



# Effect of Temperature Abuse on Physico-Chemical, Instrumental, Microbiological and Sensory Parameters of Frozen Chicken Meat

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## Abstract

*A study was conducted to evaluate the quality changes in frozen chicken meat under various temperature abuse conditions. The conditioned meat was packed in polypropylene trays covered with polypropylene film with overwrap and stored in a deep freezer (-18±10 °C) for 24 hours. The frozen chicken meat was temperature abused to different temperatures of 0±1 °C, 5±1 °C, 10±1 °C and refrozen to -18 ±10 °C. One sample kept at -18 ±10 °C served as control. All the samples, including control were evaluated for Physico-chemical parameters [pH, Drip loss, Extract Release Volume (ERV), Total Volatile Basic Nitrogen (TVBN)], Instrumental (color) and Microbiological evaluation (Total Plate Count, Psychrophilic count, Yeast and mould count) and various sensory attributes. Of the various meat quality parameters evaluated pH, Drip loss, Total Volatile Basic Nitrogen (TVBN), Total Plate Counts (TPC), Psychrophilic count and Yeast and mould count were increased significantly with an increase in thawing temperature. Whereas ERV and sensory attributes were decreased significantly when compared to the control sample.*

**Keywords:** Meat Quality, Temperature Abuse, Total Volatile Basic Nitrogen.



## Introduction

Chicken meat is generally being exported in the frozen state. Further delay in shipment and fluctuations in market rates necessitate the storage of consignment for longer period. Sometimes due to break in cold chain during transport or storage, meat may pass through freeze thaw cycle. Under such circumstances, the meat is exposed to warm and fluctuating temperature for a period varying from 2-3 hours and sometimes even longer depending on the situation. Excessive thawing duration or unusually high temperature thawing may be the most common forms of temperature abuse in the case of meat and meat products. The most important factor concerned with preservation of quality of meat and meat products is the control of temperature throughout supply chain. Any fluctuation in temperature at any point during transport and subsequent storage in supply chain will lead to food safety issues and decreased acceptance of the product or complete rejection due to the deteriorative changes in quality that develops as a result of temperature abuse. Thus, an efficient system that can monitor any form of temperature abuses like thawing associated with accidental power failure or due to consumer negligence is required to check the food spoilage in the supply chain. The meat spoilage in supply chain creates great economic losses to producers and consumers in terms of reduced sale of products and treatment of food poisoning respectively. The objective of the present study was to evaluate quality of frozen chicken meat at different temperature abuse conditions

## Materials and Methods

### *Meat Sample*

Broiler birds were procured from local market of Chengicherla, and slaughtered in experimental abattoir of ICAR-National Research Centre on Meat, Hyderabad adopting traditional halal method. After deboning, breast muscles were collected from carcass. Then the visible fat and connective tissue residues were removed using a sterile, sharp stainless steel knife. The meat was packaged into LDPE bags and kept for conditioning at  $4\pm 1^{\circ}\text{C}$  for about 1 hour. The conditioned meat of about 250g was then packaged in polypropylene trays covered with polypropylene film overwrap and stored in deep freezer ( $-18\pm 1^{\circ}\text{C}$ ) for 24 h.

