Materials and Methods
3. MATERIALS AND METHODS

3.1. Effect of pretreatment on physico-chemical characteristics of chicken and mutton meat during frozen storage

3.1.1. Preparation of the materials

3.1.1.1. Meat Samples

Meat samples (chicken and mutton) were procured from local market of Mysore, India and all trimmable fat and connective tissues were removed, deboned and washed. Chicken and mutton were cut into of 2-3 cm$^3$. The samples are packed in LDPE pouches and stored at -18$^0$C. This was treated as control. The samples were subjected to physico-chemical analysis for at intervals of 1, 2, 3, 4, 5 and 6 months of storage at -18$^0$C

3.1.1.2. Calcium propionate solution

0.2 M (37.22g/1000ml) and 0.3 M (55.866g/1000ml) Calcium propionate solutions were prepared for the treatment of chicken and mutton samples.

The chicken and mutton (2-3 cm$^3$) were dipped in calcium propionate solution (0.2M and 0.3M) for 1 hr with uniform stirring and allowed to drain for 15 min. The samples are packed in LDPE pouches and stored at -18$^0$ C. The samples were subjected for physico-chemical analysis at intervals of 1, 2, 3, 4, 5 and 6 months of storage at -18$^0$C

3.1.1.3. Ginger-Turmeric Extract (GTE)

For the preparation of ginger-turmeric extract (GTE), fresh ginger and turmeric rhizome were peeled, sliced and blended with distilled water in a blender for 1 to 2 min. The homogenate was squeezed through 4 layers of muslin cloth. Fresh GTE was prepared for each trial. For the experiments, chicken and mutton samples were treated with 3%, 5% and 7% GTE for 24h at 4$^0$C. It is then drained for 15 min. and packed in LDPE pouches and stored at -18$^0$C. The samples were subjected for physico-chemical analysis at intervals of 1, 2, 3, 4, 5 and 6 months of storage at -18$^0$C.
3.1.4. Details of pretreatments and sample code

T1-Control (Untreated)
T2-3% Ginger-Turmeric Extract
T3-5% Ginger-Turmeric Extract
T4-7% Ginger-Turmeric Extract
T5-0.3M Calcium propionate
T6-0.2 M Calcium propionate

3.1.2. PHYSICOCHEMICAL PARAMETERS

3.1.2.1. Texture

Texture is the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch and kinesthetics (Szczesniak, 2002).

Procedure: Chilled samples were equilibrated to room temperature before texture measurement. The Warner–Bratzler shear force (WBSF) of the cores were measured using texture analyser (Model No.: 5197, Stable micro systems HD Plus, Goldalming, surrey, GU71YL, UK) with V-shaped stainless steel Blade (60° angle) and triangular hole in the middle. The samples were sheared perpendicular to the muscle fiber orientation with 75 N load ranges and a crosshead speed set at 200 mm/min. The force required to shear the samples was recorded in Newton (N). Each sample was assessed 2-4 times. The parameter recorded was the maximum shear force that is the highest peak of the curve, which is the maximum resistance of the sample to shearing (Naveena, et al., 2011).

Cubes of 1 cm$^3$ chicken sample were taken and Texture Profile analysis (TPA) was analyzed using 75 mm cylindrical probe. The force required to shear the samples was determined. Hardness determined according to Bourne (1978).

3.1.2.2. Colour (In terms of hunter values L, a* & b*)

Appearance of meat plays an important role in perceived quality of meat and color represents the freshness (Mancini & Hunt, 2005). Hunter colorimeter Lab systems measure reflected and transmitted color of food products. It gives numerical
values that correlate to what we see and are ideal for measuring raw materials through final product. A Hunter Lab colorimeter is used to measure the reflectance of batches of samples.

- $L$ measures lightness and varies from 100 for perfect white to zero for black, approximately as the eye would evaluate it.

The chromaticity dimensions $(a$ and $b$) give understandable designations of colour as follows:

- $a^*$ measures redness when positive, gray when zero, and greenness when negative.
- $b^*$ measures yellowness when positive, gray when zero, and blueness when negative.

