

**Research Article****EFFECT OF SUBZERO STORAGE ON THE QUALITY OF MUTTON FROM SHEEP AND GOATS IN D.I KHAN DISTRICT, KHYBER PAKHTUNKHWA****Ahmad Saeed<sup>1</sup> | Syed Muhammad Raihan Dilshad<sup>2</sup> | Maisarah Abdul Mutalib<sup>3</sup> | Shakeeb Ullah<sup>1</sup> | Amjad Hussain<sup>4</sup> | Kamran Safdar<sup>5</sup> | Adamu Abdul Abubakar<sup>6</sup> | Faiqah Ramzan<sup>1</sup> | Sana Ullah<sup>1</sup> | Hamza Maris<sup>1</sup> | Saqib Ali Rustam<sup>1</sup> | Sumera Ali<sup>7</sup> | Muhammad Shuaib Khan<sup>\*1</sup>**

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**ABSTRACT:**

**Background:** Mutton is widely recognized for its excellent nutritional value, containing essential components such as proteins, vitamins, and minerals. **Aim:** The objective of this research was to investigate the effects of subzero storage on the freshness and quality of mutton. **Material & Methods:** To evaluate the attributes of frozen and stored mutton, we employed fresh control samples that underwent standard freezing procedures at -18°C. Determining the quality and freshness of the product required the evaluation of numerous parameters over a five-month storage period. Drip loss (comprising boiling and thawing loss), water-holding capacity (WHC), texture profile analysis (TPA), thiobarbituric acid reactive substances (TBARS), and total volatile basic nitrogen encompassed these parameters. Temperature influenced leak loss and water holding capacity (WHC), with severe freezing resulting in a reduction in moisture loss during frozen storage in comparison to standard freezing conditions. **Findings:** Extremely low-temperature freezing and subsequent storage of lamb resulted in a notable enhancement in tenderness when compared to standard freezing conditions. More precisely, sheep that underwent freezing at temperatures below -60°C exhibited tenderness akin to that of recently slaughtered mutton. Following a five-month period, frozen samples of mutton exhibited no indications of lipid oxidation, irrespective of temperature. The storage temperature of the samples, nevertheless, had an impact on the TVBN (total volatile basic nitrogen) concentration. In conclusion, our research indicates that mutton can maintain its freshness for a period of five months through the utilization subzero temperatures. A temperature of -60°C is determined to be the most favorable condition for freezing and storing frozen mutton, taking into account both economic and qualitative considerations.

**KEYWORDS:**

Frozen, Mutton, Subzero, Water-holding Capacity, thiobarbituric acid

**1| INTRODUCTION**

It is well known that mutton has a high nutritional value, comprising necessary components such as proteins, vitamins, and minerals<sup>1</sup>. The basic properties of meat, such as its texture, flavor, and tenderness, as well as its color and nutritional content, are reliant on a variety of variables as well as hygienic storage conditions<sup>2</sup>. The most

abundant chemical component that can be found in mutton is water. Many of the mutton's chemical and physical transformations may be traced back to water. The flavor, texture, and overall quality of the mutton will all be impacted as a result of these chemical and physical changes<sup>3</sup>. Mutton, with all of the numerous benefits it offers to one's health and the irresistible flavor it possesses, today accounts for the majority of our daily nutrient consumption<sup>4</sup>. Sheep meat makes up around 7% of the total amount of meat that is produced across the globe<sup>5</sup>. In most cases, full carcasses or individual pieces of sheep meat are sold in order to satisfy demand for the product. The freezing process is an alternative that can be used to increase the shelf life of meat cuts. This is because the subzero temperature inhibits the appearance of new degrading agents and slows down the growth of microbes, both of which can cause changes in the meat<sup>6</sup>.

Additionally, the subzero temperature slows down the proliferation of germs. The freezing process is utilized by the companies that deal in meat in an effort to maintain price stability at times when there is a scarcity of animals available for slaughter<sup>6</sup>. In international commerce of sheep meat, the distance between the producer and the consumer necessitates chilling or freezing the meat at subzero temperature to preserve its quality during transport and storage. The consumption of goat meat is lower than that of beef on a global scale<sup>7</sup>; yet, goats are undeniably an important source of red meat for humans, especially in less developed countries<sup>8</sup>. Meat from goats is a significant source of nutrients, in especially for people living in developing countries, which are primarily located in tropical areas. Goats are primarily raised in subtropical and tropical climates. These regions are home to more than 90 percent of the world's total goat population, which is estimated to be around 650 million.

The technique of freezing meat cuts is an alternative that can be utilized to improve the length of time that they can be preserved after being prepared. This may be done to enhance the amount of time that they can be stored for. Temperatures below zero inhibit the development of new degrading agents and slow down the growth of bacteria<sup>9</sup>. If either of these processes is allowed to continue, they can cause changes in the tissue. Refrigeration is an environmental protection technique that can be utilized when there is no need for a change in storage temperature<sup>10</sup>. In order to inhibit the proliferation of spoiling organisms, it is recommended to thaw meat, poultry, and fish within the controlled temperature environment of a refrigerator. In order to mitigate the risk of uneven cooking, it is advisable to partially thaw some types of meat, such as large roasts, prior to the cooking process. This precautionary measure ensures that the outside layers do not become excessively cooked while the interior remains undercooked. In order to ensure proper adherence of the coating, it is recommended to partly defrost meat, poultry, or fish before applying flour or breading prior to cooking.