### *Temperature Abuse Study*

The conditioned, frozen chicken breast meat samples were used for the temperature abuse study. Temperature abuse conditions were simulated in the freezer. Freezer was subjected to power failure after 24h for a considerable time period so that internal temperature of frozen chicken meat ( $-18\pm 1^{\circ}\text{C}$ ) reaches  $0\pm 1^{\circ}\text{C}$ ,  $5\pm 1^{\circ}\text{C}$  and  $10\pm 1^{\circ}\text{C}$ . The temperature abuse conditions were chosen so as to keep the meat above its freezing point i.e.  $-1.5^{\circ}\text{C}$ . The temperature abuse conditions were monitored by using probe thermometer (BT-2 Digi-thermo, China). It took 12h to reach an internal temperature of  $-18\pm 1^{\circ}\text{C}$  in meat. The time taken for attaining the internal temperature of frozen chicken meat from  $-18\pm 1^{\circ}\text{C}$  to  $0\pm 1^{\circ}\text{C}$ ,  $5\pm 1^{\circ}\text{C}$  and  $10\pm 1^{\circ}\text{C}$  were 6 hours 45 minutes, 7 hours 50 minutes and 8 hours 40 minutes respectively. Once the frozen meat reached the thawing temperatures the samples were drawn immediately for analysis of various quality parameters. One control sample was kept at  $-18\pm 1^{\circ}\text{C}$  without any temperature abuse exposure and compared with the exposed samples. The meat samples were also refrozen to  $-18\pm 1^{\circ}\text{C}$  after exposing to various temperature abuse conditions. The samples were analyzed after 24h of refreezing.

The various groups of samples used for the study are as follows:

- C Frozen meat ( $-18\pm 1^{\circ}\text{C}$ ) maintained at  $-18\pm 1^{\circ}\text{C}$
- T1 Frozen meat ( $-18\pm 1^{\circ}\text{C}$ ) thawed up to an internal temperature of  $0\pm 1^{\circ}\text{C}$
- T2 Frozen meat ( $-18\pm 1^{\circ}\text{C}$ ) thawed up to an internal temperature of  $0\pm 1^{\circ}\text{C}$  and refrozen to  $-18\pm 1^{\circ}\text{C}$
- T3 Frozen meat ( $-18\pm 1^{\circ}\text{C}$ ) thawed up to an internal temperature of  $5\pm 1^{\circ}\text{C}$
- T4 Frozen meat ( $-18\pm 1^{\circ}\text{C}$ ) thawed up to an internal temperature of  $5\pm 1^{\circ}\text{C}$  and refrozen to  $-18\pm 1^{\circ}\text{C}$
- T5 Frozen meat ( $-18\pm 1^{\circ}\text{C}$ ) thawed up to an internal temperature of  $10\pm 1^{\circ}\text{C}$
- T6 Frozen meat ( $-18\pm 1^{\circ}\text{C}$ ) thawed up to an internal temperature of  $10\pm 1^{\circ}\text{C}$  and refrozen to  $-18\pm 1^{\circ}\text{C}$

## **Meat Quality Evaluation**

### **pH and Extract Release Volume (ERV)**

The pH of meat sample was determined as per Trout *et al.*, (1992). The homogenate was prepared by blending 10g sample with 90ml distilled water using an Ultra Turrax tissue homogenizer (Model IKA T-25, Janke and Kenkel, IKA Labor Technik, Germany) for one minute. Then the pH was recorded by immersing combined glass electrode of digital pH meter (Thermo Orion, Model 420 A<sup>+</sup>, USA) into the meat homogenate. The ERV was estimated according to the procedure described by Strange *et al.* (1977) with suitable modifications.

### **Drip Loss**

Drip loss of meat samples were determined by the method of AOAC (1995). Drip was measured after thawing the frozen meat sample and comparing the thawed meat sample weight with the initial weight of the frozen meat.

$$\text{Drip loss (\%)} = \frac{\text{Initial weight of the frozen meat sample} - \text{Final weight of meat sample after thawing}}{\text{Initial weight of the frozen meat sample}} \times 100$$

### **Total Volatile Basic Nitrogen (TVBN)**

Total volatile basic nitrogen (TVBN) of chicken meat samples were determined by the procedure of Pearson (1968) following micro-diffusion technique with slight modifications.

TVBN content was calculated by using the formula:

$$\text{TVBN (mg/100g of meat)} = \frac{\text{Volume of 0.02N H}_2\text{SO}_4 \text{ consumed}}{\text{Normality of acid used for titration}} \times 14 \times 100$$

### **Instrumental Hunter lab colour values**

The instrumental colour was measured using Hunter Lab apparatus (Lovibond-RT-500 series, serial number: 35177, WO-A26424, Dortmund, Germany). Meat sample was placed below the disc of Hunter Lab apparatus. The Lightness (L\*), Redness (a\*), Yellowness (b\*) and Colour difference ( $\Delta E$ ) colour units were recorded by comparing sample with that of standard.

