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Investigation of superchilling in a customized chamber on physicochemical and quality parameters of raw beef tenderloin and lamb loin

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ABSTRACT

Superchilling, a preservation strategy maintaining temperatures slightly below the product's initial freezing point, is a promising method to extend the shelf life of red meat without the harmful effects of traditional freezing. This study examined the impact of superchilling at −2.5±0.8°C in a specially designed compartment compared to chilling at +0.5±0.1°C on the physicochemical, microbiological, sensory, and microstructural properties of raw beef *(Psoas major)* and lamb *(Longissimus dorsi)* over 16 days. Lipid oxidation, measured by TBARS, remained lower in superchilled samples, with beef values between 0.023–0.053 mg MDA/kg and lamb peaking at 0.295 mg MDA/kg, while chilling reached 0.283 and 0.385 mg MDA/kg, respectively. Microbiological analyses showed that total viable mesophilic aerobic counts (TVC) surpassed the spoilage threshold (≈7 log CFU/g) at least 10 days later under superchilling, with lamb samples maintaining 6.48 log CFU/g by day 16. Sensory evaluations indicated that superchilled beef and lamb retained overall acceptability scores above 3 for more than 16 days, whereas chilled samples dropped below this level after 5 and 6 days. Microscopic imaging revealed smaller, localized ice crystals under superchilling, contrasting with extensive crystal growth in frozen samples (−18°C). Overall, the results show that superchilling slows lipid oxidation and microbial growth while maintaining sensory quality and limiting structural damage, offering a practical way to extend the shelf life of red meat beyond conventional chilling. These findings highlight the industrial potential of superchilling and suggest that customized compartments could also help households store fresh meat longer with better quality.

Contribution/Originality: This study offers a perspective on the literature by systematically evaluating superchilling effects on beef and lamb quality using a customized chamber ensuring stable subzero control. This study employs a new estimation methodology by integrating physicochemical, microbiological, sensory, and microstructural analyses, providing novel insights into preservation with academic and industrial relevance.

1. INTRODUCTION

Consumers are placing greater emphasis on sourcing foods that are safe, of high quality, and sustainably produced, in addition to appreciating their sensory qualities. Red meat, one of the most perishable foods, remains challenging to preserve since storage temperature is crucial for maintaining both quality and safety (Duun, Hemmingsen, Haugland, & Rustad, 2008).

To increase storage life and maintain product quality, various temperature-based preservation methods have been studied for fresh red meat. Superchilling is one of these methods that has gained increasing interest. A portion

of the product's water freezes when the temperature is lowered to around 1-2°C below the initial freezing point, a process known as superchilling (Chang, Chang, Shiau, & Pan, 1998; Farouk, Kemp, Cartwright, & North, 2013). Surface freezing encourages consistent temperature regulation and controlled ice development, which limit microbial activity and slow down physical and chemical changes. Therefore, compared to traditional chilling, superchilling can prolong the shelf life of products. According to earlier research studies, superchilling can extend the shelf life of red meat by 1.4 to 4 times when compared to traditional refrigeration (Kaale, Eikevik, Rustad, & Kolsaker, 2011).

The two primary parts of the superchilling process are (i) bringing the product's temperature down to the point at which freezing begins, and (ii) drawing out latent heat to enable the development of a surface layer of ice. Product quality during storage is significantly impacted by the size, distribution, and shape of ice crystals (Kaale & Eikevik, 2014). Strict process control and steady temperature fluctuations can prevent the microstructural changes that partial freezing may cause, such as dehydration, contraction, and water loss upon thawing (Cheftel, Levy, & Dumay, 2000). Consequently, for superchilling applications to be efficient, a thorough understanding of the thermophysical characteristics of foods and precise control over storage temperatures are necessary (Magnussen, Haugland, Hemmingsen, Johansen, & Nordtvedt, 2008).

Research on superchilling has increasingly focused on its effects on food quality, particularly in relation to physicochemical changes and microbial development, including lipid oxidation, microbial counts, water-holding capacity, and textural changes (Kaale & Eikevik, 2014; Lan, Shang, Song, & Dong, 2016; Lu, Zhang, Zhu, Luo, & Hopkins, 2019). Kaale and Eikevik (2014) demonstrated in salmon fillets that ice crystals enlarged during prolonged superchilling, whereas stable temperature control minimized the extent of recrystallization. Similarly, Lan et al. (2016) observed reduced lipid oxidation and extended microbial stability in rabbit meat stored under superchilling conditions relative to conventional refrigeration. In cattle meat, Lu et al. (2019) demonstrated that superchilled storage effectively slowed microbial growth and lipid oxidation relative to chilling, thereby extending shelf life. El-Abdi, Alvarez, and Ndoye (2025) investigated the microstructure and quality of chicken breast meat and found that it seems evident that superchilling could prolong the preservation quality of chicken breast meat.