Meat's physicochemical characteristics improve significantly after refrigeration. The fundamental purpose of refrigeration is to lower the temperature of meat to a point below which the rate of microbial growth can no longer be sustained by further cooling or freezing the product. This is accomplished by lowering the temperature to a level that is lower than 0 degrees Celsius<sup>11</sup>. The quality of meat held under frozen conditions is influenced by various factors, including storage temperature, temperature fluctuations, packaging methods, and environmental humidity. Temperature fluctuations might potentially arise during several periods of food storage, including freeze-thaw cycles within the cold chain and when food is stored in residential freezers and subjected to door opening or defrosting events. Frequent temperature fluctuations in residential refrigerators are caused by the flow of air between the interior and exterior of the refrigerator, as well as physical processes such as defrosting<sup>12</sup>.

It is possible to rapidly freeze food by reducing the freezing point of liquid nitrogen, which has been shown to have a significant impact on the quality of frozen meat. The drawbacks of utilizing liquid nitrogen, however, are the possibility of cold denaturing protein and surface splitting in meat<sup>13</sup>. According to research by Grujic<sup>14</sup> from 1993, meat storage at a temperature below the eutectic point (-70°C) experienced higher drip loss than mutton storage at a temperature that was substantially higher<sup>14</sup>. As a result, studies examining the relationship between meat quality and freezing conditions have found that the ideal range is between -18°C and -40°C, although a variety of deep freezers with a range of freezing temperatures from -50°C to -100°C are now readily available due to technological advancement.

The chemical component that is most abundant in mutton is water. Numerous chemical and physical transformations occurring in mutton exhibit a direct correlation with the presence of water. The chemical and physical alterations will have an impact on the overall quality, texture, and taste characteristics of mutton<sup>11,15</sup>. Currently, conventional approaches for detecting food moisture often involve the utilization of the drying method, distillation method, and Karl Fischer method<sup>15</sup>. The current investigation sought to examine how low temperatures (below zero) and long

periods of storage (up to five months) affect the quality of meat derived from sheep and goats.

## **2 | MATERIAL AND METHODS**

### **2.1 | DESCRIPTION OF THE STUDY AREA**

The current research was carried out at District Dera Ismail Khan, which is located in Khyber Pakhtunkhwa, province of Pakistan, from December 2022 to June 2023. The district of D.I. Khan is the most southern district in Khyber Pakhtunkhwa (KPK), and it is located approximately 300 kilometers away from Peshawar, the capital of the province to the south. The provinces of Punjab, Sindh, and Balochistan are all located on its border. According to the census completed in 2023, the total population of the district is greater than 1.8 million, with the number of people who speak Saraiki being the majority of the population.

### **2.2 | SAMPLE COLLECTION**

Random sampling procedures were used in the course of the study. Dera Ismail Khan and its five tehsils are recommended for the aforementioned study. The collection of mutton from five tehsils was done by 15 butcher shops. Therefore, every shop was providing five samples of mutton. These selected butchers were provided mutton from the Longissimusdorsii muscle.

### **2.3 | SAMPLING AND ANALYTICAL PROCEDURES**

A total of 75 samples of mutton was collected in aseptic containers, labeled, and transported in an ice box. This number is comprised of 40 samples from sheep and 35 samples from goats. The Longissimus dorsi muscle act as the research project's representative sample. The sample was brought to the lab and kept there until it is time for analysis in a refrigerator at -80°C. Two portions of the muscle sample were used: one for cooking and the other for estimating the quality of the raw muscle. Cooking techniques included roasting and boiling. On a burner with a temperature maintained at 180 C, the meat typically roast or boil for 12 to 25 minutes. According to the AOAC (1990), the amounts of moisture, protein, fat, and ash was measured. Using a digital pH meter, the determination of pH in accordance with the Official Methods of Analysis AOAC<sup>16</sup>.

### **2.4 | PLAN OF WORK**

Each sample of mutton was divided into three groups: Group-A (the raw, uncooked control group), Group-B (the frozen group), and Group-C (the cooked, thawed group). The physical and chemical features of Group-A mutton samples was analyzed on the first day (fresh meat) of collection, whereas Group-B and Group-C samples were examined at intervals of one week to four weeks. In order to evaluate the effects of thawing cycles, samples were taken out of the freezer cabinet after each week of freezing and kept at ambient temperature until the meat returns to its typical posture. After that, a sample was collected for additional analysis.

### **2.5 | MEAT QUALITY TESTS**

The pH was checked by inserting a calibrated pH probe into the flesh at two different random spots along the uncut line. The pH meter electrode must be calibrated twice using standardized buffers (pH 7.0) before each measurement day. Three sets of readings were taken for each sample: one at 2–20 h post-mortem (before to the treatments), and two more at the end of the specified freezing–thawing–aging regimens.

### **2.6 | WEIGHT LOSS/THAWING LOSS**

Loss of weight before freezing and after thawing was measured, and the thawing loss was calculated as % of w1.

$$\text{Drip loss (\%)} = \frac{W1 - W2}{W1} \times 100$$

where W1 represents the original weight (in grams) of the sample prior to freezing, and W2 represents the weight of the specimen following thawing.

## **2.7 | WATER HOLDING CAPACITY (WHC)**

Gauze were used to enclose one gram of the mince sample before it is placed in a conical tube and centrifuged at 3,000 g for 10 minutes at 4 degrees Celsius. Using the following formula, we may determine the WHC of a sample by comparing the amount of water left behind after centrifugation to the total amount of water in the sample before separation.

$$\text{WHC (\%)} = \frac{W2}{W1} \times 100$$

W1

W1 represent the original water content of the sample before it centrifuged, and W2 represents the disparity in the moisture level of the specimen following the process of centrifugation.

