Material and Methods
CHAPTER-3
MATERIAL AND METHODS

Experimental studies were carried out to examine the effects on various treatments namely curing, antioxidant treatments and smoking individually and in combination, packaging materials and storage conditions on certain physico-chemical, microbial and textural characteristics and shelf-life of buffalo meat.

3.1 Equipment Used.

A number of experimental study set up were required to conduct the present study. The equipments and instruments namely Texture analyzer (TAHD), Kjeldhal plus, digital pH meter, deep freezers etc. were extensively used.

3.2 Sources of Buffalo Meat Samples.

Buffalo meat was collected from the local meat shop. Generally, male animals of about 2 years age was slaughtered according to the traditional halal method at buffalo slaughter house of Aligarh Municipal Corporation, Aligarh after pre-slaughter holding in the lairage for a period of 16-19 hours. Although weight, sex, source, method of slaughtering and time of collection of carcass were same for the entire experiment. Proximate composition of meat especially with respect to moisture and fat content was found to vary from one carcass to the other. Meat samples from round portions (Comprising mostly semimembranosus, semitendinosus, bicepsfemoris and quadriceps muscles) part of carcasses of good finish were obtained from meat shop within 3 hours of slaughter. For each trial of the experiments similar meat samples in required quantity were procured from round cut of a carcass. The meat chunks were packed in low-density
polyethylene (LDPE) bags and brought to the laboratory within 10 minutes. The temperature of meat was 25°C±3 on arrival at the laboratory.

3.3 Preparation of meat samples.

Buffalo meat was evaluated for physico-chemical properties soon after obtaining the samples within 5-6 hours of post mortem. 4 kg meat sample were taken and cut into eight small cubes each weighing 500 gms. Each samples weighing 500 grams was cut in to small cubes and made ready for control and treated samples.

For curing of meat ,a curing solution was made of 80 gram common salt (Iodine free), 20 gram sugar, 1.16 gram salt peter (Potassium nitrate) and 540 ml distilled water as suggested by Sahoo (1995). The solution was thoroughly mixed to dissolve the ingredients. The meat samples were submerged into solution and curing was allowed for 48 hours at 4° C in an ultra low temperature cabinet. After curing , the meat pieces were removed , and left for 2 hours in open to allow the meat surface to dry off. Cured meat samples were packed in HDPE and Al foil bags, and stored at 0° C and −4° C as described earlier.

Meat samples were also cured and treated with antioxidants i.e. Sodium Ascorbate and Sodium Hexa Meta Phosphate. For this purpose a solution containing 500 ppm of SA or SHMP was used in curing solution.

3.4 Estimation of Physico-Chemical Properties.

Raw meat samples were analyzed for moisture, ash, protein, fat and pH. These properties of raw buffalo meat were evaluated before and after giving different treatments (curing, curing combined with two different antioxidants like Ascorbic Acid and Sodium Hexa meta
phosphate and smoking). Samples were packed and wrapped in different packaging material, kept under refrigerated storage temperature (0±1°C and -4±1°C) for the study of shelf-life.

3.5 Experimental Methods.

3.5.1 Estimation of Moisture.

10 gm each raw and treated buffalo meat samples were weighed into a flat bottom dried tared dish. The dishes and its content were placed in hot air oven (Yorco Hot Air Sterilizer), thermo statistically controlled at 150±5°C and heated until successive weighing showed no further loss in weight. At the end the dishes were removed from the oven and placed in a desiccators and allowed to cool and thereafter weighed. Following formula was used for the estimation of moisture content in meat samples:

$$\text{Moisture content (\%) = \frac{\text{Loss in weight}}{\text{Initial weight}}} \times 100$$

3.5.2 Estimation of Ash.

Dried sample was weighed in a crucible and ignited at the temperature of 350°C for 6 hours in muffle furnace (Make-Tanco, Delhi). It was then taken out and allowed to cool for a moment and placed in desiccators until cooled and finally weighed to a constant weight. The ash content was calculated as shown below,

$$\text{Ash content (\%) = \frac{\text{Final weight of ash}}{\text{Initial weight of ash}}} \times 100$$
3.5.3 **Protein estimation.**