Despite these advantages, issues such as structural damage, water loss, and decreased nutritional quality may arise from ice crystallization (Liu, Liang, Xia, Regenstein, & Zhou, 2013). To determine how superchilling can restrict microbial growth, decrease oxidation, and maintain microstructural integrity, more research is required. This study used a specialized compartment intended for reliable temperature control to examine superchilling in raw lamb (*Longissimus dorsi*) and beef (*Psoas major*). When compared to traditional chilling storage, quality indices such as physicochemical, microbiological, sensory, and structural factors were assessed. The findings show that superchilling is a viable method for prolonging red meat's shelf life while maintaining its essential quality attributes.

2. MATERIALS AND METHODS

2.1. Chemicals

For the microbiological assays, culture media including CASO Agar Base (Tryptic Soy Agar, Merck KGaA, Darmstadt, Germany), CFC Agar Base with its Modified Selective Supplement (HiMedia, Germany), together with standard laboratory reagents such as distilled water, ethanol, and sodium chloride (Merck KGaA, Darmstadt, Germany), were utilized. Lipid oxidation (TBA) determinations involved 7.5% trichloroacetic acid (TCA), thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane (Sigma Aldrich Chemie GmbH, Germany), and hydrochloric acid solution (0.1 N, Merck KGaA, Darmstadt, Germany).

2.2. Superchilling Process and Sampling

All experiments were conducted within a specialized compartment designed for superchilling. For this study, a customized cooling compartment was developed to perform the superchilling process. It was built to maintain stable, adjustable temperatures at defined target levels. The unit was designed as an isolated section within the cooling device

and operated with an air-based control algorithm. This system enabled the compartment to regulate temperature and other conditions independently of the main chamber.

Thermocouples were used to measure temperature, and the signals were collected by a data acquisition system that provided continuous monitoring. The system ensured reliable detection of fluctuations and supported the evaluation of temperature stability within the compartment. To ensure accurate data collection, the thermocouples and data acquisition system were calibrated regularly, with calibration checks carried out before every experiment.

The superchilling process was carried out at an average temperature of –2.5°C with fluctuations of ±0.8°C. To avoid the adverse effects of repeated freezing and thawing, temperature fluctuation was minimized through the cooling device's control algorithm (Tao et al., 2023), and fluctuations were kept below 1°C. For comparison, conventional chilling was performed in a separate cooling device at +0.5°C with ±0.1°C fluctuation.

Beef tenderloin (M. *psoas major*) and lamb loin (*Longissimus dorsi*) muscle were used in the experiments. The meat used in this study was acquired from a local butcher in Istanbul. Raw samples were cut to dimensions of 10.0 x 4.0 x 4.0 cm, with an approximate weight of 150 g. They were wrapped with transparent cling film and placed inside the superchilling and chilling environments. Raw beef and lamb cuts were sampled on days 0, 2, 5, 7, 9, 12, 14, and 16. Three replicates were used for each analysis. A total of 25 samples were placed, with 5 samples on each side of the compartment. The samples were positioned with equal spacing within each compartment.

2.3. Thiobarbituric Acid (TBA) Analyses for Lipid Oxidation in Raw Beef and Lamb

TBA analyses were carried out to evaluate lipid oxidation in the samples. TBA analyses for lipid oxidation in raw meat were performed using a reference method (Pikul, Leszczynski, & Kummerow, 1989). The samples were ground and homogenized in a laboratory-type blender (Waring 8010 EB, USA), and 15 g of the ground sample was taken for further analysis. To homogenize the raw meat sample, 30 mL of 7.5% trichloroacetic acid was added, and the mixture was shaken. A blank sample was prepared using 15 mL of distilled water. The mixture was filtered through Whatman No. 1 (Merck KGaA, Darmstadt, Germany) filter paper. The filtered sample was transferred into 10 mL empty glass tubes, and 5 mL of 20 mM TBA (0.72 g of TBA + 250 mL of distilled water) was added. The tubes were shaken for 1 minute at 1500 rpm using a vortex device (Velp TX4, Italy). The tubes were then placed in a water bath (Memmert WNB22, Germany) for 45 minutes at 85°C. After the water bath, the samples were immediately cooled to room temperature. Following cooling, the samples were centrifuged using a centrifuge equipped with a cooler (Nüve NF800R, Turkey) for 10 minutes at 4100 rpm and 4°C. The supernatants were collected, and absorbance was measured at 532 nm using a UV-Vis spectrophotometer (Perkin Elmer Lambda 35, USA).

The TBA value was calculated using absorbance and the coefficient K obtained from the standard curve. The results of the TBA assay were reported in terms of mg malondialdehyde (MDA) per kg of beef (Equation 1). TBA analyses were performed with 3 replications and 3 parallels.

$$TBARS\ (mg\ MDA/kg) = K \times A532 \quad (1)$$

A532: Absorbance of the test sample at 532 nm.

K: The coefficient obtained from the standard curve.