### **Microbiological Analysis**

All the microbiological parameters of meat sample including total plate count, yeast and mould count, and psychrophilic count were determined as per the methods described by APHA (2001).

### **Sensory Evaluation**

The sensory quality of control as well as treatment samples was judged on the basis of appearance, colour, odour and sliminess characteristics. The samples were subjected to sensory evaluation by sensory panel consisting of a minimum of seven members. A 5-point descriptive scale was used for scoring the sensory attributes of frozen-thawed meat. A minimum of seven values were collected for each sample and the sensory evaluation was repeated thrice.

### **Statistical Analysis**

The experiment was repeated a minimum of three times in duplicate and the data generated for different meat quality parameters were compiled and analyzed using SPSS (version 20.0 for Windows; SPSS, Chicago, 111., U.S.A.). The data were subjected to analysis of variance, (one way ANOVA for different temperature abuse), least significant difference and Duncan's multiple range test for comparing the means to find the difference

between groups and different temperature abuse. The smallest difference ( $D_5\%$ ) for two means was reported as significantly different ( $P < 0.05$ ).

## Results

### Physico-Chemical Parameters

The pH, Drip loss, TVBN of frozen chicken meat sample was found to be increased significantly ( $P < 0.05$ ) but ERV value of frozen chicken meat sample was found to be decreased significantly ( $P < 0.05$ ) when subjected to temperature abuse conditions (Table 1).

**Table 1:** Physico - chemical parameters of frozen chicken meat subjected to different temperature abuse conditions (Mean  $\pm$  SE)

PARAMETER	C	T1	T2	T3	T4	T5	T6
pH	5.65 $\pm$ 0.01 <sup>d</sup>	5.75 $\pm$ 0.02 <sup>c</sup>	5.73 $\pm$ 0.01 <sup>c</sup>	5.97 $\pm$ 0.01 <sup>b</sup>	5.94 $\pm$ 0.01 <sup>b</sup>	6.10 $\pm$ 0.03 <sup>a</sup>	6.06 $\pm$ 0.02 <sup>a</sup>
DRIP LOSS (%)	0.00 $\pm$ 0.00 <sup>d</sup>	1.37 $\pm$ 0.02 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	2.72 $\pm$ 0.07 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	5.81 $\pm$ 0.01 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>d</sup>
ERV (ml)	34.50 $\pm$ 0.80 <sup>a</sup>	31.00 $\pm$ 0.81 <sup>b</sup>	30.83 $\pm$ 0.54 <sup>b</sup>	27.83 $\pm$ 0.60 <sup>c</sup>	27.66 $\pm$ 0.55 <sup>c</sup>	20.33 $\pm$ 0.71 <sup>d</sup>	21.00 $\pm$ 0.63 <sup>d</sup>
TVBN (mg/100g)	7.93 $\pm$ 0.29 <sup>e</sup>	9.10 $\pm$ 0.31 <sup>cd</sup>	8.86 $\pm$ 0.29 <sup>d</sup>	10.26 $\pm$ 0.29 <sup>bc</sup>	9.80 $\pm$ 0.36 <sup>c</sup>	11.90 $\pm$ 0.70 <sup>a</sup>	11.43 $\pm$ 0.56 <sup>ab</sup>

$n=6$ ; C=Frozen meat maintained at  $-18\pm 1^\circ\text{C}$ ; T1= Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $0\pm 1^\circ\text{C}$ ; T2 =Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $0\pm 1^\circ\text{C}$  and refrozen to  $-18\pm 1^\circ\text{C}$ ; T3=Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $5\pm 1^\circ\text{C}$ ; T4=Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $5\pm 1^\circ\text{C}$  and refrozen to  $-18\pm 1^\circ\text{C}$ ; T5= Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $10\pm 1^\circ\text{C}$ ; T6=Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $10\pm 1^\circ\text{C}$  and refrozen to  $-18\pm 1^\circ\text{C}$ . Means with different superscripts in the same row indicate significance difference ( $P < 0.05$ )