**Principle:**

Colorimeters measure color using three or four filters that match human color receptors. Colorimeters can show $L$, $a$, $b$ or $L^*$, $a^*$, $b^*$ numbers, but can only measure in one light source. Colorimetry is based on reflection measurements. The monochromatic colorimeter measures the amount of light (in arbitrary units) which is reflected in a narrow spectral area of the visible light. It is basically color-blind sees only one color, e.g., red, yellow, or green. The tristimulus colorimeter measures the true colors and correlates them to what the eye sees, using specialized glass color filters and light detectors (up to 10 million different shades of color can be quantified (Kim, *et al*., 2011).

**Procedure:** Instrumental surface colour (CIE $L^*a^*b^*$) of fresh and freeze thawed samples were evaluated using a Hunter Lab Mini Scan XE Plus Colour Meter (Illuminant D65, 2.5 cm diameter aperture, 10° standard observer; Hunter Associates Laboratory, Inc., Reston, VA). Calibration was performed by using standard black and white tiles prior to the colour measurement. CIE $L^*$ $a^*$ $b^*$ values were used to calculate saturation index/ chroma $[(a^*+b^*)^{1/2}]$ and hue angle $[\tan^{-1}(b^*/a^*)]$ (AMSA, 1991).
3.1.2.3. Determination of Water holding capacity (WHC)

Juiciness is one of the most important quality attribute to the consumer, while WHC is a more functional quality parameter as it is regarding the water and weight losses during storage, manufacturing, processing and preparation of meat (Van Oeckel, et al., 1999). For the estimation of the WHC, a force such as pressure (filter paper press method) or suction (filter paper method) has to be applied to the meat sample and the amount of released water determined. The force applied removes unbound or loosely bound water and the force can be centrifugal, compression, capillary action or gravity (Zhang, et al., 1995).

**Principle:** The capacity of meat to retain its water during the application of physical forces is known as water holding capacity. This property of meat has a special significance because it contributes to the juiciness of cooked meat besides influencing the texture and color.

Meat samples evaluated for WHC using the procedure reported by Wardlaw *et al* (1973).

**Centrifugal method:**

This method measures the ability of meat to absorb added water. When centrifugal force is applied the loosely bound water is removed. This method is effective in predicting drip losses from raw muscle during storage. Results of this method depend on: (a.) centrifugal force in g;

(b.) the time of centrifugation;
(c.) plasticity of the meat influenced by state of meat (pre or post rigor);
(d.) sample size;
(e.) temperature;
(f.) salt content.

Centrifugal force results in a supernatant decanted as exudate. The muscle sample is weighed before and after centrifugation to determine the amount of moisture lost and the corresponding water-holding capacity can be predicted (Hamm, 1986; Warriss, 2000).
Procedure: Meat samples evaluated for WHC using the procedure reported by Wardlaw et al (1973). Twenty gram (20g) minced meat samples were stirred with 30 ml of 0·6 M sodium chloride in a centrifuge tube. The tube was then kept at 4°±1°C for 15 min, stirred again and centrifuged at 3000 g for 25 min. The supernatant was measured and WHC was expressed as a percentage.

\[
WHC (\%) = \frac{[\text{Initial weight of NaCl (g)} - \text{Supernatant weight (g)}]}{\text{Weight of meat (g)}} \times 100
\]

3.1.2.4. Thaw drip and Cook loss Percentage

Thawing loss of the thawed meat was determined from the known weights of meat cubes before and after thawing (Xia, et al., 2009) and expressed as:

\[
\text{Thawing loss (\%) = } \frac{\text{Weight of frozen sample (g) - Weight of thawed sample (g)}}{\text{Weight of frozen sample (g)}} \times 100
\]

All measurements were made on triplicate samples, and the mean values from these triplicates were reported.

