Veterinary World, EISSN: 2231-0916
Available at www.veterinaryworld.org/Vol.7/November-2014/2.pdf

RESEARCH ARTICLE
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# Effect of higher temperature exposure on physicochemical properties of frozen buffalo meat

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**Received:** 15-07-2014, **Revised:** 03-10-2014, **Accepted:** 08-10-2014, **Published online:** 04-11-2014

**doi:** 10.14202/vetworld.2014.909-915. **How to cite this article:** Vishnuraj MR, Kandeepan G, Shukla V (2014) Effect of higher temperature exposure on physicochemical properties of frozen buffalo meat, *Veterinary World* 7(11): 909-915.

## Abstract

**Aim:** The aim was to study the changes in various physicochemical parameters of frozen buffalo meat undergone temperature abuse at two different isothermal storage temperatures (37±1°C, 25±1°C) using a simulated model.

**Materials and Methods:** Frozen buffalo meat was evaluated after exposing to various temperature abuse conditions over selected durations for different meat quality parameters including pH, extract release volume (ERV), flourescein diacetate (FDA) hydrolysis, free amino acid (FAA), total volatile basic nitrogen (TVBN) and D-glucose value and compared against a control sample maintained at 4±1°C.

**Results:** Of the various meat quality parameters evaluated pH, FDA hydrolysis, FAA content and TVBN content showed a significant ($p<0.05$) increase in temperature abused samples after temperature abuse and on subsequent refrigerated storage. However, ERV and D-glucose content decreased significantly ($p<0.05$) in temperature abused buffalo meat during the same period of study.

**Conclusions:** The present study featured the influence of exposure temperature and duration in various physicochemical parameters and the rate of spoilage development in frozen buffalo meat after temperature abuse.

**Keywords:** buffalo meat, physicochemical parameters, spoilage, temperature abuse.

## Introduction

Buffalo meat production has gained an appreciable attention in world meat market currently due to its export potential. Since deboned and deglanded buffalo meat is exported mainly by application of low temperature methods [1], the most demanding factor to maintain the meat quality is the temperature control in supply chain. Any fluctuations in cold chain temperature at any point during transport or on subsequent storage will cause accelerated deteriorative reactions, which may result in decreased acceptance or complete rejection of meat [2]. Temperature abuse can also leads to food safety issues by enhancing pathogen growth [3]. Therefore quality of any meat food products and its practically attainable shelf life is strongly dependent on its temperature exposure history from gate to plate in a continuum.

Freshness of meat has been assessed chemically on basis of protein break down and fat spoilage along with measurement of physical changes, which can be suitable spoilage indicators [4]. Microorganisms that produce putrefactive odor compounds, such as *Enterobacteriaceae, Brochothrix thermosphacta* and *Shewanella putrefaciens* grow better at a pH >6 [5]. Extract release volume (ERV) appears to have a considerable possibility in assessing the spoilage of beef [6]. A mean ERV value of 19.3 ml was reported in buffalo meat on day 4 of chiller storage, which was significantly lower than the zero day value of 24 ml [7]. As bacterial load increased from $10^2$ cfu/cm$^2$ to $10^8$ cfu/cm$^2$, in biceps femoris steaks during thawing and exposure to 25°C, flourescein diacetate (FDA) hydrolysis activity (A490) increased from 0.1 to 0.6 units [8]. Sum of free amino acids (FAA) along with water soluble protein content increased in meat during storage and this corresponded well with colony counts, particularly with meat having high glucose concentration [2]. Beef stored at 4°C for 12 days has shown a final volatile basic nitrogen (VBN) concentration of 29.9 mg/100 g compared to 19.2 mg/100 g for beef stored at 0°C for same duration and also compared different parameters in fresh pork and beef under different storage conditions and found high correlation between D-glucose as well as L-lactate with different bacterial counts [9].

In the present study, the quality and maximum attainable practical shelf life of frozen buffalo meat was evaluated after exposing to two different isothermal abuse storage temperatures (25±1°C and 37±1°C) for different durations using some of the physicochemical parameters that are indirect indicators of buffalo meat spoilage.