Protein was analytically estimated by determining the amount of total nitrogen in the sample as suggested by Ranganna (1994) using following formula:

\[
\text{Amount of protein in the sample} = \text{total nitrogen} \times 6.25
\]

Following regents were used:

(a) Sulphuric acid, 98% pure (Merk, 98%, B.No. C3 3124)
(b) 0.1 N hydrochloric acid (Qualikems, B.No. QX 030217)
(c) 2% Boric acid solution (SRL, B.No. TI822004)
(d) 30% Sodium hydroxide (Merk, B.No. DA1DR 51088/A)
(e) Catalyst mixture (Potassium Sulphate and Copper Sulphate)
(f) Mixed indicator (Bromo cresol green 0.1% + Methyl red 0.1%)

**Apparatus:** Kjeldhal apparatus

**Method:**

5g of finely minced meat was transferred into a digestion flasks and 2g catalyst mixture was also added. Then 10 ml concentrated sulphuric acid was poured into the mixture and kept for gentle heating. The heating was continued until frothing ceased, further it was boiled and digestion was continued for some time until the mixture became colourless. The complete digestion required at least 2 hours. The flask was cooled after digestion and digested liquid was filtered. The volume of this sample was made up to 250 ml. The water was boiled in the steam generator gently.

A 10 ml of sample was taken and transferred into the distillation tube through the small funnel and 40 ml of 30% NaOH was also added into the same tube. The stop cork connecting to small funnel was
closed. The steam trap thus compelled the steam to pass through the distillation tube. The Ammonia liberated from the reaction mixture was absorbed in 10 ml of 2% boric acid solution. Distillation was continued for five minutes. This solution was treated against N/10 HCl using mixed indicator. The blank was run in the second test of experiment and the titration was done in a similar way.

**Calculation:**

Percentage nitrogen was calculated as

\[
N(\%) = \frac{(\text{Sample blank}) \times N \times \text{of HCl} \times 14 \times \text{volume made up of digested}}{\text{Aliqot of digest} \times \text{weight of sample} \times 1000} \times 100
\]

3.5.4 **pH Measurement.**

The pH value of the finely minced meat samples were determined after homogenizing 10 g of the sample with 50 ml of distilled water. The pH of the suspensions were recorded using reference and glass electrode portable type Digital pH meter (Model Khera, Delhi)

3.6 **Microbiological Quality.**

Test for total plate count was conducted before and after treatments (namely curing, curing combined with different antioxidants, smoking curing followed by smoking and finally treated with antioxidants and followed by smoking) in all samples and at regular intervals during storage at two different temperature of 0°C & -4°C. Microbial quality is considered to be the most important attribute of shelf -life of meat. There is no distinct line of demarcation of microbial population for meat beyond which it is considered to be unsafe for the human consumption. However, microbial population exceeding 10⁷/ g of meat samples was taken as unsafe for human
consumption at which unpleasant odour in raw meat samples was observed (Ranken and Kill, 1993).

**Total plate count:**

In each test 1 g of meat was taken with the help of sterile knife spatula and forceps from samples and mixed in the cyclo mixer (Make Remi model CM-101). 9 ml of distilled water was added in the sample. Serial dilutions were made and suitable dilutions were poured using plate count agar medium (Composition: Peptone 5g, Yeast extract 2.5 g, meat extract 2.5 g, NaCl 5 g, Agar 10 g, Distilled water 500 ml).

Duplicate plating of sample was followed. The plates were allowed to set and were incubated (Model: BOD incubator Super Deluxe make Yorco Co.) at 30°C for 40 hours. Viable colony forming units were counted under digital colony counter from suitable dilution and the average counts were expressed in log number per gram of samples.

### 3.7 Organoleptic Properties

Colour, odour and texture of meat samples were evaluated organoleptically for all samples based on 8 point hedonic scale where in 8 was extremely desirable and 1 was extremely undesirable. The organoleptic evaluation of meat samples were done frequently as there was lack of experts and scores were entirely based on the evaluation of 3 or 4 individuals only.

