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Quality, Storage Stability, and Effect of Cryoprotectants on Ultrastructure of Superchilled Chicken

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ABSTRACT

The concept of superchilling, an alternative to traditional chilling and freezing methods, is to reduce the temperature 1-2 °C below the initial freezing point of the food enabling safe, and high-quality products. This work was aimed to explore the effects of superchilling (-2±0.5°C), chilling (4±1°C), and freezing (−18±1°C) on the quality and storage stability of dressed chicken and also to evaluate the role of cryoprotectants during superchilling for enhanced meat quality. In superchilled samples, improved water holding capacity (WHC) and lower shear force values were observed relative to frozen chicken. SDS-PAGE analysis revealed 29 kDa protein bands in superchilled samples on 7th and 14th day, indicating troponin degradation and tenderization. Scanning electron microscopy (SEM) studies indicated minimal damage to muscle fibre ultrastructure in superchilled chicken relative to frozen samples. Integrity of fibre structure was well maintained in superchilled samples treated with sorbitol as a cryoprotectant relative to sucrose and polyphosphate. Results demonstrated the potential use of superchilling process along with cryoprotectants for improved quality and enhanced shelf-life of chicken.

Key words: Superchilling, Chicken meat, Shelf-life, SDS-PAGE, Cryoprotectants, Ultrastructure

INTRODUCTION

Chilled and frozen storage are undoubtedly the most used methods to prevent meat quality deterioration. Chilled meat commands a premium over frozen meat as the former is perceived to be more fresh, less processed, and more convenient for cooking (Stonehouse and Evans, 2015). However, shelf-life of chilled (2±1°C) poultry meat is approximately 1 week (Jiménez *et al.*, 1997), whereas frozen storage at -18°C extends it upto 7-18 months (Taub and Singh, 1998). Limited storage life of chilled meat restricts its distribution and market penetration (Banerjee and Naveena, 2017). In spite of the fact that frozen storage is typically used for long-term preservation, few undesirable changes such as lipid oxidation and protein denaturation still occur (Coombs *et al.*, 2017), leading to discoloration, loss of flavor and nutrients along with drip upon thawing. Moreover, freezing and thawing of frozen meat is more energy intensive and consumes higher space and labour. Therefore, the challenge lies in finding an alternative method to chilling or freezing without compromising the quality characteristics of meat.

Superchilling is a novel preservation process which implies storage of food products at temperature between their initial freezing point (−0.5°C to −2.8°C for most food) and 1–2°C below this, specifically in the borderline between chilling and freezing temperatures (Duun and Rustad, 2007; Magnussen *et al*., 2008). Storage of foods at superchilling temperature helps to maintain freshness of food, retain food quality and suppress growth of microorganisms (Banerjee and Naveena, 2017). Superchilling, as a commercial practice, can reduce the use of freezing/thawing of foods leading to increased yield, reduction of energy cost in terms of transportation and retailing and environmental impact compared with freezing technology (Zhou *et al*., 2010). Although, the shelf-life of superchilled foods is relatively shorter than that of frozen food, when compared with traditional chilling technology, superchilling can retain better food quality and prolong the shelf life of most stored food by at least 1.5–4 times (Duun *et al*., 2008; Kaale *et al*., 2011).

Superchilling process has been reported mainly for extending the shelf-life of fish and the studies on its effect in meat and meat products are very much limited. Recently, few researchers have reported the use of superchilling to improve the quality and extend the shelf-life of rabbit meat (Lan *et al*., 2016), lamb (Bellés *et al*., 2017), pork (Ding *et al*., 2019), and beef (Qian *et al*., 2018, Lu *et al*., 2019, 2020). Freeze damage characterised by the formation of large intra- and extracellular ice crystals at the upper layer of superchilled (-1.5°C) Atlantic salmon fillets has been reported (Bahuaud *et al*. 2008). In order to minimize the damage to myofibrils and quality changes caused by ice-crystals during superchilling, few researchers have reported the use of cryoprotectants to stabilise the protein structure, prevent the loss of protein solubility and to ensure the maximum protein quality during superchilling or frozen storage (Liu *et al*., 2013, 2014). Addition of 4% mixture of sorbitol and sucrose (1:1) as a commercial cryoprotectant decreased the freezing point of common carp surimi from -1°C to -3°C (He *et al*., 2012). Use of cryoprotectants in superchilling proved to be an effective strategy in reducing protein degradation and maintaining the texture of common carp and surimi (Liu *et al*., 2013, 2014).

Considering the demand for fresh and chilled chicken relative to frozen chicken, superchilling could be an effective and energy-efficient alternative to freezing method. To the best of our knowledge, no reports are available on the potential effect of superchilling storage on quality, protein degradation and ultrastructural changes of chicken. Hence, the current study was undertaken to investigate the effects of superchilling relative to chilling and frozen storage on the quality and storage stability of chicken and to comprehend the role of cryoprotectants on microstructural changes during storage.