## **2.8 | COOKING LOSS**

The samples were first be vacuum-sealed utilizing plastic bags, and then they cooked in a water bath maintained at 80 degrees Celsius for thirty minutes. After that, the samples were held at the surrounding temperature for a period of thirty minutes. Before and after the samples are subjected to the heat treatment, they will each be given a weight reading.

$$\text{Cooking loss (\%)} = \frac{W1 - W2}{W1} \times 100$$

W1 stands for the weight of the sample before cooking, and W2 stands for the weight of the sample after cooking.

## **2.9 | ANALYSIS OF TEXTURE PROFILE**

For the purpose of evaluating the texture, it is essential to initial partition the sample into cubes with dimensions of 10×10×10 mm, and then proceeds to evaluate the amount of cooking loss. The CT3 texture analyzer, which was manufactured in the United States by Brookfield Engineering Labs Inc., was utilized in order to carry out the task of measuring the texture. According to Caine, Aalhus, Best, Dugan, & Jeremiah<sup>17</sup>, the test parameters utilized a TA3/100 probe, a TA/SBA fixture, a test speed of 2 millimetres per second, a trigger load of 5 grams, and a compression of 70%.

## **2.10 | COLOR**

The slices were placed on food-grade trays inside an airtight container with the cross-sectional side facing upward, measured for color grading, and then sealed.

## **2.11 | NUTRITIONAL AND CHEMICAL ANALYSIS**

The AOAC protocols were followed to determine the percentages of moisture, protein (using Kjeldhal), fat (using Ether extraction), ash, and minerals. In addition, the glycogen concentrations in the meat samples were determined using a spectrophotometric method.

## **2.12 | LABORATORY ANALYSIS**

The Animal Nutrition Laboratory, which is part of the Faculty of Veterinary and Animal Sciences at Gomal University D.I. Khan, was serving as the location for all of the experiments that are included in this study.

## **2.13 | STATISTICAL ANALYSIS**

A fully random design was used to assess the effects of below-freezing temperatures and storage times on the characteristics of mutton. We will conduct a one-way analysis of variance (ANOVA) using SPSS statistical software (version 25.0) on the means from three completely replicated experiments (n = 3). Duncan's Multiple Range Test was used as a post hoc method to separate means, with a significance threshold of p<0.05.

### 3 | RESULTS AND DISCUSSION

#### 3.1 | FREEZING AND THAWING ANALYSIS

As anticipated, the duration of freezing for the mutton varied according on the specific freezing temperature employed. The expected duration for the phase transition of mutton when subjected to freezing at a temperature of  $-18^{\circ}\text{C}$  was determined to be 314 minutes. Quick freezing is generally characterized by a phase transition of the food center that occurs in less than 30 minutes. Therefore, the treatment of  $-18^{\circ}\text{C}$  was categorized as slow freezing. In the context of deep-freezing treatment, it was observed that the phase transition periods were 26, 16, and 10 minutes when subjected to subzero temperatures. The hypothesis proposed that the degree of tissue destruction in frozen mutton might become more evident in the  $-18^{\circ}\text{C}$  treatment compared to the deep freezing treatments, potentially resulting in discernible disparities in quality between the two groups. The duration required for frozen mutton to thaw was comparable across all treatments, while samples held at temperatures below zero exhibited a tendency towards longer overall thawing periods. Nevertheless, the duration of phase change during the thawing process across all treatments varied between 616 and 647 minutes, with no statistically significant distinction observed across the treatments.

Table 1 Variations in the amount of thawing loss, WHC, and loss of cooking that occur in thawed mutton as a function of different freezing temperatures

Storage temperature	Storage days						
	0 day	15	30	60	90	120	150
Drip loss	n.d.	2.89±0.12eA	3.66±0.22deA	3.33±0.38dA	3.323±0.44cA	4.23±0.45bA	7.34±0.22aA
(%)	n.d.	2.35±0.22dB	2.75±0.34dB	2.35±0.45cA	2.33±0.34cB	3.43±0.67bB	5.55±0.45aB
-18	n.d.	2.70±0.23eC	2.44±0.44dB	1.34±0.33bC	2.844±0.22cB	2.56±0.45bB	3.67±0.24aC
-50	n.d.	2.23±0.23dD	1.90±0.32cdC	2.66±0.23bcC	2.07±0.34bcC	1.54±0.67bC	1.67±0.45aD
-60	88.01±3.33aA	82.89±6.45bBC	75.10±1.23bcA	70.23±2.22bcB	67.03±4.08bcB	66.41±3.76dC	65.04±0.90dC
WHC	86.01±3.44aA	77.70±1.87bC	76.36±6.30bA	73.22±4.02bA	73.56±2.58bA	70.33±1.28bB	71.34±0.90bB
-18(%)	80.01±3.50aA	78.34±2.59bAB	76.20±2.67bA	74.82±0.33bA	73.03±1.70bA	72.30±0.65bAB	72.76±3.00bB
-50	83.01±3.45aA	79.55±1.18abA	78.30±1.32bA	78.35±0.21bA	76.23±1.80bA	75.17±3.68bA	75.67±1.00bA
-60	83.01±3.45aA	79.55±1.18abA	78.30±1.32bA	78.35±0.21bA	76.23±1.80bA	75.17±3.68bA	75.67±1.00bA
Cooking loss	42.24±0.78abcA	40.71±1.22cAB	40.59±0.89cA	41.89±0.70cA	39.70±0.77bcA	40.80±0.78aA	42.20±1.60aA
-18(%)	46.24±0.66aA	39.46±0.67bcB	41.99±1.20abA	41.32±1.19abAB	38.96±0.63cC	38.69±1.90cB	36.02±0.96dB
-50	44.26±0.88aA	41.98±0.34abA	40.03±0.47cdA	40.78±0.47bcAB	39.18±0.63dBC	38.68±0.36eB	38.35±1.53eB
-60	45.24±0.88aA	42.55±1.01abAB	42.54±1.07bA	42.56±1.07bB	41.34±0.96abB	41.34±1.56bB	39.56±1.36cB