## Materials and Methods

### Ethical approval

The study was conducted after the approval of Institutional Animal Ethics Committee.

### Buffalo meat sample

The meat samples were collected from buffalo slaughter house, Bareilly district, Uttar Pradesh State, India. Thigh muscles without excessive fat and connective tissue were collected from nine different buffalo carcasses, slaughtered according to traditional halal method and brought to the lab within 4 h of slaughter. Meat collected from nine different carcasses was packed separately in low-density polyethylene bags and kept in a chiller room maintained at 7-10°C for 24 h for the rigor mortis to complete so as to avoid cold shortening and excessive drip loss. After the initial chilling period, the total meat was divided into 3 replicates and each replicates represents meat from three randomly selected animals. All the three replicates were packed in polyethylene bags with zip lock and stored in deep freezer at −18±1°C (Vest Frost, Denmark). These replicates of frozen meat were used for further experiments.

### Experimental design

Simulated temperature abuse conditions of 25±1°C and 37±1°C were produced in laboratory within an incubator and temperature was strictly monitored by using a probe thermometer (Digi-thermo, WT-2, China). Each replicates of meat was divided into seven experimental group/subsets namely C, $T_1$, $T_2$, $T_3$, $T_4$, $T_5$ and $T_6$.

- C-Control, stored at 4±1°C without abuse temperature exposure
- $T_1$, $T_2$, $T_3$ are kept at 25±1°C for 6 h, 12 h and 18 h respectively
- T4, T5, T6 are kept at 37±1°C for 4 h, 8 h and 12 h respectively.

Definite portions of each experimental group (C, T1-T6) were removed and analyzed for various meat quality parameters shortly after the experiment and reported as zero-day results. All the experimental group (C, T1-T6) were further stored at refrigerated temperature (4±1°C) and analyzed for various meat quality parameters on alternate days (3 day, 5 day and 7 day). And each experimental analyzes were performed twice in all three replicates (n=3).

### pH and ERV

The tissue homogenate was prepared by blending 10 g meat sample with 90 ml distilled water using an Ultra Turrax tissue homogenizer (Ultra Turrax IKA, Model T18 Basic, IKA Wares Inc., Willmington, USA) for 1 min. The pH was recorded by immersing combined glass electrode of digital pH meter into the meat homogenate (Model CP 901, Century Instrument Ltd, Chandigarh). The ERV was estimated according to the procedure described by Strange *et al*. [10] with suitable modifications. 25 g of minced meat was blended with 100 ml distilled water in an Ultra Turrax tissue homogenizer. The homogenate was transferred into a funnel, equipped with a Whatman filter paper No. 1. The volume of filtrate collected in first 15 min was recorded as ERV of the respective sample.

### FDA hydrolysis

The FDA hydrolysis of meat samples were measured according to the procedure described by Venkitanarayanan *et al*. [8] with suitable modifications. One gram of meat was drawn from each sample, observing necessary aseptic precautions. The samples were expressed into a tube containing 10 ml sterile peptone water (0.1%). The tubes were centrifuged at 100 ×*g* for 30 s to sediment meat particles. The supernatant was transferred into another tube and centrifuged at 3000 ×*g* for 30 min to pellet the bacterial cells. The supernatant was decanted and the bacterial pellet was washed and resuspended in 5 ml sterile sodium phosphate buffer (pH 7.6). The resultant 5 ml solution was sonicated in a bath sonicator (Soniprep 150 plus, MSE, U.K) in 4 episodes each of 15 s. To 3 ml of the resultant clear solution, 100 µL FDA reagent (500 µg FDA/ml acetone) was added. The mixture was incubated at 25°C for 3 h and the absorbance of the solution at 490 nm was recorded using a spectrophotometer (Model DU 640, Beckman, U.S. A). A tube containing 3 ml sterile phosphate buffer and 100 µl FDA reagent designated as "blank" was incubated simultaneously. The FDA hydrolysis was expressed as the mean absorbance at 490 nm.