For cook loss, 20g meat sample was sealed in a Polypropylene (PP) bag and cooked in a water bath at 100˚C for 20min. Each piece was cooled, removed from the bag and then weighed. The weights of samples were recorded before and after cooking and the cook loss was expressed as a percentage (Young and Lyon 1997).

\[
\text{Cook loss (\%) = } \frac{\text{Initial weight of meat (g) - Weight of meat after cooking (g)}}{\text{Initial weight of meat (g)}} \times 100
\]

3.1.2.5. Determination of pH

The pH of the meat was assessed by inserting a pH probe into meat and reading the pH value on a pH meter (Young and Lyon 1997).

pH is defined as the negative logarithm of the hydrogen ion activity, \(a_h\) (Sorensen & Linderstrom- Lang, 1924).

\[
pH = -\log_{10} a_h
\]

pH of a sample helps to predict the stability, stage of rigor mortis as well as it also has an influence on water holding capacity and several other processing characteristics. The flesh of animals prior to slaughter has a pH value of 7.1. After
slaughtering, some of the glycogen in the meat turns into lactic acid. As a result, the pH value is lowered. The increasing acidity of the maturing carcass varies in its speed, depending on a number of factors such as type of animal, breed, rearing characteristics and treatment of the animal prior to slaughter.

**Procedure:** The pH of fresh and frozen samples was measured with the aid of a pH meter equipped with an electrode (Model No.:1595414, Cyber Scan, Eutech Instruments, and Netherlands). The probe was inserted into the geometric center of the thickest part of the muscle and pH recorded when a stable reading on the meter was observed for 10 s.

### 3.1.2.6. Estimation of Moisture

The moisture content of the meat samples were determined by drying approximately 10-15g of the chopped sample in replications and dried in petri dishes (which were previously weighed) at 102°C in hot air oven. The weight of the product was noted after 4hrs, and then at an interval of 1 hr till the weight of samples in third decimal place were constant and the final weight of the dishes containing samples were noted down (AOAC, 1990).

\[
\text{Moisture content (\%) = } \frac{\text{Initial weight - final weight}}{\text{Initial Weight}} \times 100
\]

### 3.1.2.7. Moisture Estimation (Vacuum oven Method)

Moisture content of freeze dried chicken samples was determined using vacuum drying to a constant weight as per Ranganna (2004).

2–3g of dried sample was taken in a previously tarred flat bottom dish in dehumidified room where relative humidity was maintained at 23 ± 2% to avoid moisture uptake. Dish was covered with a lid and kept it in a desiccators containing P₂O₅ and transferred quickly to a vacuum oven for drying after uncovering the dish. The product was heated under vacuum for 12 to 16h at 70°C. After drying, vacuum was broken through the trap containing calcium oxide. The product was taken from the oven and stored for 30 min in desiccators and weighed. The moisture content of the sample was calculated as above.
3.1.2.8. Estimation of Free fatty acids (FFA) (AOAC, 1975)

About 10 g of sample was blended with anhydrous sodium sulphate in a mortar. The blend was shaken well with chloroform and filtered. 20 ml of extract was taken into a clean beaker. Chloroform was evaporated off on a water bath and weight of fat determined. Another 20ml of extract was transferred into another conical flask. Chloroform was evaporated off. To this 10 ml of neutral alcohol was added and warmed. It was titrated against 0.01N NaOH using phenolphthalein as indicator. FFA was calculated as given below as the percentage of oleic acid.

\[
\text{Volume of NaOH used} \times 0.01 \times 0.28 \times 100 \\
\text{Weight of Fat}
\]

3.2. Repeated freezing and thawing on physico-chemical quality

3.2.1. Samples preparation

For the study of repeated freezing and thawing on physico-chemical quality parameters of chicken and mutton, meat samples (chicken and mutton) were procured from local market of Mysore, India and all trimmable fat and connective tissues were removed. Chicken and mutton were cut into cubes of 2 cm³. Samples were stored at -18±2 ºC for freezing. After every 24 hours, samples were thawed under two conditions i.e., under air (at room temperature 25± 1ºC for 1 hour) and water (25± 1ºC for 45 minutes in LDPE packs). After complete thawing, samples were stored again in deep freezer for next 24 hrs. Similar procedure was repeated for 4 cycles of freezing and thawing.