**Table 2:** Hunter lab colour values of frozen chicken meat subjected to different temperature abuse conditions (Mean  $\pm$  SE)

PARAMETER	C	T1	T2	T3	T4	T5	T6
Lightness (L value)	44.65 $\pm$ 1.63 <sup>a</sup>	40.10 $\pm$ 0.57 <sup>b</sup>	40.92 $\pm$ 1.06 <sup>b</sup>	37.78 $\pm$ 0.42 <sup>c</sup>	38.64 $\pm$ 1.12 <sup>c</sup>	33.08 $\pm$ 0.53 <sup>d</sup>	34.72 $\pm$ 0.84 <sup>d</sup>
Redness (a value)	5.65 $\pm$ 0.29 <sup>a</sup>	4.99 $\pm$ 0.23 <sup>a</sup>	5.01 $\pm$ 0.24 <sup>a</sup>	3.69 $\pm$ 0.23 <sup>b</sup>	3.72 $\pm$ 0.23 <sup>b</sup>	2.81 $\pm$ 0.33 <sup>c</sup>	2.83 $\pm$ 0.39 <sup>c</sup>
Yellowness (b value)	20.17 $\pm$ 0.48 <sup>d</sup>	21.48 $\pm$ 0.40 <sup>c</sup>	21.02 $\pm$ 0.40 <sup>c</sup>	23.83 $\pm$ 0.33 <sup>b</sup>	23.22 $\pm$ 0.28 <sup>b</sup>	25.55 $\pm$ 0.44 <sup>a</sup>	25.03 $\pm$ 0.27 <sup>a</sup>
Colour difference ( $\Delta E$ value)	28.26 $\pm$ 0.33 <sup>a</sup>	26.61 $\pm$ 0.36 <sup>b</sup>	27.02 $\pm$ 0.27 <sup>b</sup>	22.94 $\pm$ 0.28 <sup>c</sup>	22.77 $\pm$ 0.44 <sup>c</sup>	19.37 $\pm$ 0.41 <sup>d</sup>	20.47 $\pm$ 0.58 <sup>d</sup>

$n=6$ ; C=Frozen meat maintained at  $-18\pm 1^\circ\text{C}$ ; T1= Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $0\pm 1^\circ\text{C}$ ; T2 =Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $0\pm 1^\circ\text{C}$  and refrozen to  $-18\pm 1^\circ\text{C}$ ; T3=Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $5\pm 1^\circ\text{C}$ ; T4=Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $5\pm 1^\circ\text{C}$  and refrozen to  $-18\pm 1^\circ\text{C}$ ; T5= Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $10\pm 1^\circ\text{C}$ ; T6=Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $10\pm 1^\circ\text{C}$  and refrozen to  $-18\pm 1^\circ\text{C}$ . Means with different superscripts in the same row indicate significance difference ( $P < 0.05$ )

### Hunter Lab Colour Values of Meat Sample at Different Temperature Abuse Conditions

Temperature abused frozen chicken meat samples showed a significantly ( $P < 0.05$ ) lower lovibond tintometer lightness (L), redness (a) and colour difference ( $\Delta E$ ) units, immediately after temperature abuse study as compared to control sample (Table 2). But the yellowness (b) showed a significantly ( $P < 0.05$ ) higher value in temperature abused frozen chicken meat samples than control sample.

### Microbiological and Sensory Parameters

Total plate count, psychrophilic count and yeast and mold count of temperature-abused chicken meat samples showed significantly ( $P < 0.05$ ) higher values than control samples (Table 3). Temperature abused frozen chicken meat samples showed a significantly ( $P < 0.05$ ) lower appearance score, colour score and odour score compared to control sample immediately after the temperature abuse study. There is no sliminess was observed in all groups

(Table 4).