### FAA content

The FAA was determined in accordance with the procedure described by Rosen [11] following colorimetric ninhydrin method with suitable modifications. The α-amino acid present in the meat was estimated as nihydrin reactive substance. Finely minced 10 g meat sample was homogenized with 100 ml distilled water for 2 min in an Ultra Turrax tissue homogenizer. The homogenate was kept overnight in refrigerator at 4°C. Then the homogenate was transferred into a polycarbonate centrifuge tube and centrifuged at 3000 rpm for 15 min in a REMI research centrifuge. After the first centrifugation, 10 ml of supernatant was drawn into another centrifuge tube and 10 ml of 10% trichloroacetic acid (TCA) was added. This was again centrifuged at 3000 rpm for 15 min. 1 ml aliquot in duplicates were drawn from the clear supernatant. To each 1 ml solution, 1 ml of both 80% phenol in ethanol and 10% ninhydrin reagent in acetone was added. A volume of 10 µl of pyridine solution was added to both tubes to improve the sensitivity of color reaction. In blank, 1 ml of distilled water was added instead of aliquot. This mixture was stirred and kept at boiling water bath for 10 min for proper color development. The mixture was cooled in running tap water, and 5 ml of ethanol was added to all the tubes. The optical density was determined with a spectrophotometer (Model DU 640, Beckman, USA) at 570 nm and converted by using a standard curve (leucine standard curve) to mg FAA per ml of aliquot. The FAA content of meat was expressed as mg per 100 g of meat.

### Total volatile basic nitrogen (TVBN) estimation

TVBN of buffalo meat samples were determined by the procedure of Pearson [12] following

micro-diffusion technique with slight modifications. 10 g of the meat sample was weighed and blended for 2 min with 90 ml distilled water in a tissue homogenizer. It was filtered through a muslin cloth. 5 ml of the filtrate was taken in a test tube and 5 ml of 10% TCA was added to the tube. The test tubes were covered with aluminium foil and kept at room temperature for 30 min. It was then filtered through a Whatman filter paper No. 1 using glass funnels. Thoroughly cleaned, dried Conway micro-diffusion unit was taken and 2 ml of boric acid reagent (prepared in the laboratory) was added in its center compartment. One ml of meat filtrate was accurately pipetted into the outer compartment. Cover lid was then put in such a way that only a small portion of outer compartment sufficient to insert the pipette remained open and 1 ml of saturated potassium carbonate solution was then added through the gap. Lid was immediately closed without leaving any space. Dish was rotated manually to ensure proper mixing of meat extract with saturated potassium carbonate solution and then incubated at 37°C for 3-4 h. During incubation dishes were rotated 2-3 times. After incubation boric acid solution in the center compartment (faint reddish color of boric acid changed to green color) was titrated with 0.02 N sulfuric acid. Diffusion was carried out in duplicate along with a blank. TVBN content was calculated by using the formula:

TVBN (mg/100 g of meat) = Reading of burette (volume of 0.02 N $H_2SO_4$ consumed) × Normality of acid used for titration × 14 × 100

#### D-glucose estimation

D-glucose concentration in different meat samples were estimated using glucose oxidase/peroxidase assay kit (Sigma-Aldrich, USA). 1 g meat sample was weighed and blended with 10 ml distilled water for 2 min in an Ultra Turrax tissue homogenizer. It was then filtered through a Whatman filter paper No. 1 using glass funnels. One ml aliquot in duplicates was drawn from the clear supernatant. One ml of distilled water was taken in one test tube marked as blank. To all the tubes, 2 ml of assay reagent was added and kept at 37°C for 30 min. The reaction was stopped after 30 min by addition of 2 ml of 12 N $H_2SO_4$ into each tube. The optical density was determined with a spectrophotometer (Model DU 640, Beckman, USA) at 540 nm and converted by using D-glucose standard curve to mg D-glucose per ml of aliquot. The D-glucose content of meat was expressed as mg per 100 g of meat.