2.4. Microbiological Analyses in Raw Beef and Lamb

The microbiological evaluation was carried out in accordance with an established reference method (Argyri, Panagou, Tarantilis, Polysiou, & Nychas, 2010). For analyses, 10 g of raw beef or lamb sample was added to 90 mL of peptone salt solution (PSS) and homogenized for 90 seconds in a stomacher (Interscience Bagmixer, France). Serial decimal dilutions were prepared in peptone salt solution. Subsequently, 0.1 mL aliquots were spread onto agar surfaces with a Drigalski spatula. Enumeration of total viable mesophilic aerobic bacteria was performed using CASO Agar Base (Tryptic Soy Agar), while *Pseudomonas spp* was quantified on CFC agar medium supplemented with Modified CFC Selective Supplement (FD281), applying the spread plate technique. These agar media were incubated

for 48 hours at 37°C for total viable mesophilic aerobic bacteria and *Pseudomonas spp* growth. Microbiological analyses were performed in 3 repetitions and 3 parallels. The growth was expressed as log cfu g^{-1}. Microorganism count was calculated with Equation 2 as shown below.

$$Microorganism\ count\ (cfu) = a \times b \div c \quad (2)$$

a: Microbial colony counts determined on agar plates.

b: Dilution factor.

c: Volume inoculated onto petri dish (mL).

2.5. Sensory Evaluations

The sensory evaluations were conducted following the procedure of a reference quantitative descriptive analysis (QDA) method with some modifications. (Yenipazar & Şahin-Yeşilçubuk, 2023). The sensory evaluation panels consisted of six trained panelists on sampling days. Panelists were instructed to score the appearance (redness), odor, and overall acceptability of the samples. The research involved only sensory evaluations of food products, conducted under standard controlled conditions. Participants were not exposed to any hazardous materials, medical treatments, or interventions that could pose physical or psychological risks. All participants were informed about the purpose and procedures of the study before their involvement. Each participant voluntarily agreed to take part in the study and provided informed consent prior to the evaluations, and no personal or sensitive data were collected from participants. The study was conducted in compliance with relevant data protection regulations, ensuring participant anonymity and privacy.

Descriptive sensory evaluations were conducted on the test samples, and quality characteristics were assessed using a scale ranging from 0 to 5 for both beef and lamb samples. The sensory analysis parameters included redness (0 = almost brown color to 5 = bright red), typical meat odor (0 = completely foreign odor to 5 = characteristic meat odor), and overall acceptability (0 = dislike extremely to 5 = like extremely) for the meat samples. Samples scoring below 3 in overall acceptability were deemed as rejected.

2.6. Microscopic Imaging of Ice Crystals

Microscopic imaging of beef samples preserved in different environments was visualized on days 0, 1, 2, 3, and 4 to observe ice crystal formation. Samples stored in different environments were sequentially taken, and small cross-sections were excised for microscopic examination of cellular tissues. Microscopic visualizations were performed using a Zeiss brand microscope with a 100x zoom ratio. The area of the sample examined by the microscope was 100µm.

2.7. Statistical Analyses

Means and standard deviations were computed for all measurements. The General Linear Model (GLM) was applied to the dataset, and Tukey's test was used for post-hoc multiple comparisons. A threshold of $p<0.05$ was adopted to indicate statistical significance. Statistical analyses were conducted with Minitab 21 software (Minitab Inc., USA). Statistical analyses were conducted to assess whether there were significant differences among the results, with a continuous focus on the repeatability and reproducibility values of the tests performed in multiple iterations. These analyses aimed to determine if the values observed across different days exhibited statistically significant changes from one another.

Table 1. The results for lipid oxidation of raw beef and lamb for 16 days of storage in superchilling and chilling compartments.

Conditions	Day 0	Day 2	Day 5	Day 7	Day 9	Day 12	Day 14	Day 16
Superchilling (Beef) (-2.5°C±0.8°C)	$0.023\pm0.000_{a}^{a}$	$0.046\pm0.010_{a}^{bc}$	$0.050\pm0.000_{a}^{bc}$	$0.050\pm0.010_{a}^{bc}$	$0.049\pm0.010_{a}^{bc}$	$0.053\pm0.010_{a}^{c}$	$0.023\pm0.010_{a}^{a}$	$0.037\pm0.010_{a}^{ab}$
Chilling (Beef) (+0.5°C±0.1°C)	$0.023\pm0.000_{a}^{a}$	$0.074\pm0.010_{b}^{bc}$	$0.075\pm0.010_{b}^{bc}$	$0.077\pm0.010_{b}^{c}$	$0.081\pm0.010_{b}^{c}$	$0.283\pm0.050_{b}^{d}$	$0.038\pm0.010_{b}^{ab}$	$0.050\pm0.010_{b}^{abc}$
Superchilling (Lamb) (-2.5°C±0.8°C)	$0.004\pm0.000_{a}^{a}$	$0.065\pm0.030_{a}^{ab}$	$0.077\pm0.010_{a}^{ab}$	$0.076\pm0.020_{a}^{ab}$	$0.295\pm0.060_{a}^{c}$	$0.250\pm0.150_{a}^{c}$	$0.275\pm0.030_{a}^{c}$	$0.138\pm0.050_{a}^{b}$
Chilling (Lamb) (+0.5°C±0.1°C)	$0.004\pm0.000_{a}^{a}$	$0.047\pm0.010_{a}^{ab}$	$0.084\pm0.010_{a}^{ab}$	$0.091\pm0.010_{a}^{ab}$	$0.350\pm0.180_{a}^{d}$	$0.350\pm0.080_{a}^{cd}$	$0.385\pm0.070_{b}^{d}$	$0.173\pm0.040_{a}^{bc}$

Note: Different subscript letters within the same column for a given food indicate significant differences between storage conditions on the same day ($p<0.05$). Outliers were screened using Grubbs' test, and no missing data were detected. Values represent the mean of three replicates per condition per day.
Different superscript letters within the same row indicate significant differences between days for a given condition ($p<0.05$). Values represent the mean of three replicates per condition per day.