**Table 3:** Microbiological characteristics of frozen chicken meat subjected to different temperature abuse conditions (Mean  $\pm$  SE)

PARAMETER	C	T1	T2	T3	T4	T5	T6
TPC (log cfu/g)	2.85 $\pm$ 0.28 <sup>e</sup>	3.00 $\pm$ 0.01 <sup>cd</sup>	2.98 $\pm$ 0.02 <sup>d</sup>	3.15 $\pm$ 0.03 <sup>b</sup>	3.12 $\pm$ 0.03 <sup>b</sup>	3.41 $\pm$ 0.06 <sup>a</sup>	3.37 $\pm$ 0.03 <sup>a</sup>
Psychrophilic count (log cfu/g)	1.08 $\pm$ 0.05 <sup>c</sup>	1.52 $\pm$ 0.26 <sup>bc</sup>	1.44 $\pm$ 0.15 <sup>bc</sup>	1.76 $\pm$ 0.24 <sup>abc</sup>	1.63 $\pm$ 0.35 <sup>abc</sup>	2.26 $\pm$ 0.15 <sup>a</sup>	2.13 $\pm$ 0.16 <sup>ab</sup>
Yeast and mold count (log cfu/g)	1.10 $\pm$ 0.05 <sup>b</sup>	1.24 $\pm$ 0.10 <sup>ab</sup>	1.29 $\pm$ 0.16 <sup>ab</sup>	1.56 $\pm$ 0.29 <sup>ab</sup>	1.60 $\pm$ 0.27 <sup>ab</sup>	1.88 $\pm$ 0.18 <sup>a</sup>	1.79 $\pm$ 0.19 <sup>a</sup>

n=6; C=Frozen meat maintained at  $-18\pm 1^\circ\text{C}$ ; T1= Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $0\pm 1^\circ\text{C}$ ; T2 =Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $0\pm 1^\circ\text{C}$  and refrozen to  $-18\pm 1^\circ\text{C}$ ; T3=Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $5\pm 1^\circ\text{C}$ ; T4=Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $5\pm 1^\circ\text{C}$  and refrozen to  $-18\pm 1^\circ\text{C}$ ; T5= Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $10\pm 1^\circ\text{C}$ ; T6=Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $10\pm 1^\circ\text{C}$  and refrozen to  $-18\pm 1^\circ\text{C}$   
Means with different superscripts in the same row indicate significance difference ( $P<0.05$ )

**Table 4:** Sensory attributes of frozen chicken meat subjected to different temperature abuse conditions (Mean  $\pm$  SE)

PARAMETER	C	T1	T2	T3	T4	T5	T6
Appearance	4.97 $\pm$ 0.20 <sup>a</sup>	4.89 $\pm$ 0.05 <sup>a</sup>	4.91 $\pm$ 0.05 <sup>a</sup>	4.16 $\pm$ 0.07 <sup>b</sup>	4.20 $\pm$ 0.07 <sup>b</sup>	2.33 $\pm$ 0.14 <sup>c</sup>	2.41 $\pm$ 0.14 <sup>c</sup>
Colour	5.00 $\pm$ 0.00 <sup>a</sup>	4.54 $\pm$ 0.12 <sup>b</sup>	4.62 $\pm$ 0.12 <sup>b</sup>	4.08 $\pm$ 0.05 <sup>c</sup>	4.12 $\pm$ 0.06 <sup>c</sup>	4.00 $\pm$ 0.00 <sup>c</sup>	4.02 $\pm$ 0.02 <sup>c</sup>
Odour	4.95 $\pm$ 0.12 <sup>a</sup>	4.77 $\pm$ 0.03 <sup>b</sup>	4.79 $\pm$ 0.04 <sup>b</sup>	4.45 $\pm$ 0.04 <sup>c</sup>	4.50 $\pm$ 0.02 <sup>c</sup>	4.02 $\pm$ 0.02 <sup>d</sup>	4.06 $\pm$ 0.04 <sup>d</sup>
Sliminess	5.00 $\pm$ 0.00 <sup>a</sup>	5.00 $\pm$ 0.00 <sup>a</sup>	5.00 $\pm$ 0.00 <sup>a</sup>	4.97 $\pm$ 0.02 <sup>a</sup>	5.00 $\pm$ 0.00 <sup>a</sup>	4.95 $\pm$ 0.04 <sup>a</sup>	5.00 $\pm$ 0.00 <sup>a</sup>