#### Statistical analysis

A randomized block design with three completely random replicates was used for experiments and the data generated for different meat quality parameters were compiled and analyzed using SPSS (version 20.0 for Windows; SPSS, Chicago, 111, USA). The data were subjected to analysis of variance, (two-way ANOVA for storage data) and least significant difference for comparing the means to find the difference between groups and storage periods. The smallest difference ($D_{5\%}$) for two means was reported as significantly different ($p<0.05$).

## Results

#### pH

pH of the buffalo meat samples increased significantly ($p<0.05$) when exposed to temperature abuse conditions (Table-1). It was observed that the sample subjected to T6 showed a high significant ($p<0.05$) pH value compared to the control and other treatment groups immediately after temperature abuse. On subsequent refrigerated storage of the temperature abused meat sample it was observed that the pH increased significantly ($p<0.05$) with increase in storage period. Similar trend was also observed in control samples. The pH of control group was always significantly ($p<0.05$) lower than other treatment groups during the storage period. The pH values reached beyond the marginally acceptable value for samples T3 and T6, T2 and T5, and T1 and T4 on day 3, 5 and 7 of storage period respectively, which is indicating the development of spoilage in stored buffalo meat.

#### ERV

ERV of the buffalo meat samples also showed significant ($p<0.05$) difference when exposed to temperature abuse conditions (Table-1). The samples subjected to T6 and T3 showed a significantly ($p<0.05$) lower ERV value compared to the control and other treatment groups immediately after temperature abuse. ERV also decreased significantly ($p<0.05$) with increase in storage period under refrigerated storage for the temperature abused meat samples. Similar trend was also observed in control samples, but the degree of decrease was more pronounced in treatments samples. ERV values of treatments T3 and T6, T2 and T5, T1 and T4 reached the lowest values on day 3, 5 and 7 respectively indicating the development of spoilage in stored buffalo meat.

#### FDA hydrolysis

FDA hydrolysis value which is an indirect evaluation of the developed microbiota in meat was found to be increased significantly ($p<0.05$) on buffalo meat samples when exposed to temperature abuse conditions (Table-1). The samples subjected to T3 and T6 showed a higher significant ($p<0.05$) FDA hydrolysis value compared to the control and other treatment samples immediately after temperature abuse (Figure-1). On subsequent refrigerated storage at 4±1°C for the temperature abused meat sample the FDA hydrolysis increased significantly ($p<0.05$) with storage period.

#### FAA

FAA content of the buffalo meat samples differed significantly ($p<0.05$) when exposed to temperature abuse conditions (Table-2). It was observed that the samples subjected to T3 showed a highly significant ($p<0.05$) FAA content compared to control and other

**Table-1:** pH, ERV and FDA hydrolysis values of control and treatment samples immediately after temperature exposure study and on subsequent refrigerated storage (mean±SE).