MATERIALS AND METHODS

Commercially reared market age (6 weeks) broilers obtained from local market were slaughtered at Institute's Poultry processing plant according to standard procedures. Poultry slaughtering was approved by the Institutional Animal Ethics Committee. After bleeding, scalding, defeathering, evisceration and washing, the dressed carcasses along with skin were chilled at 4 ± 1 °C in a chiller room for 8 h to allow the completion of rigor-mortis. Chilled carcasses were wrapped with a polyvinylchloride (PVC) film and randomly divided into three lots. The first lot of carcasses labelled as "chilled" were stored at 4±1°C. The second lot of carcasses were labelled as "frozen" and stored at −18°C. For "superchilling" storage, third lot of carcasses were subsequently cooled in a blast chiller (Sirman, Model: CERVINO 5T, Italy) until the core temperature of the carcass was brought down to approximately 0°C (in about 30 min) and then again divided into 2 batches. The first batch was maintained at a temperature of -2±0.5°C for temperature equalization and storage. The second batch of carcasses were immersed for 30 min in 3 different solutions containing cryoprotectants (sucrose and sorbitol 40 mg ml^{-1} distilled water each and tri-sodium polyphosphate 3 mg ml^{-1} distilled water) and stored at −2 ± 0.5 °C in superchilling cabinet. The day of processing was denoted as day '0. Prior to analysis, frozen samples were thawed at 4°C for 12 h. Superchilled samples were analyzed on days 0, 7, 14, 21, 28 and 35, whereas chilled samples stored at 4°C were analyzed on days 0, 4 and 7^{th} day of storage. Frozen samples were analyzed on 0, 14, 28, 60 and 90^{th} days. At each storage interval, samples were analyzed for different physico-chemical parameters.

Analytical procedure
Physoco-chemical properties

The pH was determined using a standardized electrode attached to a digital pH meter (Hanna Instruments, Model HI2216, USA). Water holding capacity (WHC) of samples was estimated according to Wardlaw *et al*. (1973). The color measurement (Hunter L^*, a^* and b^*) was performed using a Hunter lab Miniscan XE Plus colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA) with a 25 mm aperture set for illumination D65, 10°standard observer angle. Measurements were performed at six different positions for each sample, and at least three samples were measured to obtain an average value (Hunter and

Harold, 1987). Myoglobin (Mb) was extracted from meat samples using a modified procedure of Warris (1979). The % metmyoglobin (MetMb) was calculated as below (Trout, 1989):

$$\text{Mb (mg/g)} = (A_{525} - A_{700}) * 2.303 * \text{dilution factor}$$

$$\%\ \text{MetMb} = 1.395 - [(A_{572} - A_{700}) / (A_{525} - A_{700})] * 100$$

Lipid oxidation was determined by the thiobarbituric acid reactive substances (TBARS) method according to the extraction method described by Witte *et al.* (1970) with slight modifications and expressed in mg malonaldehyde kg^{-1}. The Warner-Bratzler shear force (WBSF) of the samples were measured using Texturometer (Tinius Olsen, Model H1KF, 6 Perrywood Business park, Redhill, RH1 5DZ, England) with V-shaped stainless-steel Blade (60° angle) and triangular hole in the middle.

Total protein extraction and SDS-PAGE analysis

Total protein was extracted from 100 mg of samples by mixing with 1 ml of extraction buffer (7 M Urea, 2 M Thiourea,4% w/v CHAPS, 2% carrier ampholytes pH4–7, 40 mM DTT and protease inhibitor) using a BeadBug microtube homogenizer (Model: D1030E, Benchmark scientific, Edison, NJ08818, USA) for 2x30 s of 4000 cycle min^{-1}. The homogenized samples were held at room temperature for 1 h with alternate vortexing followed by centrifugation using a refrigerated ultracentrifuge (Eppendorf centrifuge 5430R, Germany) at 10000 g for 1 h. The supernatant was collected (avoiding fat layer) and stored at –80°C till further analysis (Montowska and Pospiech, 2013). The SDS-PAGE was carried out using the method of Laemmli (1970), with a midi-electrophoresis apparatus (GE Healthcare, Uppsala, Sweden, Model: SE600Ruby). The gel scanned using an Image Scanner III, LabScan 6.0 (GE Healthcare, Uppsala, Sweden) and an IQTL calibration converter was used to obtain image.

Scanning Electron Microscopy

Samples were fixed in 2.5% gluteraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h at 4°C and post-fixed in 2% aqueous osmium tetroxide for 4 h. Later samples were dehydrated in series of graded alcohols, freeze fractured and dried with t-butylalcohol. The processed samples were mounted over the stubs with double sided carbon conductivity tape, and a thin layer of gold coat over the samples was applied by using an automated sputter coater (Joel JFC1600) and scanned under a scanning electron microscope (Joel JSM 5600, USA) at required magnification as per the standard procedure (Bozzola and Russel, 1998).