3. RESULTS AND DISCUSSIONS

3.1. Lipid Oxidation in Raw Beef and Lamb

Lipid oxidation reactions result in the formation of hydroperoxides and the degradation of hydroperoxides into aldehydes, ketones, and carbonyl compounds, causing pungent, rancid, and off-flavor development (Lan et al., 2016). TBA analysis is the most frequently used test to quantify lipid oxidation levels in meat (Ganhão, Estévez, & Morcuende, 2011). The results for all conditions in which raw beef and lamb samples were preserved for 16 days are presented in Table 1. Although no legal limit has been established for TBA values, 1 mg MDA/kg meat is generally considered the threshold for sensory detection of rancidity (Limbo, Torri, Sinelli, Franzetti, & Casiraghi, 2010). In the present study, the initial TBA levels were approximately 0.02 mg MDA/kg meat for both beef and lamb. During the first two days, a marked increase in TBA values was observed in beef under both superchilling and chilling conditions, as shown in Table 1. Although the values remained stable after the 2nd day, the oxidation values of the beef samples stored in the chilling condition remained higher than those of the beef samples stored in the superchilling compartment throughout the entire storage period. On the other hand, TBA values increased substantially over time for lamb samples under both conditions, except on day 16, as can be seen from Table 1. Raw lamb samples preserved in chilling conditions have a higher degree of lipid oxidation than lamb samples preserved in superchilling temperatures after the 5th day until the end of the storage period. TBA values of raw beef and lamb preserved in both superchilling and chilling conditions did not exceed the 1 mg MDA/kg meat threshold during 16 days of storage. It was evaluated that the decrease in TBA values on the 16th day was sample-based. A study on the superchilling application for meat was conducted by Lu et al. (2019). The shelf life and quality attributes of yellow calf meat in China after superchilling were examined by Lu et al. (2019). Lipid oxidation (TBA) was analyzed throughout the storage processes of the meats. In the study of Lu et al. (2019), superchilling storage was conducted at -4°C, while comparative analyses were performed under traditional chilling (2°C) and freezing (-18°C) conditions. The TBA values of all beef samples steadily increased throughout the storage period. Samples subjected to superchilling storage showed less increase in TBA values compared to samples stored at 2°C. These findings from this research study indicate that superchilling application can limit lipid oxidation. The present results align with those of Lan et al. (2016), who demonstrated that superchilled samples exhibited a slower rise in TBA values compared with chilled controls. Using the TBA assay, they evaluated lipid oxidation in rabbit meat stored under different conditions and reported that values increased from 0.05 mg/kg to 0.6 mg/kg at 4°C, whereas superchilling at −2.5°C limited the increase to 0.2 mg/kg by day 8. These findings confirm that lower temperatures effectively suppress lipid oxidation and that superchilling extended the shelf life of rabbit meat at least three times relative to conventional refrigeration.

When the results of TBA in beef and lamb are compared, it is observed that the TBA value in lamb is higher than in beef. This is because lamb has a higher lipid content than beef and is more susceptible to lipid oxidation (Liu et al., 2025). Similar results are seen in the studies of James et al. (2025).

3.2. Microbiological Analyses in Beef and Lamb

Total viable mesophilic aerobic bacteria counts (TVC) and *Pseudomonas spp.* The growth of raw beef slices and lamb samples was analyzed, and the analysis results were examined.

According to the results of beef and lamb samples from day 0 to day 16, it was observed that the total viable mesophilic aerobic bacteria count in samples preserved under chilling conditions was higher than in samples preserved under superchilling conditions (Table 2). According to Fernández, Aspé, and Roeckel (2009) acceptable threshold limit is 6.0 log CFU/g for TVC. As defined in the Turkish Food Codex Regulation of Microbiological Criteria (2011), the permissible limit is 6.7 log CFU/g. In the present study, beef slices stored under chilling conditions reached values above this limit by day 2.8. TVC was above 6.7 log CFU/g on day 13.1 for the samples stored in superchilling conditions, while TVC was around 8.3 log CFU/g with a 95% CI of approximately [8.197, 8.495] in beef samples preserved under chilling conditions.