| Groups | Parameters | | | |
|---|---|---|---|---|
| | Storage period (days) | | | |
| | 0 | 3 | 5 | 7 |
| pH | | | | |
| C | 5.27±0.01$^{d3}$ | 5.39±0.01$^{c6}$ | 5.55±0.03$^{b4}$ | 5.71±0.02$^{a3}$ |
| T1 | 5.28±0.01$^{d3}$ | 5.47±0.01$^{c5}$ | 5.93±0.03$^{b3}$ | 6.17±0.01$^{a2}$* |
| T2 | 5.29±0.02$^{c3}$ | 5.89±0.02$^{b3}$ | 6.31±0.03$^{a2}$* | ND |
| T3 | 5.31±0.02$^{b3}$ | 6.29±0.04$^{a1}$* | ND | ND |
| T4 | 5.29±0.01$^{d3}$ | 5.54±0.02$^{c4}$ | 5.95±0.03$^{b3}$ | 6.25±0.01$^{a1}$* |
| T5 | 5.45±0.01$^{c2}$ | 6.19±0.01$^{b2}$ | 6.5±0.01$^{a1}$* | ND |
| T6 | 5.54±0.01$^{b1}$ | 6.21±0.01$^{a2}$* | ND | ND |
| ERV (ml) | | | | |
| C | 29.00±0.77$^{a1}$ | 28.23±0.29$^{a1}$ | 24.00±0.12$^{b1}$ | 18.12±0.16$^{c1}$ |
| T1 | 24.58±0.24$^{a2}$ | 20.17±0.34$^{b3}$ | 17.33±0.17$^{c3}$ | 14.50±0.18$^{d2}$* |
| T2 | 23.58±0.20$^{a2}$ | 17.42±0.16$^{b4}$ | 15.07±0.30$^{c4}$* | ND |
| T3 | 21.18±0.46$^{a3}$ | 12.98±0.25$^{b6}$* | ND | ND |
| T4 | 23.73±0.31$^{a2}$ | 22.48±0.24$^{b2}$ | 19.3±0.20$^{c2}$ | 14.65±0.26$^{d2}$* |
| T5 | 23.25±0.71$^{a2}$ | 19.27±0.71$^{a3}$ | 13.92±0.28$^{b5}$* | ND |
| T6 | 20.98±0.20$^{a3}$ | 14.08±0.17$^{b5}$* | ND | ND |
| FDA hydrolysis | | | | |
| C | 0.24±0.02$^{d6}$ | 0.30±0.02$^{c6}$ | 0.35±0.01$^{b5}$ | 0.49±0.01$^{a2}$ |
| T1 | 0.27±0.02$^{d4}$ | 0.31±0.03$^{c5}$ | 0.41±0.02$^{b4}$ | 0.50±0.03$^{a1}$* |
| T2 | 0.40±0.02$^{c3}$ | 0.48±0.02$^{b3}$ | 0.51±0.03$^{a2}$* | ND |
| T3 | 0.48±0.02$^{b1}$ | 0.56±0.04$^{a1}$* | ND | ND |
| T4 | 0.26±0.03$^{d5}$ | 0.36±0.03$^{c4}$ | 0.47±0.02$^{b3}$ | 0.51±0.03$^{a1}$* |
| T5 | 0.42±0.01$^{c2}$ | 0.49±0.01$^{b2}$ | 0.53±0.02$^{a1}$* | ND |
| T6 | 0.48±0.02$^{b1}$ | 0.56±0.02$^{a1}$* | ND | ND |

n=3, C=Control, T1=Treatment 1 (exposed at 25±1°C for 6 h), T2=Treatment 2 (exposed at 25±1°C for 12 h), T3=Treatment 3 (exposed at 25±1°C for 18 h), T4=Treatment 4 (exposed at 37±1°C for 4 h), T5=Treatment 5 (exposed at 37±1°C for 8 h), T6=Treatment 6 (exposed at 37±1°C for 12 h), ND=Not detected. *The spoiled meat sample was analyzed to study the extent of variation in parameters due to prolonged storage. Means with different superscripts (letters in the same row and numbers in the same column) indicate significance (p<0.05). ERV=Extract release volume, FDA=Flourescein diacetate, SE=Standard error

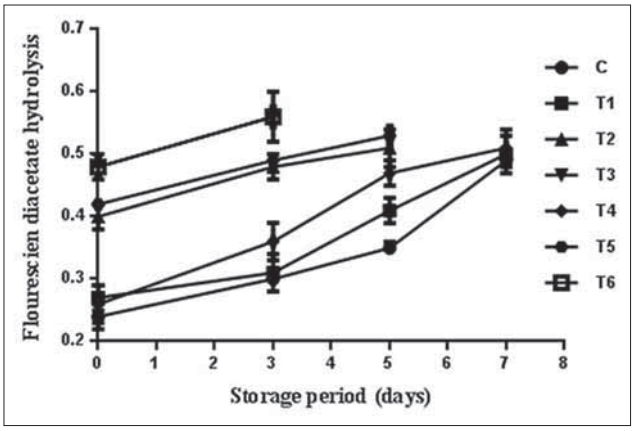


**Figure-1:** Changes in flourescein diacetate hydrolysis of control and treatment samples immediately after exposure study and on subsequent refrigerated storage (samples T3 and T6 was studied up to day 3 and T2 and T5 up to 5 days of refrigerated storage since meat was spoiled beyond these days for the respective samples).

treatment samples immediately after temperature abuse. During the refrigerated storage of the temperature abused meat sample it was observed that the FAA content increased significantly (p<0.05) with increase in storage period. Similar trend was also observed in control samples. The FAA contents turned to an unacceptable level for samples T3 and T6 on day 3, T2 and T5 on day 5 and T1 and T4 on day 7 respectively. However, the FAA content of control sample was within an acceptable limit during the entire storage study period of the present experiment.