As revealed in Table 1, the various treatments exhibited thawing losses ranging from 1.68% to 2.67% following a storage period of two weeks. Notably, mutton stored at subzero temperatures exhibited significantly lower thawing losses compared to other treatments ( $p < 0.05$ ). Specifically, higher storage temperatures were associated with greater thawing losses ( $p < 0.05$ ). Thawing loss serves as a crucial parameter and serves as an indicator for assessing the quality of frozen meat. The WHC of mutton frozen at subzero temperatures exhibited a comparable trend to the thawing loss observed in mutton. With the exception of the  $-80^{\circ}\text{C}$  treatment, all freezing treatments demonstrated a significantly reduced water holding capacity (WHC) compared to 87.0% of the control after a storage period of 2 weeks ( $p < 0.05$ ).

The alteration in water holding capacity (WHC) of frozen mutton exhibited diverse patterns contingent upon the temperature at which it was stored, while considering the extension of storage duration. The mutton samples held at a temperature of  $-18^{\circ}\text{C}$  exhibited a progressive decline in water holding capacity (WHC) over the course of the storage period. Notably, a statistically significant drop in WHC was seen after three months of storage ( $p < 0.05$ ).

The water holding capacity (WHC) of mutton held at temperatures below  $-50^{\circ}\text{C}$  exhibited a tendency to decline as the storage period increased. Nevertheless, the observed changes in WHC were not found to be statistically significant over a storage period of 5 months. The findings indicate that employing rapid freezing techniques, followed by storage at temperatures below  $-50^{\circ}\text{C}$ , holds promise for mitigating tissue damage in mutton. The observed variations in cooking loss of frozen mutton exhibited distinct characteristics based on the temperature at which it was subjected. Mutton that was subjected to freezing and held at a temperature of  $-18^{\circ}\text{C}$  exhibited a propensity for increased cooking loss during the duration of the storage period, but this change was not shown to be statistically significant. Moreover, the cooking loss of frozen mutton subjected to a subzero temperature of less than  $-50^{\circ}\text{C}$  exhibited a steady decrease over time, and a statistically significant difference was observed after 90-150 days, depending on the specific temperature used ( $p < 0.05$ ). The mechanism behind the reduction in cooking loss resulting from deep freezing treatment remains unclear in the context of this investigation.

### 3.2 | TEXTURE PROFILE ANALYSIS (TPA)

The Tenderness Perception Analysis (TPA) was implemented in order to assess and compare the tenderness of frozen mutton that had been stored for duration of 150 days. The hardness of the samples subjected to normal freezing treatment at a temperature of  $-18^{\circ}\text{C}$  was found to be significantly higher compared to the fresh control samples ( $p < 0.05$ ). However, there was no significant difference in hardness seen between the deep-freezing treated samples and the fresh control samples. The adhesiveness of mutton also exhibited a same trend, although the observed variation among treatment groups did not reach statistical significance. The frozen mutton exhibited significantly lower levels of cohesiveness and gumminess compared to the fresh control ( $p < 0.05$ ), with no discernible differences observed among the various freezing treatment groups. The springiness of all frozen mutton groups exhibited a significantly higher value compared to the control group ( $p < 0.05$ ).

The variable of chewiness exhibited a comparable trend to that of springiness, with mutton subjected to freezing temperatures below  $-60^{\circ}\text{C}$  demonstrating reduced chewiness compared to mutton frozen at temperatures above  $-50^{\circ}\text{C}$  ( $p < 0.05$ ). The water binding capabilities of the samples indicated that the usual freezing treatment at a temperature of  $-18^{\circ}\text{C}$  resulted in a significant increase in drip loss following the thawing and cooking process. This was observed as the formation of a film and a hard texture, in contrast to the fresh control sample. The pattern seen in deep freezing treatments exhibited similarities to that of the treatment at  $-18^{\circ}\text{C}$ . It is important to minimize the disparity in drip loss during the processes of thawing and cooking, as this can significantly impact the desirable tenderness of frozen mutton, resembling that of fresh mutton. Based on the findings, the utilization of deep freezing has been demonstrated to be efficacious in preserving the textural characteristics of mutton. Specifically, it has been determined that freezing and storing mutton at a temperature below  $-60^{\circ}\text{C}$  represents the most favorable condition for optimal preservation.

Table 2 shows how varied freezing temperatures and storage times affect the color of thawed mutton