Table 2. TVC analysis results of raw beef samples preserved for 16 days in different conditions (log CFU/g)

Conditions	Day 0	Day 2	Day 5	Day 7	Day 9	Day 12	Day 14	Day 16
Superchilling (Beef) (-2.5°C±0.8°C)	$5.726\pm0.140_{a}^{a}$	$6.401\pm0.210_{a}^{c}$	$5.940\pm0.080_{a}^{b}$	$5.734\pm0.080_{a}^{a}$	$6.204\pm0.060_{a}^{c}$	$6.872\pm0.010_{a}^{d}$	$6.921\pm0.020_{a}^{d}$	$6.959\pm0.010_{a}^{d}$
Chilling (Beef) (+0.5°C±0.1°C)	$5.726\pm0.140_{a}^{a}$	$6.455\pm0.090_{a}^{b}$	$7.582\pm0.040_{b}^{c}$	$7.807\pm0.020_{a}^{c}$	$7.703\pm0.060_{b}^{cd}$	$8.346\pm0.060_{a}^{e}$	$8.558\pm0.030_{b}^{f}$	$8.675\pm0.040_{b}^{f}$
Superchilling (Lamb) (-2.5°C±0.8°C)	$5.481\pm0.060_{a}^{a}$	$5.517\pm0.040_{a}^{a}$	$5.856\pm0.030_{a}^{b}$	$5.920\pm0.010_{a}^{bc}$	$5.955\pm0.020_{a}^{c}$	$6.112\pm0.050_{a}^{d}$	$6.325\pm0.030_{a}^{e}$	$6.481\pm0.020_{a}^{f}$
Chilling (Lamb) (+0.5°C±0.1°C)	$5.481\pm0.060_{a}^{a}$	$6.244\pm0.050_{b}^{b}$	$7.007\pm0.180_{b}^{c}$	$7.413\pm0.160_{b}^{d}$	$7.581\pm0.040_{b}^{d}$	$7.862\pm0.020_{b}^{e}$	$8.441\pm0.030_{b}^{f}$	$8.530\pm0.040_{b}^{f}$

Note: Different subscript letters within the same column for a given food indicate significant differences between storage conditions on the same day ($p<0.05$). Outliers were screened using Grubbs' test, and no missing data were detected. Values represent the mean of three replicates per condition per day.
Different superscript letters within the same row indicate significant differences between days for a given condition ($p<0.05$). Values represent the mean of three replicates per condition per day.

The values of TVC of lamb slices preserved in chilling conditions exceeded the threshold limit by day 4.1. TVC was not above 6.7 log CFU/g for the samples stored in superchilling conditions for all storage times. However, it was above 6.0 log CFU/g at the 9th day for superchilling conditions, while TVC was around 7.6 log CFU/g in lamb samples preserved in chilling conditions.

Pseudomonas spp. are psychrotrophic microorganisms and are generally associated with the main cause of spoilage in meat and meat products at chilling conditions. An increase in *Pseudomonas spp.* numbers initiate the initial signs of spoilage, resulting in off-odor (Pellissery, Vinayamohan, Amalaradjou, & Venkitanarayanan, 2020). According to Olaoye and Ntuen (2011), *Pseudomonas spp.* cause slime and off-odor when the population reaches the level of 7-8 log CFU/g. Despite this, there was no evidence of *Pseudomonas spp.* count throughout the entire test. Although more *Pseudomonas spp.* growth was expected in the chilling condition than in the superchilling condition; microbiological evaluation was carried out via TVC.

Similar experiments were reported by Bellés, Alonso, Roncalés, and Beltrán (2017), who examined the combined influence of superchilling and packaging on the shelf life of lamb. Packaging conditions were O_2 enriched and vacuum packaging. In line with findings from similar studies, packaging did not significantly enhance microbial growth during storage. However, lamb slices stored under superchilling exhibited lower microbial loads than those kept under refrigeration, regardless of packaging type.

3.3. Sensory Evaluations

Sensory evaluations of beef and lamb samples preserved under superchilling and chilling conditions were conducted, assessing the parameters of redness, odor, and overall acceptability.

The sensory evaluation scores for beef and lamb samples are presented in Table 3 and 4, respectively. While all scores for different evaluation parameters of beef and lamb slices preserved under superchilling conditions remained above 3.0 throughout the storage period, scores for beef samples preserved under chilling conditions began to decrease below 3.0 by day 5 for all parameters. Overall acceptability scores indicated that beef samples were no longer consumable after day 5. In lamb meat samples stored under chilling conditions, this decline below 3.0 was observed on the 6th day. Higher temperatures accelerate spoilage mechanisms, as evidenced by the TBA and microbiological results. Care must be taken to avoid temperature abuse during superchilling, as high fluctuations can cause the melting of small ice crystals, increasing the tendency for spoilage and creating a conducive environment for microbial growth. Additionally, the fresh appearance of meat may be compromised due to the destruction of muscular tissues caused by recrystallization during significant temperature fluctuations, as reported by Anese et al. (2012).

Table 3. Sensory evaluation scores of beef samples preserved at superchilling and chilling conditions (0-5 range).