#### TVBN

TVBN content of the buffalo meat samples also showed significant (p<0.05) variations after temperature abuse (Table-2). The TVBN content of the sample subjected to T3 showed the highest significant (p<0.05) value compared to all other samples of buffalo meat studied in the experiment. TVBN content also increased significantly (p<0.05) with an increase in storage period. The TVBN content of the control group was significantly (p<0.05) lower than other treatment groups during the storage period. The TVBN values reached beyond the threshold level for samples T3 and T6, T2 and T5, T1 and T4 on day 3, 5 and 7 respectively parallels with spoilage in stored buffalo meat. TVBN increased significantly (p<0.05) with storage time and were significantly (p<0.05) higher for higher temperature abused meat samples.

#### D-glucose content

Buffalo meat samples showed significant (p<0.05) decrease in D-glucose concentration after exposed to temperature abuse (Table-2). Both the samples subjected to T3 and T6 showed a significantly

**Table-2:** FAA, TVBN and D-glucose content of control and treatment samples immediately after temperature exposure study and on subsequent refrigerated storage (mean±SE).

| Groups | Parameters | | | |
|---|---|---|---|---|
| | Storage period (days) | | | |
| | 0 | 3 | 5 | 7 |
| FAA (mg/100 g) | | | | |
| C | 4.80±0.07$^{d7}$ | 19.72±0.04$^{c6}$ | 27.28±0.44$^{b5}$ | 42.18±0.54$^{a2}$ |
| TI | 5.30±0.04$^{d6}$ | 23.90±0.46$^{c5}$ | 38.03±0.44$^{b4}$ | 57.87±0.55$^{a1}$* |
| T2 | 8.68±0.04$^{c4}$ | 45.17±0.40$^{b4}$ | 58.17±0.48$^{a2}$* | ND |
| T3 | 18.83±0.21$^{b1}$ | 56.20±0.51$^{a2}$* | ND | ND |
| T4 | 6.57±0.14$^{d5}$ | 23.98±0.26$^{c5}$ | 42.60±0.43$^{b3}$ | 57.40±0.44$^{a1}$* |
| T5 | 9.48±0.19$^{c3}$ | 48.07±0.32$^{b3}$ | 62.87±0.32$^{a1}$* | ND |
| T6 | 16.27±0.13$^{b2}$ | 62.03±0.20$^{a1}$* | ND | ND |
| TVBN (mg/100 g) | | | | |
| C | 8.13±0.09$^{d6}$ | 11.48±0.13$^{c6}$ | 13.70±0.07$^{b5}$ | 16.55±0.14$^{a3}$ |
| T1 | 8.70±0.04$^{d5}$ | 13.82±0.11$^{c5}$ | 17.00±0.08$^{b3}$ | 21.33±0.16$^{a2}$* |
| T2 | 10.72±0.04$^{c4}$ | 15.45±0.20$^{b4}$ | 24.48±0.50$^{a2}$* | ND |
| T3 | 15.63±0.09$^{b1}$ | 25.93±0.22$^{a2}$* | ND | ND |
| T4 | 9.03±0.12$^{d5}$ | 12.17±0.33$^{c6}$ | 15.33±0.14$^{b4}$ | 21.95±0.32$^{a1}$* |
| T5 | 12.02±0.27$^{c3}$ | 16.18±0.39$^{b3}$ | 28.08±0.32$^{a1}$* | ND |
| T6 | 14.77±0.10$^{b2}$ | 28.73±0.17$^{a1}$* | ND | ND |
| D-glucose concentration (mg/100 g) | | | | |
| C | 142.00±1.03$^{a1}$ | 122.50±1.26$^{b1}$ | 53.23±0.72$^{c1}$ | 33.77±0.73$^{d1}$ |
| T1 | 123.25±0.91$^{a3}$ | 96.53±1.11$^{b3}$ | 45.90±0.65$^{c2}$ | 30.37±0.67$^{d2}$* |
| T2 | 115.00±0.98$^{a5}$ | 76.37±0.99$^{b4}$ | 33.20±0.50$^{c3}$* | ND |
| T3 | 95.13±0.81$^{a6}$ | 17.58±0.39$^{b6}$* | ND | ND |
| T4 | 135.30±0.88$^{a2}$ | 105.42±0.61$^{b2}$ | 46.30±0.93$^{c2}$ | 29.33±0.64$^{d2}$* |
| T5 | 118.41±0.69$^{a4}$ | 64.17±0.56$^{b5}$ | 32.78±0.45$^{c3}$* | ND |
| T6 | 95.69±0.79$^{a6}$ | 17.33±0.40$^{b6}$* | ND | ND |