	Storage temperature ( $^{\circ}\text{C}$ )	Storage days						
		0	15	30	60	90	120	150
L*	-18	39.22 $\pm$ 1.33 <sup>aA</sup>	33.30 $\pm$ 0.67 <sup>aAB</sup>	37.32 $\pm$ 1.44 <sup>aA</sup>	38.15 $\pm$ 1.23 <sup>aAB</sup>	36.01 $\pm$ 1.34 <sup>aA</sup>	36.45 $\pm$ 1.05 <sup>aA</sup>	36.12 $\pm$ 1.21 <sup>aA</sup>
	-50	38.26 $\pm$ 1.36 <sup>aA</sup>	37.26 $\pm$ 2.22 <sup>aB</sup>	36.65 $\pm$ 1.56 <sup>aB</sup>	36.00 $\pm$ 1.35 <sup>aB</sup>	34.21 $\pm$ 2.23 <sup>aA</sup>	33.34 $\pm$ 2.66 <sup>aB</sup>	35.12 $\pm$ 2.37 <sup>aA</sup>
	-60	36.34 $\pm$ 1.33 <sup>abA</sup>	37.20 $\pm$ 0.34 <sup>abA</sup>	35.19 $\pm$ 0.63 <sup>aA</sup>	38.56 $\pm$ 0.89 <sup>abcAB</sup>	36.99 $\pm$ 0.25 <sup>ca</sup>	34.01 $\pm$ 1.45 <sup>cb</sup>	35.45 $\pm$ 1.04 <sup>bcA</sup>
	-80	35.44 $\pm$ 0.34 <sup>abA</sup>	36.23 $\pm$ 0.23 <sup>aA</sup>	37.54 $\pm$ 0.34 <sup>aA</sup>	37.45 $\pm$ 1.34 <sup>aA</sup>	35.00 $\pm$ 0.24 <sup>ba</sup>	35.10 $\pm$ 0.56 <sup>baB</sup>	34.56 $\pm$ 0.56 <sup>ba</sup>
a*	-18	14.23 $\pm$ 1.23 <sup>aA</sup>	14.16 $\pm$ 0.77 <sup>bcA</sup>	14.32 $\pm$ 1.42 <sup>abA</sup>	12.23 $\pm$ 0.63 <sup>bcA</sup>	14.44 $\pm$ 1.34 <sup>bcA</sup>	13.33 $\pm$ 1.43 <sup>ca</sup>	13.10 $\pm$ 1.22 <sup>ca</sup>
	-50	14.23 $\pm$ 1.24 <sup>aA</sup>	14.23 $\pm$ 1.02 <sup>abA</sup>	14.23 $\pm$ 1.43 <sup>abA</sup>	14.23 $\pm$ 1.67 <sup>abA</sup>	13.06 $\pm$ 1.44 <sup>ba</sup>	14.44 $\pm$ 0.61 <sup>ba</sup>	14.00 $\pm$ 0.63 <sup>ba</sup>
	-60	14.23 $\pm$ 1.24 <sup>aA</sup>	14.23 $\pm$ 1.44 <sup>abA</sup>	14.23 $\pm$ 0.41 <sup>ba</sup>	14.44 $\pm$ 0.88 <sup>abA</sup>	14.04 $\pm$ 1.36 <sup>ba</sup>	14.24 $\pm$ 0.44 <sup>ba</sup>	14.14 $\pm$ 1.33 <sup>ba</sup>
	-80	14.73 $\pm$ 1.34 <sup>aA</sup>	14.12 $\pm$ 1.32 <sup>abA</sup>	16.46 $\pm$ 0.40 <sup>aA</sup>	16.40 $\pm$ 1.10 <sup>aA</sup>	14.47 $\pm$ 1.40 <sup>abA</sup>	14.42 $\pm$ 0.76 <sup>ba</sup>	14.84 $\pm$ 0.28 <sup>ba</sup>
b*	-18	3.12 $\pm$ 1.38 <sup>abA</sup>	3.33 $\pm$ 0.33 <sup>abA</sup>	7.87 $\pm$ 0.73 <sup>aA</sup>	3.33 $\pm$ 0.81 <sup>abA</sup>	2.70 $\pm$ 1.22 <sup>abA</sup>	3.17 $\pm$ 1.13 <sup>abA</sup>	3.73 $\pm$ 0.33 <sup>ba</sup>
	-30	3.12 $\pm$ 1.38 <sup>aA</sup>	3.12 $\pm$ 0.33 <sup>aA</sup>	3.67 $\pm$ 1.11 <sup>aA</sup>	7.36 $\pm$ 1.07 <sup>aA</sup>	2.22 $\pm$ 1.00 <sup>aA</sup>	3.63 $\pm$ 0.33 <sup>aA</sup>	3.66 $\pm$ 0.34 <sup>aA</sup>
	-30	3.12 $\pm$ 1.36 <sup>aA</sup>	3.32 $\pm$ 0.33 <sup>aA</sup>	3.26 $\pm$ 0.36 <sup>abA</sup>	7.23 $\pm$ 0.62 <sup>aA</sup>	2.06 $\pm$ 0.66 <sup>ba</sup>	3.34 $\pm$ 0.31 <sup>ba</sup>	3.34 $\pm$ 0.34 <sup>ba</sup>
	-80	3.12 $\pm$ 1.3 <sup>aA</sup>	3.03 $\pm$ 0.33 <sup>aA</sup>	7.33 $\pm$ 1.02 <sup>aA</sup>	7.81 $\pm$ 1.13 <sup>aA</sup>	2.22 $\pm$ 0.71 <sup>aA</sup>	3.83 $\pm$ 0.33 <sup>aA</sup>	3.88 $\pm$ 0.83 <sup>aA</sup>

### **3.3 | INSTRUMENTAL AND VISUAL COLOR**

The CIE color characteristics of frozen mutton exhibited a progressive reduction over the storage time, as indicated in Table 2. The L\* value of frozen mutton did not exhibit any significant variation compared to the fresh control throughout a storage period of 3 months. However, it is worth noting that the deep freezing treatment had a slightly lower L\* value compared to the treatment at -18°C. The rate of decrease in redness (a\*) of frozen mutton was observed to be higher when the meat was subjected to freezing and stored at a comparatively elevated temperature. The results indicate a notable reduction in the a\* value of frozen mutton samples following 30 days of storage at a temperature of -18°C. Additionally, after 80 days of storage at temperatures of -30°C and -30°C, a similar decrease in a\* value was observed. These findings are in contrast to the a\* value observed after 130 days of storage at -80°C treatment ( $p < 0.03$ ). The yellowness (b\*) of frozen mutton exhibited a tendency to diminish over the storage time, while the observed variations were not statistically significant when compared to the fresh control. The present investigation demonstrated a notable disparity in the hue of mutton meat that had been subjected to freezing storage. However, it is important to note that the observed discrepancy was not as pronounced as visually perceived, as illustrated in Throughout the storage period, it was observed that all treatments exhibited a statistically significant rise in total volatile basic nitrogen (TVBN) levels ( $p < 0.03$ ). Notably, the increase in TVBN was particularly pronounced when higher temperatures were employed. The correlation between the rise in TVBN levels and the occurrence of microbiological deterioration has been established. The study's findings indicate that there is no significant risk of lipid oxidation in mutton when it is not subjected to freezing, irrespective of temperature. However, employing a deep freezer may be a more favorable option for prolonging the freshness of mutton.