Statistical Analysis

The overall experiment was replicated on three separate occasions. The experimental data were analyzed as a randomized block design with storage temperature, storage time and their interaction as fixed factors, and carcass as a random factor. For experiments dealing with cryoprotectants, storage temperature, storage time, cryoprotectant levels and their interaction served as fixed factors whereas carcasses as random factors. Duplicate subsamples used for all the parameters were averaged for statistical analysis. Statistical analysis was performed with the analysis of variance (ANOVA) using SPSS (SPSS version 13.0 for windows; SPSS, Chicago, IL, USA). Least significant differences (LSD) at the 5% level were used to test the difference between means and differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Physico-chemical properties

The changes in pH, WHC and shear force values of chilled, superchilled and frozen samples were presented in Table 1. The pH of superchilled samples decreased ($p < 0.05$) on day 7, remained stable till day 21 and later increased significantly ($p < 0.05$) during storage. Initial decrease in pH value may be attributed to the accumulation of inorganic phosphoric acid resulting from the depletion of muscle ATP and the production of lactic acid (Scherer *et al.*, 2005). The changes in the pH value of superchilled sample may also be associated with the formation and growth of ice crystals during storage (Lan *et al.*, 2016; Ding *et al.*, 2019). Later during storage period, endogenous enzymatic and microbial reactions generating ammonia and other alkaline substances might be responsible for increased pH (Muela *et al.*, 2010). The pH value of frozen samples deceased significantly ($p < 0.05$) initially but remained stable during the entire storage time. Similar trends in pH of superchilled rabbit meat was reported by Lan *et al.* (2016).

Overall, the WHC of all stored samples decreased gradually as storage time increased. In the present study, the WHC of superchilled meat was higher ($p < 0.05$) than the chilled samples on day 7, which might be due to more severe proteolysis at higher temperatures in chilling caused by the activity of bacterial and endogenous enzymes (Olsson *et al.*, 2003, 2007). On day 28, a lower ($p < 0.05$) WHC was observed in frozen meat when compared to superchilled samples, indicating that freezing could destroy muscular tissues by causing mechanical damage to cell membranes and thus a loss in WHC (Anese *et al.*, 2012).

Table 1: Effect of chilling (4±1°C), superchilling (-2±0.5°C) and freezing (-18±1°C) on pH, water-holding capacity (WHC) and Warner-Bratzler shear force of chicken meat.

Storage time (days)	4±1°C	-2±0.5°C	-18±1°C
		pH	
0	6.38 ± 0.07^{a}	6.38 ± 0.07^{a}	6.38 ± 0.07^{a}
4	5.70 ± 0.05^{b}	-	-
7	5.72 ± 0.07^{b}	5.65 ± 0.03^{c}	-
14	-	5.55 ± 0.06^{c}	5.66 ± 0.003^{d}
21	-	5.60 ± 0.03^{c}	-
28	-	5.97 ± 0.06^{b}	5.71 ± 0.006^{cd}
35	-	6.32 ± 0.05^{a}	-
60	-	-	5.77 ± 0.01^{c}
90	-	-	5.89 ± 0.003^{b}
		WHC (%)	
0	76.83 ± 1.30^{a}	76.83 ± 1.30^{a}	76.83 ± 1.30^{a}
4	66.33 ± 1.72^{b}	-	-
7	60.16 ± 1.13^{cB}	71.50 ± 1.08^{bA}	-
14	-	69.66 ± 1.74^{bcd}	68.16 ± 1.57^{b}
21	-	71.0 ± 0.85^{bc}	-
28	-	65.83 ± 1.37^{dA}	48.66 ± 0.21^{cB}
35	-	66.66 ± 2.33^{cd}	-
60	-	-	45.83 ± 0.30^{d}
90	-	-	42.83 ± 1.07^{e}
		Shear force (N)	
0	10.15 ± 0.34^{a}	10.15 ± 0.34^{a}	10.15 ± 0.34^{a}
4	7.09 ± 0.57^{b}	-	-
7	6.64 ± 0.31^{b}	7.94 ± 0.22^{b}	-
14	-	7.16 ± 0.19^{bcB}	9.35 ± 0.84^{aA}
21	-	6.24 ± 0.40^{cd}	-
28	-	5.26 ± 0.46^{deB}	10.0 ± 1.24^{aA}
35	-	5.03 ± 0.59^{e}	-
60	-	-	8.76 ± 0.93^{ab}
90	-	-	5.75 ± 0.15^{b}

^{a,b,c,d,e} Means within column (between storage) without common letters are significantly different at $P < 0.05$.

^{A,B} Means between column (between temperature) without common letters are significantly different at $P < 0.05$.

In the current study, the WBSF tended to decrease ($p < 0.05$) with storage time for all samples and it is in agreement with the findings of Lan *et al.* (2016). Breakdown of myofibrillar tissues may be the main reason why the shear force decreased sharply and this may be attributed to the activity of enzymes, including bacterial enzymes and endogenous enzymes that breakdown the key proteins. Superchilled meat showed significantly ($p< 0.05$) higher WBSF values than chilled samples on day 7, which was consistent with the findings of Lu *et al.* (2019, 2020) in superchilled (- 4 °C) beef. In the present study, WBSF values of frozen samples remained stable upto 60 days and significantly decreased ($p < 0.05$) on day 90.