n=3, C=Control, T1=Treatment 1 (exposed at 25±1°C for 6 h), T2=Treatment 2 (exposed at 25±1°C for 12 h), T3=Treatment 3 (exposed at 25±1°C for 18 h), T4=Treatment 4 (exposed at 37±1°C for 4 h), T5=Treatment 5 (exposed at 37±1°C for 8 h), T6=Treatment 6 (exposed at 37±1°C for 12 h), ND=Not detected. *The spoiled meat sample was analyzed to study the extent of variation in parameters due to prolonged storage. Means with different superscripts (letters in the same row and numbers in the same column) indicate significance ($p<0.05$). FAA=Free amino acid, TVBN=Total volatile basic nitrogen, SE=Standard error

($p<0.05$) lower D-glucose content compared with the control and other treatment groups immediately after temperature abuse. All the samples showed consistently lower D-glucose content on refrigerated storage. The lowest D-glucose content were noted on buffalo meat samples on 3$^{rd}$ day for T3 and T6, 5$^{th}$ day for T2 and T5 and 7$^{th}$ day for C, T1 and T4.

## Discussion

The pH of frozen and chilled buffalo meat post slaughter showed an increasing trend with increasing storage life. Up to day 4 of chiller storage there was no significant increase in pH, but on day 7 meat showed considerably higher pH with a mean pH of 6.2 [7]. An increase in pH during the storage of beef at 4°C was reported by Galgano *et al*. [13]. Buffalo meat keema prepared from young animals showed a mean pH value of 6.13±.02 on 3$^{rd}$ day of storage at ambient temperature of 37±1°C [14]. Similarly a mean pH value of 5.90±.03 was reported in buffalo meat curry on 3$^{rd}$ day of storage at 37±1°C [15]. All these reports was in agreement with present finding that pH of buffalo meat and meat products showed consistent increase after exposure to higher temperature or on storage after such exposure. Higher pH development in packaged fresh meat is important in the context that it can enhance the growth and proliferation of psychrotrophic clostridia including *Clostridium estertheticum* [16] which can produce blown pack spoilage and this organism is a common contaminant in slaughter house environments [17]. Silva *et al*. [18] reported that the pH of spoiled meat samples was between 6.0-7.0 with putrid aroma and gas production; whereas the pH of negative control were only 5.5.

ERV is primarily based on the water holding capacity of meat proteins, which is lowest for fresh meat at its ultimate pH (close to isoelectric pH of meat proteins), but gradually increases as spoilage occurs. The present finding was in agreement with Shelef and Jay [19], where they had showed a decreased ERV value associated with higher bacterial count in beef stored at 5°C. As storage period advances a gradual decrease in ERV value of buffalo meat on chiller storage was reported by Kandeepan and Biswas [7].