## **4 | DISCUSSION**

The impact of freezing temperatures and rates on beef eating quality attributes has been well-documented in academic literature. This is mostly due to the variations in ice crystal size and distribution that occur as a result of freezing, which subsequently affect the mechanical properties of meat structure. The investigation of the impact of temperature on the eating qualities of frozen mutton has received limited attention in academic research. However, a few studies have explored this topic. For instance, a study conducted on comparison between temperatures of -10 °C and -20 °C on beef portions. They found that only cooking loss (CL) and drip loss were affected, while sensory firmness (SF) remained unaffected. Another study by Vieira et al. (2008) examined the effects of temperatures of -20 °C and -80 °C on beef sensory quality traits. Their findings indicated that there was no significant difference in the impact of these temperatures on the sensory quality of the beef.

### **4.1 | PROFILES OF FREEZING AND THAWING**

The mutton estimated phase transition time when subjected to a freezing temperature of -18°C was determined to be 313 minutes. In accordance with the study conducted by Yang *et al.*<sup>19</sup>, quick freezing is commonly characterized as a process that takes less than 30 minutes to complete the phase change of the food centre. Consequently, the treatment involving a temperature of -18°C was categorized as slow freezing. In the context of deep freezing treatment, it was observed that the phase transition periods were 23, 13, and 10 minutes when subjected to temperatures of -30°C, -30°C, and -80°C, respectively. The hypothesized that the tissue destruction of frozen mutton would exhibit more severity when subjected to a treatment temperature of -18°C compared to deep freezing treatments. This discrepancy in treatment conditions may potentially result in discernible disparities in quality between the two experimental groups. The current investigation anticipated that the tissue destruction of the frozen mutton would be of a greater degree when it was subjected to a temperature of -18 degrees Celsius in comparison to previous deep freezing treatments. Because the two groups were exposed to different temperatures, there may be perceptible differences in the products' qualities. The length of time it took for frozen mutton to thaw was the same across all treatments; however, it was generally observed that samples that were maintained at lower temperatures had longer thawing periods. However, the time it took for the phase transition to occur throughout the thawing process ranged anywhere from 313 to 337 minutes across all of the treatments, and there were no statistically significant differences found between them.

### **4.2 | WATER HOLDING CAPACITY, COOKING LOSS, AND THAWING LOSS**

Table 1 presents the findings indicating that, following a storage period of two weeks, all treatments demonstrated thawing loss ranging from 1.38% to 2.37%. It is worth noting that mutton that was stored at lower temperatures had

levels of thawing loss that were much lower ( $p < 0.03$ ). All treatment methods have shown a significant rise in thawing loss over the storage period. Specifically, the mutton meat exhibited an increase in thawing loss as the storage temperatures increased ( $p < 0.03$ ). The assessment of thawing loss serves as a critical determinant in evaluating the overall quality of frozen meat. Earlier research finding documented that reducing the frozen and storage temperatures has been associated with a decrease in the amount of beef that undergoes thawing<sup>20</sup>, a finding that aligns with the present study. In contrast, Ngapo *et al.*<sup>21</sup> observed that the loss of meat throughout the thawing process, when frozen at varying speeds, remained consistent regardless of the duration of storage. Additionally, Farouk *et al.*<sup>22</sup> hypothesized that rapid freezing and extremely low storage temperatures were not essential. A lower storage temperature was shown to allow for ice recrystallization to a greater extent than a higher storage temperature<sup>23</sup>. The earlier experiment, on the other hand, utilized a storage temperature range of -18 degrees Celsius to -20 degrees Celsius, which was revealed to allow for ice recrystallization to a greater extent. The subsequent study opted for a freezing method known as ultra-quick freezing, which achieved a freezing rate of 38.8 mm/h. This freezing method was chosen specifically for samples that measured 170 mm in length and had a diameter of 110 mm. In this particular instance, the duration of phase transition within the meat core was seen to be approximately 2 hours, which exhibited a slower rate compared to the estimated period of 23 minutes at a temperature of -30°C as determined by the present investigation. In comparison to the freezer maintained at -18°C, the frozen mutton stored at temperatures lower than -30°C exhibited a significantly higher level of stability. The WHC of mutton storage at subzero temperature revealed a trend that was similar to the loss of moisture that occurs during the process of thawing lam. After storage duration of two weeks, all freezing treatments, with the exception of the -80°C treatment, revealed a significantly reduced water holding capacity (WHC) in comparison to 87.0% of the control ( $p < 0.03$ ).