n=12; C=Frozen meat maintained at  $-18\pm 1^\circ\text{C}$ ; T1= Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $0\pm 1^\circ\text{C}$ ; T2 =Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $0\pm 1^\circ\text{C}$  and refrozen to  $-18\pm 1^\circ\text{C}$ ; T3=Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $5\pm 1^\circ\text{C}$ ; T4=Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $5\pm 1^\circ\text{C}$  and refrozen to  $-18\pm 1^\circ\text{C}$ ; T5= Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $10\pm 1^\circ\text{C}$ ; T6= Frozen meat ( $-18\pm 1^\circ\text{C}$ ) thawed up to an internal temperature of  $10\pm 1^\circ\text{C}$  and refrozen to  $-18\pm 1^\circ\text{C}$ .  
Means with different superscripts in the same row indicate significance difference ( $P<0.05$ )

## Discussion

### pH

The pH of frozen chicken meat sample was found to be significantly ( $P<0.05$ ) increasing when subjected to temperature abuse conditions.

The increase in pH of frozen-thawed chicken meat samples may be due to the bacterial activity that resulted in the production of ammonia, amines and other alkaline substances (Nychas *et al.*, 2008).

### Extract Release Volume (ERV)

ERV value of frozen chicken meat sample was found to be decreasing significantly ( $P<0.05$ ) when subjected to temperature abuse conditions. The decrease in ERV value of frozen- thawed chicken meat samples may be due to proteolysis caused by enzymatic and bacterial action.

The rapid decline in ERV value was due to the higher bacterial counts associated with them. The significantly ( $P<0.05$ ) lower ERV value during low temperature storage may be due to a comparatively higher pH and total plate count (Strange *et al.*, 1977)

### **Total Volatile Basic Nitrogen (TVBN)**

A significantly ( $P < 0.05$ ) increased TVBN was found on exposure of frozen chicken meat samples to temperature abuse conditions.

The increase in TVBN content of frozen- thawed chicken meat samples may be due to the breakdown of protein and deamination of amino acids leading to production of ammonia and other volatile bases. The total volatile basic nitrogen (TVBN) is one of the main chemical parameters related to the microbial growth of microorganisms, such as *Pseudomonas* (Fraqueza *et al.*, 2008).

### **Total Plate Count (TPC)**

TPC of the frozen chicken meat samples increased significantly ( $P < 0.05$ ) when exposed to temperature abuse conditions.

The increase in TPC of frozen-thawed and thawed-refrozen chicken meat samples may be due to the exposure of chicken meat to a temperature optimum for the growth of an already existing microflora in the meat. The temperature seems to be the most important factor that influences the spoilage as well as safety of meat (Koutsoumanis and Toukis, 2005). The first indication of spoilage in fresh chicken meat is the production of off-odours, which become apparent when microbial numbers reach around  $10^7$  cfu/cm<sup>2</sup> (Obrien *et al.*, 1995). At this point, the microorganisms have exhausted levels of glucose in the meat and amino acids as a growth substrate. Zhang *et al* (2012) reported an increasing trend in TPC for aerobically packaged poultry from 4.60 to 6.38 log cfu/g at 0-4°C.