Lightness ($L^\star$), redness ($a^\star$) and yellowness ($b^\star$) of samples were affected ($p < 0.05$) by storage temperature and storage time (Table 2). The $L^\star$ value of fresh chicken meat was 38.39 on day 0' and increased ($p < 0.05$) to 42.90 during superchilling storage, whereas $a^\star$ and $b^\star$ values of superchilled samples decreased from 6.71 and 10.77 on day 0' to 3.93 and 5.61 on 35th day of storage. Generally, $a^\star$ value tend to decrease as a function of freezing treatment and storage time. Throughout the storage period, the activity of the metmyoglobin reducing enzyme, decreases as meat is frozen, and as a result, the metmyoglobin accumulates gradually on the surface of the meat (Ballin and Lametsch, 2008). Moreover, during storage, decrease in $a^\star$ value is proposed to be related to the oxidation of myoglobin and formation of metmyoglobin (Suman *et al.*, 2014). However, in the present study, the redness of superchilled sample was stable upto day 21 of storage. The a* value was significantly higher ($p < 0.05$) than the redness of chilled meat on day 7; but no significant difference is observed with the redness value of frozen meat on day 28. Moreover, compared to chilled samples, $L^\star$ and $a^\star$ values of superchilled samples changed slowly ($p < 0.05$) during storage. A similar trend was observed by Lu *et al.* (2019) in superchilled (-4 °C) beef. These results indicate that superchilling could significantly inhibit the decrease in redness of meat, which is considered a positive aspect associated with freshness and superior quality product. Bellés *et al.* (2017) also observed a protective effect of superchilling on redness value of vacuum-packaged lamb meat.

Table 2. Effect of chilling (4±1°C), superchilling (-2±0.5°C) and freezing (-18±1°C) on instrumental color ($L^\star$, $a^\star$ and $b^\star$) of chicken meat.

Storage time (days)	4±1°C	-2±0.5°C	-18±1°C
		Lightness ($L^\star$)	
0	38.39 ± 1.10^{b}	38.39 ± 1.1^{0b}c	38.39 ± 1.10^{bc}
4	40.55 ± 0.21^{ab}	-	-
7	43.56 ± 1.43^{aA}	36.78 ± 1.25^{cB}	-
14	-	36.56 ± 1.32^{c}	36.08 ± 0.62^{c}
21	-	38.98 ± 0.84^{bc}	-
28	-	41.17 ± 0.62^{abA}	36.64 ± 1.20^{cB}
35	-	42.90 ± 0.9^{9}a	-
60	-	-	40.73 ± 0.41^{ab}
90	-	-	41.21 ± 0.64^{a}
		Redness ($a^\star$)	
0	6.71 ± 0.48^{a}	6.71 ± 0.4^{8}a	6.71 ± 0.48^{a}
4	4.62 ± 0.29^{b}	-	-
7	3.72 ± 0.10^{bB}	6.59 ± 0.18^{aA}	-
14	-	6.15 ± 0.32^{a}	5.00 ± 0.43^{b}
21	-	5.92 ± 0.37^{a}	-
28	-	4.49 ± 0.18^{b}	4.27 ± 0.41^{b}
35	-	3.93 ± 0.3^{2}b	-
60	-	-	2.93 ± 0.22^{c}
90	-	-	2.70 ± 0.08^{c}

Storage time (days)	4±1°C	-2±0.5°C	-18±1°C
	Lightness (L^*)		
	Yellowness (b^*)		
0	10.77 ± 0.97[a]	10.77 ± 0.9[7]a	10.77 ± 0.97[c]
4	8.72 ± 0.71[ab]	-	-
7	8.08 ± 0.35[bB]	11.18 ± 0.46[aA]	-
14	-	7.69 ± 0.52[bB]	18.02 ± 0.45[aA]
21	-	7.17 ± 0.32[bc]	-
28	-	6.30 ± 0.23[bcB]	16.02 ± 0.36[abA]
35	-	5.61 ± 0.5[8]c	-
60	-	-	13.82 ± 0.94[b]
90	-	-	10.09 ± 0.38[c]

[a,b,c] Means within column (between storage) without common letters are significantly different at $P < 0.05$.

[A,B] Means between column (between temperature) without common letters are significantly different at $P < 0.05$.