The samples for evaluation of the effect of repeated freezing and thawing on physico-chemical parameters of chicken and mutton were prepared as follows. The experiments were planned for four cycles with and without the incorporation of 0.5% as cryoprotectant. The breast piece of chicken was selected for the study. The samples for different cycles of experiments with respect to air thawing and water thawing were packed in LDPE pouches and kept for frozen storage. The complete set of the samples were removed from frozen storage after 24h and subjected for air thawing and water
thawing. After reaching room temperature the first cycle samples were removed for analysis and the remaining samples were kept in frozen storage. This procedure was continued for second, third and fourth cycles.

Similarly, samples were prepared for cryoprotectant treated samples, but in this case, before freezing samples were dipped in 1% glucose solution for 10 minutes then packed in LDPE. Samples were assessed for pH, water holding capacity, thawing loss, cooking loss, color and texture to compare it when subjected to different conditions such as cryoprotectant treatment.

Samples were then assessed for various properties such as pH, water holding capacity (WHC), cooking loss, thawing loss, color (hue & chroma), tenderness (Warner Bratzler shear force) and structural analysis (Scanning electron microscope).

Sample Codings:-

\[ T_{1c} \]: Chicken; air thawed; untreated
\[ T_{2c} \]: Chicken; air thawed; cryoprotectant treated
\[ T_{3c} \]: Chicken; water thawed; untreated
\[ T_{4c} \]: Chicken; water thawed; cryoprotectant treated
\[ T_{1m} \]: Mutton; air thawed; untreated
\[ T_{2m} \]: Mutton; air thawed; cryoprotectant treated
\[ T_{3m} \]: Mutton; water thawed; untreated
\[ T_{4m} \]: Mutton; water thawed; cryoprotectant treated

3.2.2. Scanning Electron Microscopy

The SEM allowed surface characterization of external or internal features (by fracture) without the need to prepare ultra thin sections this technique constitutes one of the most valuable tools in micro structural food analysis.

Samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h at 4 °C and post fixed in 2% aqueous osmium tetroxide for 4 h. Later samples were dehydrated in a series of graded concentrations of ethanol, freeze-fractured with a razor blade in liquid nitrogen, and dried with t-butyl alcohol. The processed samples were mounted over the stubs with double-sided carbon
conductivity tape, and a thin layer of gold coat over the samples was applied by using an automated sputter coater (Model: JEOL JFC-1600) for 3 min and scanned under scanning electron microscope (SEM, Model: S-340014, Hitachi, Japan) at ×200, ×400 and ×700 magnifications as per the standard procedures (Bozolla & Russell, 1998).

The electron beam is focused by one or two condensers, passes through pairs of electromagnetic scanning coils, deflecting the beam, and leaves the final lens into the specimen chamber, where it strikes the sample surface obliquely. The degree of obliqueness is termed the tilt. The deflection system moves the beam along a line, through discrete locations, and then along the next line below the first previous one, until a rectangular raster is generated. The magnification of the image is the ratio of the length of the raster on the viewing screen to the corresponding length of the raster in the specimen. Increase in magnification is achieved by decreasing the scanned area.

3.3. Moisture sorption analysis

The moisture desorption studies were carried out during repeated freezing and thawing in different cycles (Four cycles of freezing and thawing) with respect to water thaw process. Five empirical models viz, Oswin model, Iglesias and Chirife model, Smith model, Caurie model and Peleg model are used for fitting the desorption data. The goodness of fit of each model was computed in terms of coefficient of regression ($R^2$) from the plot of experimental and predicted values.