The odour of the stored meat was observed soon after opening of the packets taken out from the refrigerator. The test Performa was also developed and supplied to experts at the time of evaluation. The test Performa is given below in Table-3.1
Table-3.1 Eight point Hedonic Scale for Sensory Evaluation.

<table>
<thead>
<tr>
<th>Sensory Attributes</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>8</td>
</tr>
<tr>
<td>Very good</td>
<td>7</td>
</tr>
<tr>
<td>Good</td>
<td>6</td>
</tr>
<tr>
<td>Fair</td>
<td>5</td>
</tr>
<tr>
<td>Slightly poor</td>
<td>4</td>
</tr>
<tr>
<td>Moderately poor</td>
<td>3</td>
</tr>
<tr>
<td>Very poor</td>
<td>2</td>
</tr>
<tr>
<td>Extremely poor</td>
<td>1</td>
</tr>
</tbody>
</table>

3.8 Smoking Process.

3.8.1 Requirement for construction of smoke house

The function of a smoke house is to retain enough heat and smoke for smoking to be completed. The items used in building a smoked house must,

(a) be capable of providing a source of smoke,
(b) create a space which confines the smoke,
(c) have provision to hold the meat block, and,
(d) provide an air passage along which to direct the smoke.

3.8.2 Construction of drum smoke house.

An empty oil drum was used for the construction of drum type smoke chamber. A hole was made at the bottom of the drum while the top of the drum was kept open. This drum was then placed over a pit of diameter less than that of drum while the depth of the pit was kept 2'. For smoking, meat samples were hanged in the smoke drum with the help of metallic wires. Properly dried neem wood was used as fuel to create smoke, the temperature of which was kept between 50-60°C by
manually controlling the draft of wind in the fire pit. Smoking was done for 8-9 hr and smoked meat samples were packed in HDPE and Al-foils and stored at 0°C and −4 °C temperatures respectively. Similarly cured meat and cured and anti-oxidant treated meat samples were also smoked and stored.

3.9 Texture Analysis.

Textural analysis of raw, treated and preserved meat samples was done by TAHD type texture analyzer. Texture analyzer is an instrument, which determines the textural properties of food material. Textural properties of different food materials/products, which are indicative of its quality, are not same. For example in case of potato chips crispness and tenderness, in case of bread sponginess, in case of jam and butter spreadibility in case of meat products springiness, tenderness, juiciness and cohesiveness etc. are used for this purpose. In case of meat and meat products guillotine knife was used as the instrument of the probe, for measurement of hardness/tenderness of meat/meat products. Texture expert, a computerized package helpful in texture analysis of meat and meat products. The study on TAHD is automated through computer. Salient features of texture expert include texture analyzer setting probe selection, opening of new file and finally run the test to get the graphical representation between force and time or force vs. distance. In this type of test, the positive peak force measures the hardness of meat samples in gram. Once the probe has reached the sample, force is seen to increase at a steady rate. As the probe moves down further onto the sample the force begin to increase rapidly as the sample begins to deform or rupture or penetrate. After penetrating or rupturing has occurred the subsequent increase in force is as a result of the force required to push. Test results obtained from samples of the approximate same type give the typical average
maximum peak force (Firmness/hardness) values. The results shown in the results and discussion part (in graphs) that the different storage time, treatments with packaging material and storage temperature be repeatably differentiated by both the measured peak force and the area under the curve. The maximum force required to penetrate the sample was reported as the hardness of the sample. The highest peak on the graph was reported as the hardness value of the meat. The textural hardness or fracturability was measured and expressed in shear force as Newton (Nordyke et. al., 2000).

3.10. Statistical Analysis.

The experiment was replicated thrice. Observed data up to the entire storage for every treatment, packaging materials and refrigerated temperatures were statistically analyzed using a factorial block design. The data were subjected to Duncans new multiple range test (Saha, 1995). A regression line was drawn to predict correlation of different treatments and packaging material with storage temperature and storage time on pH and TPC with the help of a statistical software package SPSS on an SAMTRON-Compatible personal computer.
Fig. 1 Raw buffalo meat sample
Fig.2. TAHD type texture analyzer
Fig. 3 Drum type smoke chamber
Fig. 4. Meat sample hanged from copper wire during smoking.