Changes in the MetMb % in chicken meat during refrigerated, superchilled and frozen storage were shown in Table 3. Irrespective of treatment, MetMb % of all the samples increased significantly during storage, though the values changed slowly in superchilled samples compared to the chilled meat. In the present study, regarding superchilled storage, the MetMb% considerably increased ($p < 0.05$) with the storage time up to 21st day and thereafter remained constant till 28th day. At the initial stage of storage, the MetMb content of chilled samples exhibited higher rates of increase than those of superchilled or frozen samples. The observed increase in MetMb indicated that the ferrous form of myoglobin (OxyMb) underwent oxidation to ferric state (MetMb) during storage. Typically in fresh meat, ferric MetMb can be reduced back to deOxyMb and OxyMb due to the existence of MetMb-reducing enzyme system. However, the activity of MetMb-reducing enzyme in meat decreases in frozen storage (Alonso *et al*. 2016). Moreover, the increase in MetMb content coincided with the increase in TBARS (Table 3) during storage, supporting the fact that lipid oxidation also contributes to accelerated ferrous myoglobin oxidation (Lan *et al*., 2016). The TBARS increased significantly ($p < 0.05$) in all the treatments during storage period, however, the chilled samples presented a higher rate of increase than the superchilled or frozen samples, indicating that superchilling could restrain lipid oxidation for prolonged time. Moreover, TBARS was stable in superchilled samples in the present study upto 21st day and increased significantly ($p < 0.05$) only after day 28. These results were in agreement with the findings reported by Lan et al. (2016), Wang *et al*. (2018) and Lu *et al*. (2019). Researchers have also argued that frozen storage is not necessarily sufficient to prevent oxidation from occurring. Xia *et al*. (2009) reported that freezing and thawing of muscle tissues accelerated lipid oxidation, which was attributed to the fact that formation of ice crystals in superchilled or frozen storage may irreversibly disrupt the structure of muscle cells and tissues causing the subsequent release of pro-oxidants for lipid oxidation, particularly non-heme iron and accelerating oxidation (Banerjee and Maheswarappa, 2017). However, in the present study, the TBARS values remained at a low level and did not reach the threshold levels of rancidity.

Table 3. Effect of chilling (4±1°C), superchilling (-2±0.5°C) and freezing (-18±1°C) on metmyoglobin (%) and lipid oxidation of chicken meat.

Storage time (days)	4±1°C	-2±0.5°C	-18±1°C
	Metmyoglobin (%)		
0	26.50 ± 0.15[c]	26.50 ± 0.15[e]	26.50 ± 0.15[e]
4	31.60 ± 0.35[b]	-	-
7	43.26 ± 1.11[aA]	32.30 ± 2.30[dB]	-
14	-	39.96 ± 0.85[cA]	29.11 ± 0.41[dB]
21	-	45.31 ± 0.63[b]	-
28	-	47.47 ± 0.69[bA]	39.46 ± 1.01[cB]
35	-	52.41 ± 0.87[a]	-
60	-	-	41.5 ± 0.95[b]
90	-	-	48.11 ± 0.41[a]
	TBARS (mg malondialdehyde kg^{-1})		
0	0.06 ± 0.005[b]	0.06 ± 0.005[c]	0.05 ± 0.005[c]
4	0.08 ± 0.007[ab]	-	-
7	0.12 ± 0.02[aA]	0.06 ± 0.01[cB]	-
14	-	0.068 ± 0.01[c]	0.07 ± 0.009[c]
21	-	0.074 ± 0.01[c]	-
28	-	0.095 ± 0.003[b]	0.10 ± 0.011[b]
35	-	0.133 ± 0.01[a]	-
60	-	-	0.134 ± 0.013[a]
90	-	-	0.154 ± 0.035[a]

[a,b,c,d,e] Means within column (between storage) without common letters are significantly different at $P < 0.05$.

[A,B] Means between column (between temperature) without common letters are significantly different at $P < 0.05$.

Protein degradation

The SDS-PAGE pattern of proteins extracted from chicken meat under different storage conditions is presented in Fig. 1. Major protein components, including myosin light chains (MLC) (~16 to 27.5 kDa), actin (42 kDa), and troponin (70 kDa) did not show any significant change during superchilled (-2°C) storage. However, storage temperature had a significant effect on intermediate filament protein desmin (53 kDa). Band intensities of desmin reduced in chilled storage on 4th and 7th day, whereas both superchilled and frozen samples exhibited distinct desmin bands, which supported slower rate of proteolysis in superchilled meat com-