Redness	**Day 0**	**Day 2**	**Day 5**	**Day 7**	**Day 9**	**Day 12**	**Day 14**	**Day 16**
Superchilling (-2.5°C±0.8°C)	$4.70\pm0.40_{a}^{abc}$	$4.95\pm0.20_{a}^{a}$	$4.90\pm0.30_{a}^{ab}$	$4.45\pm0.50_{a}^{bcd}$	$4.06\pm0.20_{a}^{d}$	$4.00\pm0.00_{a}^{d}$	$4.13\pm0.20_{a}^{d}$	$4.38\pm0.60_{a}^{cd}$
Chilling (+0.5°C±0.1°C)	$4.70\pm0.40_{a}^{a}$	$4.30\pm0.40_{b}^{a}$	$3.55\pm0.40_{b}^{b}$	$2.05\pm0.40_{b}^{c}$	$1.56\pm0.50_{b}^{cd}$	$1.17\pm0.40_{b}^{d}$	$1.00\pm0.00_{b}^{d}$	$1.19\pm0.30_{b}^{d}$
Odor	**Day 0**	**Day 2**	**Day 5**	**Day 7**	**Day 9**	**Day 12**	**Day 14**	**Day 16**
Superchilling (-2.5°C±0.8°C)	$5.00\pm0.00_{a}^{a}$	$5.00\pm0.00_{a}^{a}$	$4.95\pm0.20_{a}^{a}$	$4.85\pm0.30_{a}^{ab}$	$4.83\pm0.30_{a}^{ab}$	$4.16\pm0.60_{a}^{c}$	$3.81\pm0.80_{a}^{c}$	$4.31\pm0.50_{a}^{bc}$
Chilling (+0.5°C±0.1°C)	$5.00\pm0.00_{a}^{a}$	$4.85\pm0.20_{a}^{a}$	$4.50\pm0.60_{b}^{a}$	$3.15\pm0.80_{b}^{b}$	$1.77\pm0.40_{b}^{c}$	$1.50\pm0.80_{b}^{c}$	$1.06\pm0.20_{b}^{c}$	$1.18\pm0.40_{b}^{c}$
Overall acceptability	**Day 0**	**Day 2**	**Day 5**	**Day 7**	**Day 9**	**Day 12**	**Day 14**	**Day 16**
Superchilling(-2.5°C±0.8°C)	$5.00\pm0.00_{a}^{a}$	$5.00\pm0.00_{a}^{a}$	$4.95\pm0.20_{a}^{ab}$	$4.60\pm0.30_{a}^{bc}$	$4.16\pm0.30_{a}^{d}$	$4.00\pm0.30_{a}^{d}$	$4.12\pm0.20_{a}^{d}$	$4.37\pm0.60_{a}^{cd}$
Chilling (+0.5°C±0.1°C)	$5.00\pm0.00_{a}^{a}$	$4.50\pm0.60_{b}^{a}$	$3.65\pm1.10_{b}^{b}$	$1.70\pm0.60_{b}^{c}$	$1.27\pm0.40_{b}^{c}$	$1.05\pm0.20_{b}^{c}$	$1.00\pm0.00_{b}^{c}$	$1.06\pm0.20_{b}^{c}$

Note: Different subscript letters within the same column indicate significant differences between storage conditions on the same day ($p<0.05$). Outliers were screened using Grubbs' test, and no missing data were detected. Values represent the mean of three replicates per condition per day.
Different superscript letters within the same row indicate significant differences between days for a given condition ($p<0.05$). Values represent the mean of three replicates per condition per day.

Table 4. Sensory evaluation scores of lamb samples preserved at superchilling and chilling conditions (0-5 range).

Redness	**Day 0**	**Day 2**	**Day 5**	**Day 7**	**Day 9**	**Day 12**	**Day 14**	**Day 16**
Superchilling (-2.5°C±0.8°C)	$5.00\pm0.00_{a}^{a}$	$5.00\pm0.00_{a}^{a}$	$4.60\pm0.30_{a}^{ab}$	$4.63\pm0.40_{a}^{ab}$	$4.40\pm0.50_{a}^{b}$	$4.44\pm0.40_{a}^{b}$	$4.45\pm0.40_{a}^{b}$	$4.45\pm0.40_{a}^{b}$
Chilling (+0.5°C±0.1°C)	$5.00\pm0.00_{a}^{a}$	$4.50\pm0.30_{b}^{a}$	$3.65\pm0.30_{b}^{b}$	$2.81\pm0.70_{b}^{c}$	$2.10\pm0.30_{b}^{d}$	$2.17\pm0.80_{b}^{cd}$	$1.30\pm0.40_{b}^{e}$	$1.30\pm0.50_{b}^{e}$
Odor	**Day 0**	**Day 2**	**Day 5**	**Day 7**	**Day 9**	**Day 12**	**Day 14**	**Day 16**
Superchilling (-2.5°C±0.8°C)	$5.00\pm0.00_{a}^{a}$	$5.00\pm0.00_{a}^{a}$	$4.85\pm0.20_{a}^{a}$	$4.63\pm0.40_{a}^{ab}$	$4.55\pm0.40_{a}^{ab}$	$3.78\pm0.70_{a}^{c}$	$4.05\pm0.60_{a}^{bc}$	$3.90\pm0.20_{a}^{c}$
Chilling (+0.5°C±0.1°C)	$5.00\pm0.00_{a}^{a}$	$4.90\pm0.20_{a}^{ab}$	$4.20\pm0.60_{b}^{bc}$	$3.50\pm0.80_{b}^{c}$	$2.20\pm0.60_{b}^{d}$	$2.28\pm1.00_{b}^{d}$	$1.15\pm0.30_{b}^{e}$	$1.00\pm0.00_{b}^{e}$
Overall acceptability	**Day 0**	**Day 2**	**Day 5**	**Day 7**	**Day 9**	**Day 12**	**Day 14**	**Day 16**
Superchilling(-2.5°C±0.8°C)	$5.00\pm0.00_{a}^{a}$	$5.00\pm0.00_{a}^{a}$	$4.75\pm0.40_{a}^{a}$	$4.63\pm0.40_{a}^{ab}$	$4.30\pm0.40_{a}^{bc}$	$4.22\pm0.40_{a}^{bc}$	$4.10\pm0.30_{a}^{c}$	$4.15\pm0.30_{a}^{c}$
Chilling (+0.5°C±0.1°C)	$5.00\pm0.00_{a}^{a}$	$4.50\pm0.30_{b}^{a}$	$3.80\pm0.40_{b}^{b}$	$2.81\pm0.70_{b}^{c}$	$1.50\pm0.50_{b}^{d}$	$1.72\pm0.70_{b}^{de}$	$1.10\pm0.20_{b}^{e}$	$1.05\pm0.20_{b}^{e}$