The present study established a minimum FDA hydrolysis of 0.5±0.03 (absorbance, Mean±standard error) which coincided with an unacceptable total microbial count ($\log_{10} 10^7$ cfu/g) on buffalo meat. The FDA hydrolysis value reached beyond the acceptable value for samples T3 and T6, T2 and T5, and T1 and T4 on day 3, 5 and 7 of refrigerated storage respectively which coincide with of spoilage in stored buffalo meat. Venkitanarayanan *et al*. [8] reported a FDA hydrolysis in biceps femoris steaks under thawing and

temperature abuse condition at 25°C with an average absorbance of 0.5±0.02 when the bacterial load was log 6.8 cfu/cm$^2$.

Nychas *et al*. [2] reported that sum of FAAs along with water soluble protein content increased during storage and this corresponded well with colony counts, particularly with meat having high glucose concentration. The significant ($p<0.05$) increase in FAA content of treatment samples was due to increased breakdown of buffalo meat proteins by bacterial proteases especially in buffalo meat with higher *Pseudomonas* count. A maximum acceptable limit of 16.5mg of volatile nitrogen per 100 g of beef has been recommended [20].

Fresh meat generally contains sufficient glucose and other simple carbohydrate to support approximately $10^9$ cfu/cm$^2$ [21]. The significant decrease ($p<0.05$) in D-glucose concentration recorded for treatment and control samples in the present study was due to the growth of glucose utilizing bacterial microflora. The organisms that grow the fastest and utilize glucose at refrigeration temperature are *Pseudomonas* [22]. Low concentration of D-glucose in spoiled samples suggested the possible involvement of pseudomonads, since fresh meat spoilage under aerobic condition was usually associated with utilization of amino acids by these microbes after exhausting the available glucose in meat. Therefore proteolysis by *Pseudomonas* may be the reason for increased FAA and TVBN content in meat with decreasing glucose concentration. Doulgeraki and Nychas [23] reported that *Pseudomonas fragi* and *Pseudomonas putida* are the important spoilage species in minced beef. Bruckner *et al*. [24] established the importance of storage temperature in attaining "Microbial shelf life" in fresh meat. In his research, he demonstrated a time gap of 165.8 h at 2°C compared to 45.5 h at 15 °C to reach the an unacceptably higher *Pseudomonas* count (log 7.5 cfu/g) and sensory spoilage characteristics in fresh loin. Storage of meat by low temperature is the most common method of preservation of meat caracass and sub primal cuts [25]. And exposure of meat to higher temperature is the single most important cause for premature spoilage of meat during distribution chain. This can be prevented by maintaining accurately controlled temperature during storage and as reported by Anbalagan *et al*. [26], that storage of meat at −18±1°C will reduce the total viable count on subsequent days.

## Conclusions

Exposing buffalo meat at 25±1°C for 6 h or 37±1°C for 4 h will reduce the shelf life by 2 days and at 25±1°C for 12 h or 37±1°C for 8 h will reduce the shelf life by 4 days compared to a control sample having 7 days shelf life under refrigerator storage (4±1°C). The threshold values for various physicochemical parameters were evaluated as spoilage indicators and found that pH of 6.17±0.01, FAA of 56.20±0.51 mg/100 g, TVBN of 16.55±0.14 mg/100 g, FDA of 0.50±0.03, ERV of 15.07±0.30 ml and D-glucose of 30.37±0.67 mg/100g were indicative of spoilage on fresh buffalo meat stored under refrigerator aerobically.

## Authors' Contributions

The present work was carried out during MRV's M.V.Sc thesis program and it was an original research work. GK conceptualized the aim of the study, designed, planned and supervised the experiment and corrected the manuscript. Collection of samples, execution of the experimental study, collation and analysis of data, interpretation of the results and drafting the manuscript was done by MRV. VS helped in analyses, draft and revision of the manuscript. All authors read and approved the final manuscript.

## Acknowledgments

The authors are thankful to the Director, Indian Veterinary Research Institute, Izatnagar for the facilities provided. The work was carried out using the institute fund provided for the project approved by The Director and The Joint Director (Research), Indian veterinary Research Institute.

## Competing Interests

The authors declare that they have no competing interests.

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