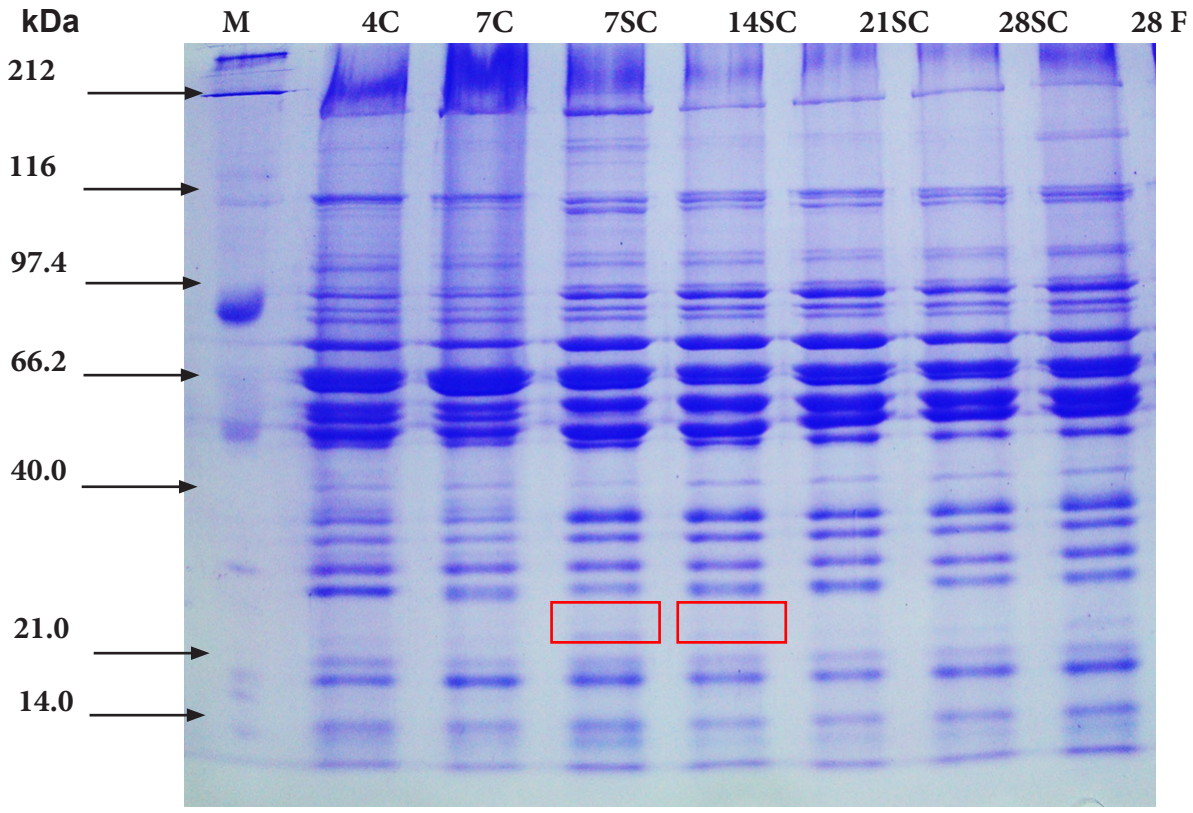

Fig. 1. SDS PAGE profile of total proteins from chicken stored under chilling (4±1°C), superchilling (-2±0.5°C) and frozen (-18±1°C) storage conditions. Lane M, High-range protein marker; Lane 4C, 7C, 4th and 7th day chilled samples respectively; Lane 7SC, 14SC, 21SC and 28SC, 7, 14, 21 and 28th day superchilled samples respectively; Lane 28F, 28th day frozen sample.

pared to chilled condition. Results also indicated that the structural changes in frozen samples may not be due to degradation of key myofibrillar proteins. Our results were in agreement with the findings of Lan *et al.* (2016) and Lu *et al.* (2020). In the present study, protein bands with molecular masses around 29 kDa became more evident on 7 and 14th day of storage at superchilling temperature (-2°C). More specifically, the 29 kDa protein band has been ascribed to the degradation product of troponin-T (Pomponio *et al.*, 2018) and the appearance of 29 kDa band is regarded as an indicator of meat tenderization (Harris *et al.*, 2001). The degradation of troponin directly reflects the breakage of filaments causing the loss of structural integrity of muscle fibres at the corresponding positions within the sarcomeres in superchilling, which can be correlated with the significantly ($p < 0.05$) lower shear force values in superchilled samples. Our findings clearly demonstrate the benefits of superchilling technology, which is low enough to substantially maintain the quality of fresh meat but high enough to avoid significant levels of ice crystal growth causing protein changes.

Ultrastructure

On day 0, the myofibres of fresh samples (Fig. 2A) were tightly in contact with one another revealing an organized structure. On day 28, the myofibres of superchilled samples (Fig. 2B) still maintained a compact shape but small gaps or spaces were observed between muscle fibres with variation in the fibre diameter. Similarly, increased myofibre separation in the superchilled Atlantic salmon fillets was reported by Bahuaud *et al.* (2008). Frozen sample showed disintegration of fibre structure with larger inter-fibre spaces on day 28 (Fig. 2C) in the present study. These effects might be the results of formation of intracellular and extracellular ice crystals within muscle fibre during freezing (Anese *et al.*, 2012; Bahuaud *et al.*, 2008). Our results were in agreement with the findings of Lan *et al.* (2016) in rabbit meat with increased storage time and decreased storage temperature, where frozen meat revealed more severe damage to the structure of myofibrils, including the shrinking and breaking myofibrils compared to chilling and superchilling.