Note: Different subscript letters within the same column indicate significant differences between storage conditions on the same day ($p<0.05$). Outliers were screened using Grubbs' test, and no missing data were detected. Values represent the mean of three replicates per condition per day.
Different superscript letters within the same row indicate significant differences between days for a given condition ($p<0.05$). Values represent the mean of three replicates per condition per day.

As a conclusion of sensory evaluations for raw beef and lamb samples preserved in superchilling and chilling conditions for 16 days, overall acceptability scores of samples in chilling conditions decreased faster than in superchilling conditions. According to the results, raw beef had an overall acceptability score above 3.0 for 5 days in chilling, and more than 16 days in superchilling conditions. The results for lamb samples are 6 days in chilling and more than 10 days in superchilling conditions. In conclusion, when sensory evaluation results were investigated for raw beef and lamb, overall acceptability limits exceeded more than four times later in superchilling storage.

Ganhão et al. (2011) reported that lipid oxidation initially generates hydroperoxides, which subsequently degrade into secondary compounds such as aldehydes, ketones, and other carbonyl derivatives. These molecules are responsible for undesirable sensory attributes, including rancid and pungent odors. Hansen et al. (2004) further indicated that trained sensory panels are able to detect such off-flavors when TBA values exceed 0.5 mg/kg. In the present study, TBA values remained below this threshold throughout 16 days of chilling and superchilling (−2.5°C) storage in both beef and lamb, suggesting that lipid oxidation was not the main limiting factor. The earlier sensory changes observed compared with TBA values may be more closely related to the increase in total viable counts (TVC).

3.4. Microscopic Image of the Samples Preserved in Superchilling Conditions and Comparison Between Samples Preserved in Freezer and Chiller Temperatures

Microscopic examinations of cellular structure were performed over a four-day storage period to evaluate ice crystal formation during superchilling and to assess the impact of recrystallization on red meat quality. This analysis aimed to provide insights into how different storage conditions affect the microstructure of meat, particularly focusing on the comparison between superchilled meat and meat stored in conventional freezer and chiller compartments. Throughout the study, samples were monitored regularly to capture changes in the size, distribution, and morphology of ice crystals within the meat tissue. In order to promote partial ice formation and reduce the negative consequences usually associated with full freezing, the superchilling storage was designed to keep temperatures slightly below the freezing threshold. On the other hand, because of the more thorough freezing and recrystallization processes, which are known to result in considerable cell damage and moisture loss, the beef kept in the freezer displayed larger ice crystals. Conversely, meat kept in the traditional chiller compartment showed less ice crystal formation but more microbial growth and enzymatic activity, which could hasten the deterioration of quality. By comparing these different storage methods, the study aimed to illustrate the relative advantages of superchilling in preserving the structural integrity and overall quality of red meat, providing a balance between maintaining low temperatures and avoiding extensive cellular damage. The observations highlighted the importance of optimizing superchilling parameters to enhance the shelf life and sensory attributes of meat products while mitigating the adverse effects of ice crystal formation and recrystallization.