### **Psychrophilic Count**

The psychrophilic count of the frozen chicken meat samples (T1 to T4) did not differ significantly ( $P > 0.05$ ) when exposed to temperature abuse conditions. While, a significant ( $P < 0.05$ ) increase in the psychrophilic count was observed between Control, T5 and T6 groups.

From the present study, it was observed that psychrophiles were the most common bacteria present in the chilled meat. The microbial quality data showed a range of 50-63% of psychrophiles when the frozen meat was subjected to chilling conditions varying from 0 to 10°C. According to Jensen (1945), psychrophiles have temperature optima between -2°C and 7°C and the most important single factor governing microbial growth is temperature. At chill temperature under aerobic conditions, spoilage flora of meat is dominated by *pseudomonas* and, under anaerobic condition by *lactobacilli*. Some bacteria can grow over a wide range of temperature covering from refrigerated temperature to ambient room temperature. Initial contamination of meat with such a group of bacteria from the slaughtered animal or from slaughter premises has contributed to the higher microbial count especially psychrotrophs during chilled storage. Byrd *et al.* (2011) reported that psychrophilic count was 2.18 on 0 day, 7.05 log cfu/g on 14 days at  $2 \pm 1$ °C.

### **Yeast and Mold Count**

Yeast and mold count of the frozen chicken meat samples did not differ significantly ( $P > 0.05$ ) between groups T1 to T4 when exposed to temperature abuse conditions. Whereas, the yeast and mold counts differed significantly ( $P < 0.05$ ) between control, T5 and T6 groups.

Freshly cut meat stored in a refrigerator with high humidity invariably undergoes microbial spoilage preferential to mold spoilage. Molds tend to predominate in spoilage of beef cuts when surface is too dry for bacterial growth or when beef is treated with antibiotics (Jay, 1996). Samelis and Sofos (2003) reported yeast and mould count between log 5- log 6 cfu/g, when the meat was rejected organoleptically. Yeast and mold count varied between 1.87-2.52 log cfu/g in fresh chicken meat samples (Kumar *et al.*, 2012).

### **Hunter Lab Colour Values of Meat Sample at Different Temperature Abuse Conditions**

The lightness ( $L^*$ ) values of frozen- thawed and thawed- refrozen chicken meat samples decreased significantly ( $P < 0.05$ ) compared to the control group. The results of the present study is in agreement with the findings of Cortez-Vega *et al.* (2012) who observed reduction in brightness in raw chicken breast samples packaged in high-density

ethylene vinyl alcohol based polyethylene-nylon plastic bags under modified atmospheric conditions (0.03% CO<sub>2</sub>, 21% O<sub>2</sub> and 78% N<sub>2</sub>) during refrigerated storage at 5±1°C, the lightness values ranging from 59.1 to 54.4.

The Redness (a\*) values of frozen- thawed and thawed- refrozen chicken meat samples differed significantly (P<0.05). Decrease in redness (a\*) value may be attributed to metmyoglobin formation which imparts brownish discoloration to meat.

The yellowness (b\*) values of frozen-thawed and thawed-refrozen chicken meat samples increased significantly (P<0.05) compared to the control group. Increase in yellowness (b\*) may be due to denaturation of muscles proteins and loss of pigment in drip.

Colour difference ( $\Delta E$ ) values of frozen-thawed and thawed-refrozen chicken meat samples decreased significantly (P<0.05) compared to the control group. Angsupanich and Ledward (1998) suggested that the changes in muscle colour may be related to the denaturation of myofibrillar and sarcoplasmic proteins. Colour deterioration is measured by the total colour change and changes in lightness and redness of the meat sample (Antoniewski *et al.*, 2007). Colour changes in chicken breast are related to quality changes and are highly dependent upon the storage day and temperature.