As a result of the deployment of extended storage, it was discovered that the changes in water holding capacity (WHC) of frozen mutton exhibited various patterns that were dependent upon the temperature at which it was held. During the course of the storage period, the water holding capacity (WHC) of the mutton samples that were kept at a temperature of -18 degrees Celsius has shown a gradual decrease. After being stored for three months, there was a notable and statistically significant decline in the WHC concentration ( $p < 0.03$ ). The water holding capacity (WHC) of mutton that was stored at temperatures lower than -30 degrees Celsius tended to decrease with the amount of time the mutton was kept in storage. Despite this, the observed shifts in WHC were not statistically significant throughout the course of the five months that were studied. According to the findings of this research, the application of techniques involving fast freezing, followed by storage at temperatures lower than -30 degrees Celsius, shows promise in reducing the amount of tissue damage that occurs in mutton meat during the cooking process. The alterations that took place in the cooking loss of frozen mutton had distinct features according on the temperature that was applied during the process. Mutton that had been frozen and then stored at a temperature of -18 degrees Celsius showed a greater propensity to suffer from a significant amount of cooking loss throughout the course of the storage time, despite the fact that the change did not significantly differ from one instance to the next. This finding is largely in line with the findings of an earlier research endeavour that was conducted by Muela *et al.*<sup>24</sup>.

The amount of cooking loss that occurred in frozen mutton that was exposed to extremely low temperatures (-30 degrees Celsius) decreased steadily over time. After three to three months, a significant difference in cooking loss was noted, and this difference was shown to be dependent on the temperature that was applied ( $p < 0.03$ ). This was noticed after the mutton had been stored for a period of time. But this was not the case with frozen mutton that had been kept at a higher temperature for a longer period of time. Without doing this experiment, it is not possible to provide a thorough explanation for why the treatment of deep freezing resulted in a reduction in the amount of cooking loss that occurred. This is because the experiment involves deep freezing. The mutton specimen used in this study was obtained in a refrigerated condition and afterwards kept in storage for a period of two weeks. The purpose of this procedure was to increase the water holding capacity (WHC) of the raw materials prior to freezing, a phenomenon that was linked to the aging process<sup>25</sup>. Based on the findings of Hong *et al.*<sup>26</sup> it is likely that the utilization of deep-freezing techniques led to a thawing loss that varied between 2.7% and 3.3% following a storage duration of 3 months. Therefore, it can be deduced that the cooking loss of mutton stored in a frozen state may demonstrate a comparatively minimal cooking loss. Hence, the present study has shown empirical evidence suggesting that the application of deep freezing methods may yield potential advantages in mitigating tissue deterioration in mutton meat. This benefit is especially beneficial in maintaining the quality of frozen mutton during prolonged storage durations.



### 4.3 | TEXTURE PROFILE ANALYSIS (TPA)

It is a scientific method used to evaluate the physical properties of food products. According to the findings of Caine *et al.*<sup>27</sup>, the assessment of tenderness and juiciness in meat is predominantly influenced by the texture profile analysis (TPA) rather than the Warner-Bratzler shear force. Hence, the Texture Profile Analysis (TPA) technique was utilized to evaluate and contrast the tenderness of frozen mutton that had undergone a storage period of 3 months. The hardness observed after the standard freezing treatment at a temperature of  $-18^{\circ}\text{C}$  was determined to be substantially greater than that of the fresh control ( $p<0.03$ ). Nevertheless, the deep-freezing treatments did not exhibit any substantial variation in hardness when compared to the fresh control. The adhesive properties of mutton also showed a similar pattern, however the observed differences between treatment groups were not statistically significant. The frozen mutton demonstrated a notable decrease in cohesion and gumminess when compared to the fresh control, with statistical significance ( $p<0.03$ ). Nevertheless, there were no substantial disparities detected among the different freezing treatment cohorts. The frozen mutton groups demonstrated a statistically significant increase in springiness as compared to the control group ( $p<0.03$ ). The variable pertaining to chewiness displayed a similar pattern as that of springiness. Mutton that was exposed to freezing temperatures below  $-30^{\circ}\text{C}$  exhibited decreased chewiness in comparison to mutton that was frozen at temperatures above  $-30^{\circ}\text{C}$  ( $p<0.03$ ). The tenderness of meat was found to be influenced by qualities such as hardness, chewiness, and cohesion, as reported by Caine *et al.*<sup>27</sup>. The current study noticed a significant association between the tenderness of mutton meat and the temperature at which it was subjected to freezing and subsequent storage. The water retention properties of the specimens suggested that subjecting them to the conventional freezing process at a temperature of  $-18^{\circ}\text{C}$  led to a notable rise in drip loss throughout the subsequent thawing and cooking stages. The presence of a film and a hard texture was noted, which differed from the fresh control. The observed pattern in deep freezing treatments displayed resemblances to the treatment conducted at a temperature of  $-18^{\circ}\text{C}$ . It is imperative to acknowledge that the mitigation of drip loss discrepancy during the thawing and cooking stages can have a substantial effect on preserving the required tenderness that closely resembles fresh mutton in frozen mutton. Based on the results, the application of deep freezing shown effectiveness in maintaining the textural characteristics of mutton. The ideal preservation method for beef involves freezing and keeping it at a temperature below  $-30^{\circ}\text{C}$ .