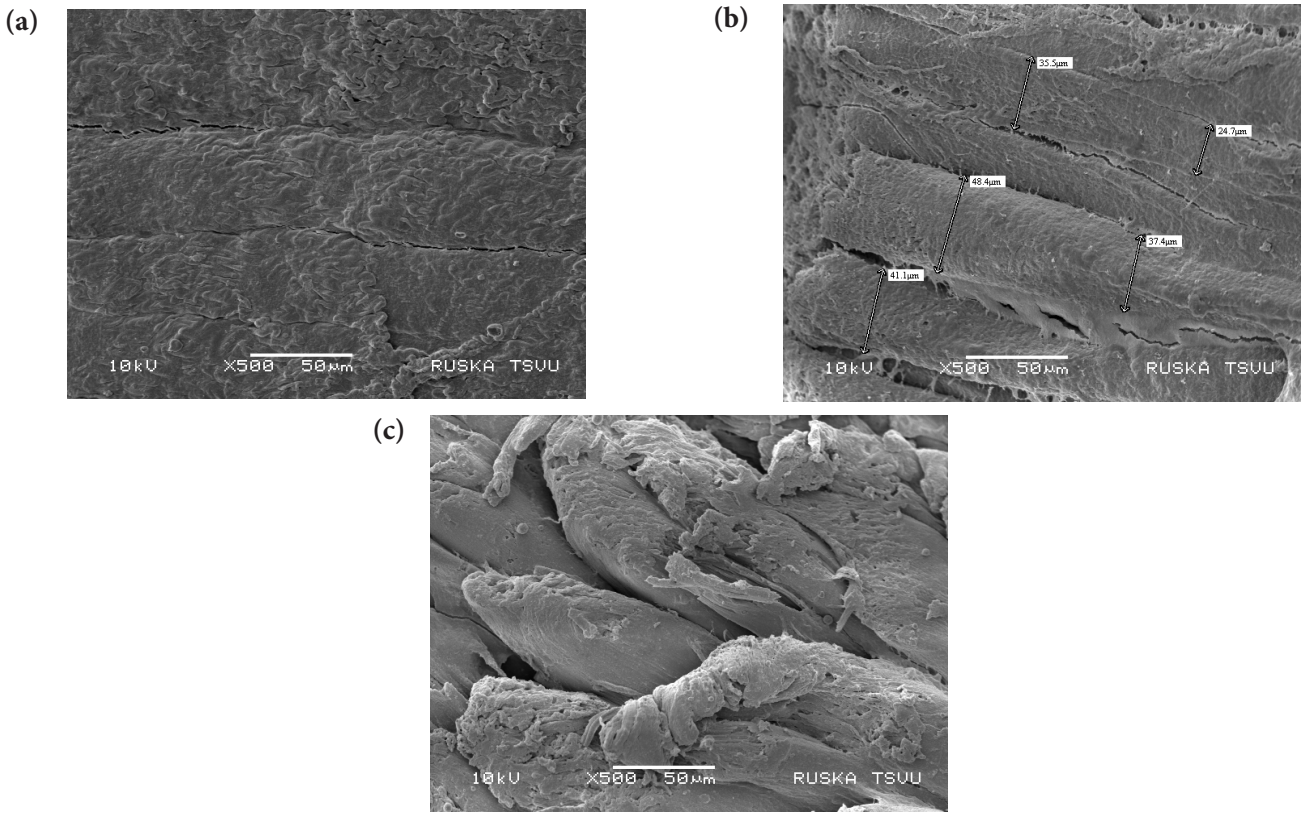

Fig. 2. Scanning electron micrographs (SEM) of chicken meat samples (a) Fresh meat sample on day 0, (b) Superchilled (-2±0.5°C) sample on day 28, (c) Frozen (-18±1°C) sample on day 28.