The significant (P<0.05) decrease in meat colour recorded for treatment samples in the present study was due to the effect of high temperature thawing on the major meat pigment myoglobin and change in the oxymyoglobin to metmyoglobin in fresh meat. Wherein the oxymyoglobin is responsible for the red colour associated with fresh meat and metmyoglobin is responsible for an undesirable brown colour. With increasing temperatures, the stability of oxymyoglobin decreases and thus discolourations occur (Belitz *et al.*, 2009). The redness (a value) showed a decreasing trend due to excessive thawing temperature causing loss of muscle pigment in meat extract and meat became dull due to decreased colour intensity (chroma). Yellowness (b value) increased during temperature abuse due to denaturation of muscle protein and loss of pigment in drip. At the refrigerated storage period, metmyoglobin accumulation in the meat tissue increased consistently (Sahoo and Anjaneyulu, 1997) which was also recorded in the present study in broiler chicken meat.

### ***Change in Sensory Attributes of Chicken Meat Sample Subjected To Temperature Abuse Conditions***

#### **Appearance**

A significantly decreased (P<0.05) appearance score was recorded for treatments in the present study at higher temperature compared to lower temperature. At higher temperature there was protein denaturation, loss of pigment in drip and more drip were accumulated which affected the appearance of meat. Gradual decrease in appearance scores was because of oxidation of myoglobin to metmyoglobin (Sahoo and Anjaneyulu, 1997).

#### **Colour**

Decreasing trend was observed for colour score of frozen chicken meat sample when subjected to temperature abuse conditions. Colour is probably the single most important appearance factor, when meat cuts are already packaged. A significantly decreased (P<0.05) colour score was recorded for treatment samples in the present study at higher temperature compared to lower temperature. Colour was the major sensory attribute affected by temperature. A decreased colour score was due to formation of surface metmyoglobin layer at higher temperature. The loss of water soluble protein, pigment in drip and denaturation of meat protein also affected the colour score of the meat. Loss of moisture from meat through drip contributed to a decreased colour score when the frozen chicken meat abused at higher temperature. As the refrigerated storage period increased, the metmyoglobin accumulation in the meat tissue increased consistently (Sahoo and Anjaneyulu, 1997). Barbut (2001) used hedonic scale for evaluating colour of fresh chicken (1=dislike, 10=like) and gave score ranging from 3.57-7.01 under different light conditions.

#### **Odour**

There was a significant (P<0.05) decrease in odour score of chicken sample on exposure to temperature abuse conditions. Psychrotrophic bacteria, like *Pseudomonas* spp. and *Shewanella* spp. had been previously identified as

the primary bacteria producing sulphurous off-odors associated with spoiled poultry (Ayres *et al.*, 1950). A significantly decreased ( $P < 0.05$ ) odour score was recorded for treatment samples in the present study. From the present study it was observed that off-odours which develop due to surface microbial contamination is one of the major cause of spoilage in meat. Off odour in meat are due to the by-product of putrefaction of protein. The decreased odour score of treatment samples immediately after temperature abuse study was associated with the higher microbial count especially *Pseudomonas*. The present work was in agreement with Skandamis and Nychas (2001), who observed decrease in odour scores from 1 to 3 for air packaged minced beef samples without essential oil during 12 days stored at 5°C, where 1 referred to acceptable, 2 referred to marginal and 3 as unacceptable.

### **Sliminess**

No sliminess was observed in all groups after temperature abuse study. Nychas *et al* (2007) has correlated the sliminess development in meat with growth of bacteria mainly belongs to *pseudomonas*, *lactobacillus* and *enterococcus*.

### **Conclusion**

It was concluded that temperature abuse during the storage of frozen chicken meat subjected to freezing-thawing-refreezing cycle leads to appreciable reduction in meat quality especially due to increased pH, drip loss, total volatile basic nitrogen, total plate count, psychrophilic count, yeast and mold count, besides, remarkable decrease in extract release volume, redness, lightness, color difference, appearance, color and odour. Higher the temperature and duration of thawing, higher was the reduction in meat quality.

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### **Contribution by Authors**

All the authors contributed equally to writing the manuscript. The final manuscript was read by all authors and consented to publication.

### **Conflict of Interests**

There is no conflict of interest.

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