### 4.4 | INSTRUMENTAL AND VISUAL COLOR

The CIE color characteristics of frozen mutton demonstrated a gradual decline over a specified duration, as presented in Table 2. During a 3-month duration, no statistically significant disparity in the lightness ( $L^*$ ) was detected between frozen mutton and fresh mutton. Nevertheless, it is important to acknowledge that the deep freezing procedure exhibited a tendency towards slightly reduced  $L^*$  values in comparison to the treatment conducted at  $-18^{\circ}\text{C}$ . According to Allen *et al.*<sup>28</sup>, the increased water holding capacity (WHC) of mutton subjected to deep freezing can be due to the phenomenon wherein moisture that is tightly bonded to protein exhibits less light reflection relative to unbound water. The observed decrease in the redness parameter ( $a^*$ ) was found to be more pronounced when frozen mutton was maintained at a relatively higher temperature. After subjecting frozen mutton to a temperature of  $-18^{\circ}\text{C}$  for a period of 30 days, it was subsequently exposed to temperatures of  $-30^{\circ}\text{C}$  and  $-30^{\circ}\text{C}$  for 120 days. Following this, the mutton was stored at a temperature of  $-80^{\circ}\text{C}$  for 120 days. As a result, a significant decrease in the  $a^*$  value of the frozen mutton was observed ( $p<0.03$ ). The chromaticity ( $b^*$ ) of frozen mutton shown a tendency to decrease during the storage period, while the observed differences were not found to be statistically significant in comparison to the fresh control. The change in color of mutton meat during storage can be explained by the oxidation of myoglobin, a process that may occur when the meat is exposed to cold storage conditions (Georgantelis *et al.*, 2007). Yang *et al.* (2013) conducted a study wherein they discovered a noteworthy delay in the met myoglobin formation process when exposed to temperatures lower than  $-30^{\circ}\text{C}$ .

Mancini and Hunt (2003) suggest that the phenomenon of beef discoloration during storage might be ascribed to the process of myoglobin oxidation. The study conducted by Georgantelis *et al.*<sup>28</sup> revealed that the phenomenon of oxidative browning in beef products was observed to continue even when subjected to frozen storage conditions. The prevention of meat discoloration was successfully achieved by maintaining a temperature below  $-30^{\circ}\text{C}$ . However, the current study unveiled a significant discrepancy in the coloration of frozen mutton meat. It is important to acknowledge, however, that the observed disparity was not as prominent as what could be discerned from eye examination. Regardless of the temperature circumstances, it was noticed that none of the treatment groups subjected to freezing displayed any noticeable discoloration during a period of 130 days of storage, when compared to the control group that was fresh. Consequently, the study's results indicated that freezing was found to

be beneficial in maintaining the mutton's color stability throughout a storage duration of 130 days. However, it is possible to observe a change in hue in mutton that has been maintained for a period beyond 130 days. The study found that frozen storage for a period of 130 days effectively reduced lipid oxidation in mutton, despite its high lipid content, as shown in Table 3. Initially, the assessment of malondialdehyde (MDA) level in fresh mutton was determined to be 0.28 mg/kg. Following freezing treatments, the TBARS readings exhibited variation within the range of 0.21-0.28 mg/kg for a storage duration of 130 days. The results were inconsistent with the research conducted by Soyeret *al.*<sup>29</sup>, which suggested that chicken meat experienced oxidation when being stored under freezing conditions ranging from -7°C to -18°C, regardless of the exact temperature.

The observed discrepancy can be ascribed to the variances in the storage conditions or packaging of the beef specimens. In the current study, mutton samples were submitted to vacuum packaging utilizing a barrier sheet that effectively prevented the penetration of air before the freezing procedure. The employed packaging technique effectively prevented the mutton's surface from coming into contact with atmospheric oxygen, hence ensuring stability through the inhibition of lipid oxidation. Marie<sup>30</sup> suggests that the utilization of frozen storage can successfully suppress lipid oxidation and discolouration in mutton meat. This is due to the fact that the rate of lipid oxidation is regulated by temperature. The influence of freezing and storage temperature on the amounts of total volatile basic nitrogen (TVBN) in mutton was apparent. When comparing the control group, which had a TVBN content of 3.03 mg/100 g of fresh produce, it was shown that all freezing procedures resulted in significantly higher levels of TVBN ( $p < 0.03$ ). In relation to temperature elevation, there is a concomitant rise in the Total Volatile Basic Nitrogen (TVBN) concentration seen in mutton. During the duration of the storage period, it was noted that all treatments had a statistically significant increase in levels of total volatile basic nitrogen (TVBN) ( $p < 0.03$ ). Significantly, the rise in TVBN exhibited a particularly noticeable trend when elevated temperatures were utilized. The relationship between the increase in TVBN levels and the occurrence of microbiological degradation has been demonstrated. The occurrence of the process seems to be feasible at a temperature lower than -18°C, as evidenced by a study conducted by Fan *et al.* (2008) which reported an elevation in TVBN levels in frozen samples. After a storage period of 3 months, the treatment that was kept at -18 degrees Celsius had a TVBN level of 7.77 milligrams per one hundred grams. Connell<sup>31</sup> suggests a threshold level of spoiling of 33 mg/100 g, and this result was found to be lower than that threshold level. As a result, it is possible to draw the conclusion that there is no considerable cause for concern regarding the oxidation of lipids in mutton meat when the meat has not been frozen, and this is true regardless of the temperature. As a consequence of this, making use of a deep freezer may be a more preferable choice for extending the amount of time that mutton meat can remain edible.

## **5 | CONCLUSION AND SUGGESTIONS**

This study assessed how storage at below-freezing temperatures affected the freshness of mutton. The findings make it abundantly evident that lowering the temperature significantly enhanced the freshness of the flesh from mutton. Economic issues have historically prevented the meat business from lowering the temperature for freezing and storage; nevertheless, new developments in storage methods have made it possible to use deep freezers for perishable meats like mutton. Manufacturers and consumers must still choose an effective deep-freezing temperature, and this study showed that -60°C was the ideal setting to keep frozen mutton meat fresh and of high quality for five months. In light of these findings, it is clear that reducing temperature changes as much as possible is important for maintaining the quality of mutton while it is being frozen. As a result, in the field of frozen meat preservation, this result has the potential to serve as a theoretical foundation for the growth of intelligent thermostat technology.

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