonged the shelf-life of chicken breast meat and maintained its quality during storage and transportation in a better manner as compared to the quality maintained using the traditional chilling technique. It is, however, crucial that the initial number of microbes in the product be controlled via hygiene processing.

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