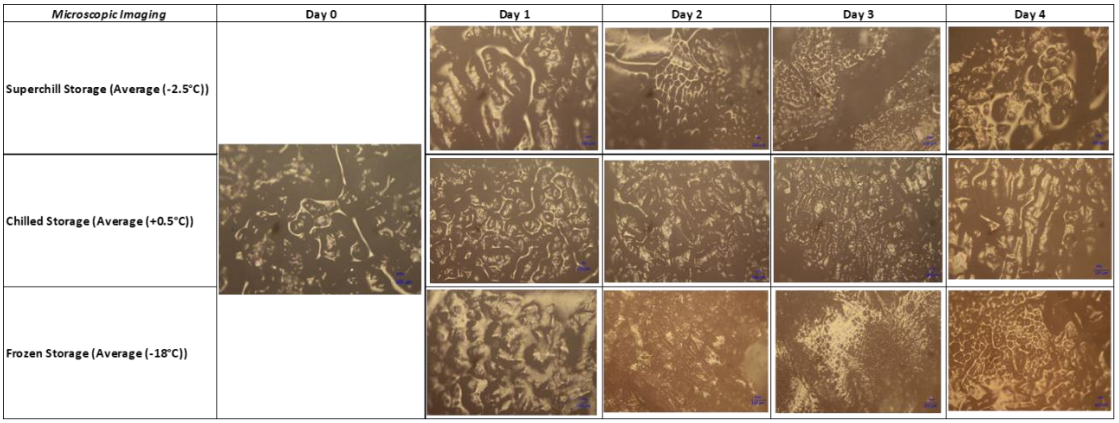

Figure 1. Microscopic imaging of beef samples preserved in different environments was visualized for 4 days (100x zoom ratio, the area of the sample 100µm).

Figure 1 displays the microscopic pictures taken throughout the investigation, which show the cellular structure and ice crystal morphology under various storage circumstances. These pictures support the above-discussed results by visually comparing the effects of freezing (-18°C), superchilling (-2.5°C), and traditional chilling (+0.5°C) on the microstructure of red meat.

To evaluate structural alterations induced by storage temperature, red meat samples were subjected to microscopic analysis following four days of storage under superchilling (−2.5°C), chilling (+0.5°C), and freezing (−18°C) conditions. As illustrated in Figure 1, the microscopic images demonstrate variation in ice crystal formation and distribution among the storage treatments. Under chilled storage (+0.5°C), no ice crystals were observed in the meat samples throughout the four days. Instead, water molecules were clearly visible under the microscope, indicating that the samples did not undergo any freezing process at this temperature. In contrast, the superchilling storage condition (average -2.5°C) displayed a progressive change in the microstructure of the meat. On day 1 of storage, the absence of ice crystals showed that the samples remained above the freezing threshold. However, starting from the second day, ice crystals began to form, and their presence became more evident on the third and fourth days. These crystals were heterogeneously distributed within the meat tissue, and their size increased over time. The growth of ice crystals from day to day indicates the occurrence of recrystallization, a process where existing ice crystals enlarge due to temperature fluctuations or slow migration of water. Recrystallization can be unfavorable to the quality of meat, as larger ice crystals can cause mechanical damage to the cellular structure, leading to cell wall rupture, moisture loss, and a progressive decrease in texture quality. The expansion of ice crystals can also increase drip loss upon thawing, further compromising the meat's sensory and nutritional properties (Kaale & Eikevik, 2015).

In frozen storage (−18°C), microscopic images revealed small ice crystals distributed evenly across the meat tissue. Their uniform size and distribution indicate that freezing limited the damage usually caused by ice crystal growth. Under these conditions, cellular structures were better preserved, with less rupture and moisture loss than in superchilling. As a result, frozen storage provided the best protection of meat structure during the study period.

Two red meat species, beef (*Psoas major)* and lamb *(Longissimus dorsi)*, were the sole subjects of this investigation, which was conducted under carefully monitored superchilling and cooling settings. The 16-day storage time used in the research might have missed longer-term preservation results. Furthermore, testing was limited to a single bespoke superchilling chamber; results may differ depending on the equipment design or scale-up to industrial applications. A more thorough grasp of the advantages and disadvantages of superchilling as a preservation technique would be possible with additional research involving a wider variety of meat types, packing techniques, and longer storage times.

4. CONCLUSION

In contrast to conventional chilling at +0.5±0.1°C, this investigation showed that superchilling at −2.5±0.8°C successfully postponed lipid oxidation, inhibited microbial growth, and maintained the sensory quality of raw beef (*Psoas major*) and lamb (*Longissimus dorsi*). Under superchilling, the total viable counts remained above the spoiling criterion for at least 10 days, and the sensory acceptability stayed above the limit for almost 16 days. Smaller and more confined ice crystals were verified by microscopic examinations during superchilling, suggesting less structural damage than during freezing. All things considered, these results demonstrate that superchilling of red meat prolongs its shelf life and preserves its quality characteristics beyond what is possible with conventional cooling, providing a viable preservation method for both commercial and scholarly purposes.

4.1. Future Work

There is a need to investigate the effects of ice crystallization on drip loss and potential loss of nutritional value in beef and lamb subjected to superchilling. This investigation should include a comparison of drip loss and nutritional values with conventional chilling methods where ice crystallization does not occur. By implementing a well-designed

and optimized superchilling process with minimal temperature fluctuations, these risks can be significantly reduced. Differences in ice crystallization formation in foods between low and high temperature fluctuation superchilling applications can be further analyzed by investigating ice crystal structures with microtomographic methods, with a comparison to conventional freezing conditions. Microtomographic methods can provide more information about the destruction of muscular tissues and mechanical damage to cell membranes, as well as the causes of drip loss and loss of nutritional value. Understanding thermo-physical processes in the food is important for the ideal operation of superchilling.

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