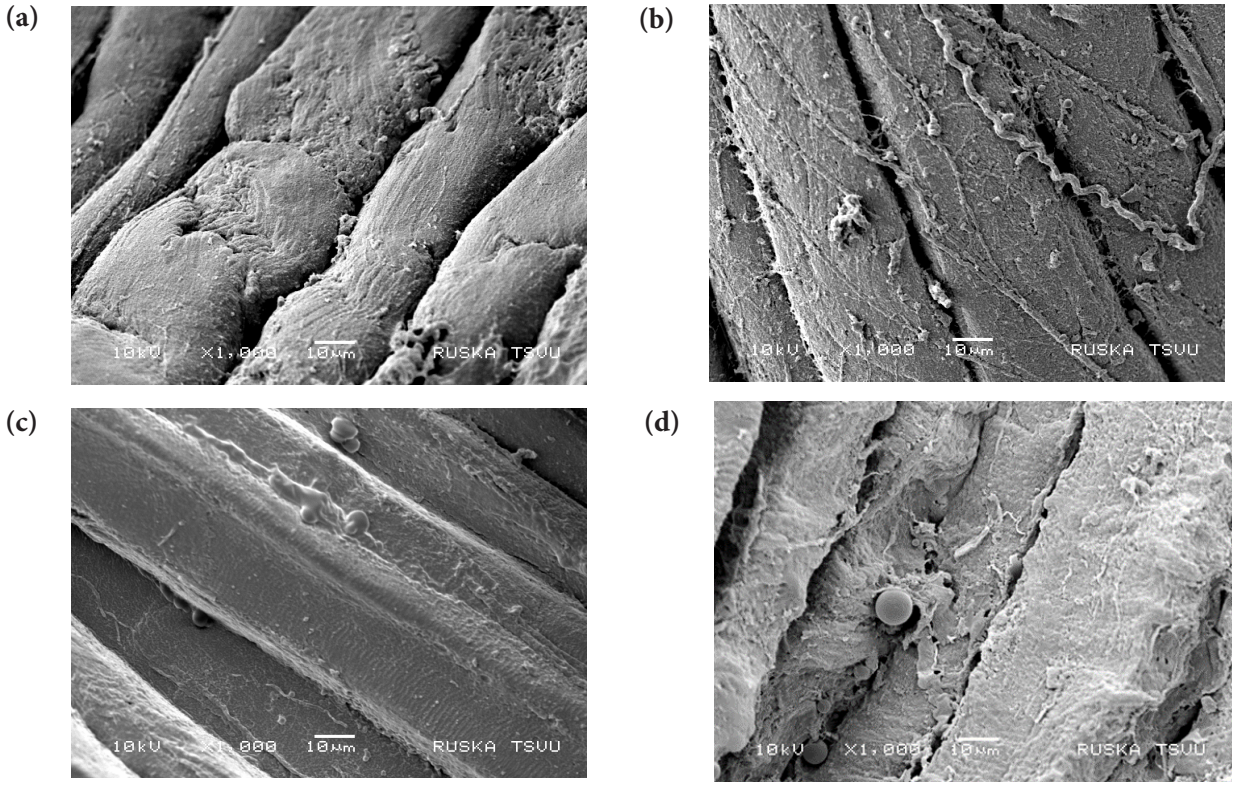

Fig. 3. Scanning electron micrographs (SEM) of superchilled (-2±0.5°C) samples treated with or without cryoprotectants on day 15, (a) Control (without any cryoprotectants), (b) Samples treated with sucrose, (c) Samples treated with sorbitol, (d) Samples treated with polyphosphate.

Effect of cryoprotectants on ultrastructure of superchilled chicken

In order to explore the role of cryoprotectants during superchilled storage of chicken meat, ultrastructure of superchilled meat with or without sucrose, sorbitol and polyphosphates were analyzed on day 15 by scanning electron microscopy (Fig. 3). Fig. 3A revealed gaps or spaces formed between myofibres in superchilled samples treated without any cryoporotectants. Compared with the sample superchilled at -2°C, the samples superchilled with cryoprotectants exhibited a marked reduction in the ultrastructure deterioration. Though smaller inter-fibre spaces were evident in samples treated with sucrose (Fig. 3B), greater integrity of the muscle structure was maintained with less separation between myofibres when the superchilled meat was treated with sorbitol under the same storage conditions (Fig. 3C). Similar cryoprotective effects of sorbitol in maintaining the integrity of the muscular microstructure of frozen yamú muscle was reported by León *et al*. (2019). Sucrose is an important cryoprotectant widely used in the frozen storage of fish products mainly surimi. In general, it is added at 4 % in combination with sorbitol (4 %) and polyphosphate (0.2 %) during the frozen storage of surimi which prevented the myofibrillar proteins from denaturation and aggregation during frozen storage (Cao *et al*., 2016). Commercial blends of sucrose and sorbitol (1:1) with polyphosphates have been stated to prevent frozen surimi proteins from denaturation (Mueller and Liceaga, 2016). Phosphates are globally recognized as an effetive cryoprotectant during storage conditions and their effects occur even at very low concentration (0.5 %). Addition of phosphate might have increased the ionic strength, supporting the immobilization of water and leading to swelling of muscle fibres in the present study (Fig. 3D). Phosphates increase the pH slightly, which also lead to improved gel-forming ability. However, the level of incorporation of polyphosphate (0.3 %) was not sufficient enough to maintain the structural integrity of muscle fibre during superchilling storage as compared to sucrose or sorbitol.

CONCLUSIONS

In conclusion, compared to chilled storage (4±1°C), superchilling (−2°C) was able to prolong the shelf life of chicken meat upto 5 times. Superchilled samples exhibited lower rate of increase of TBARS values and metmyoglobin % compared to chilled samples. The SDS-PAGE findings revealed that during superchilling, protein denaturation was minimal while also maintaining tenderness of meat. Superchilling did not seem to adversely affect the quality of meat as compared to freezing due to the damage caused by ice crystals. Cryoprotectants were effective in reducing the breakage of myofibres by inhibiting mechanical damage caused by ice crystals during superchilling storage, and the structural integrity of muscle fibre was better maintained by sorbitol compared to sucrose or polyphosphate. Future research could be directed at establishing the energy-efficiency of superchilling technology compared to freezing and identifying the growth of ice crystals during superchilling storage with or without cryoprotectants resulting in changes in the physicochemical properties and sensory attributes of meat.

COMPETING INTEREST

The authors declare no conflicts of interest regarding the materials presented in this paper.

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