Moisture sorption analysis was carried out using a moisture sorption analyzer (Q 5000 SA, TA Instruments, USA) under controlled conditions of temperature and humidity. The stepwise adsorption and desorption studies of the meat samples were carried out from 90% RH to 10% RH at a step interval of 10% RH at 25°C. At each RH level, equilibration was stopped when the relative change in sample mass remained below 0.01% for 5 min, and the next RH step was automatically applied.

3.4. Factors influencing calorimetric determination of glass transition

3.4.1. Sample preparation

Boneless chicken breast meat was brought from local market, Mysore, India. The meat was trimmed of any visible fat, chopped with a knife into small pieces and divided into two lots. The first lot was kept frozen until analyzed. The second lot
was freeze-dried and packed in Aluminium foil based packaging material and kept frozen at -18°C until analyzed. Before any experiments, the meat was thawed in a refrigerator at 5±1°C. Samples approximately below 10mg were used for thermal analysis.

3.4.2. Freeze Drying

Freeze drying was carried out in a pilot scale freeze dryer, Epsilon 1/60 (Martin Christ GmbH & Co KG, Osterode, Germany) equipped with rapid freezing and drying facilities. The samples (Chicken and mutton) were pre-frozen to – 40 ± 2°C for 4 hours (Hull corporation, USA) and drying was carried out under a chamber pressure ranging between 1-3 millibar and using a variable degree of temperatures (30-60°C) so that the final moisture content of chicken and mutton samples were reduced to 2-3%. Vacuum was released and the products were taken to low humidity room (23 ± 2%) and packing was carried out.

3.4.3. Measurement of Tg by DSC

A DSC 2010 differential scanning calorimeter (TA Instruments, New Castle, DE, USA) was used to determine the Tg values. Samples were enclosed in hermetically sealed aluminum pans just before analysis and then loaded into the equipment at room temperature. An empty pan was used as reference and nitrogen gas at a flow rate of 60ml/min was employed in the purge line to control the local environment around the sample. The calibration of the cell was made following the DSC manufactures recommendation and consisted of three procedures namely baseline, cell constant and temperature calibration. The base line calibration involved heating the cell through the entire temperature range i.e., from -50 to 20°C. The cell constant of the cell was determined by heating the standard material (indium) through its melting temperature. The temperature calibration was performed by using a multiple-point calibration with indium (mp 156.60°C, ΔH_m 28.71 J/g), distilled water (mp 0°C, ΔH_m 335 J/g) and heptane (mp, -91.0°C, ΔH_m 140 J/g). In our experiments samples were held at various annealing temperatures like –11°C, -14°C, -17°C, -20°C, -23°C and held isothermally for 1 h and cooled to -30°C. After holding isothermally for 1 min, samples were ramped at various heating rates like 0.5, 1, 2, 3, 4 and 5°C/min to 20°C. The DSC measurement was repeated in triplicate. The results of the experimental run were analyzed with the universal analysis software, which is
provided with DSC instrument. Mid point of the step change in the heat flow curve was taken as glass transition temperature. DSC was also employed to determine the freezing behavior of meat samples.

3.4.4. Thermo gravimetric analysis

Thermo gravimetric analyzer (TGA Q50, TA Instruments, DE, USA), a thermal weight change analysis instrument was used in conjunction with a thermal analysis controller. The Thermogravimetric analyzer was employed to measure the amount and rate of change of weight of the material either as a function of increasing temperature or time, in a controlled atmosphere. The initial weight of each sample was approximately 20 mg. The samples were kept in platinum crucible and heated in a furnace flushed with N₂ gas at the rate of 40 ml/min and heated from 30°C to 105°C at a rate of 10°C/min and held isothermally for 60 min. The percentage weight loss was plotted against time and this was used to determine moisture content of all samples.

3.5. Preparation of Ready To Eat (RTE) pineapple chicken curry

Broiler chicken (6-8 weeks old) were procured from the local market, dressed conventionally and were brought to the Freeze Drying and Animal Products Technology Division Lab, (FDAPT) Mysore, India. On the day of preparation, the carcasses (1.10 to 1.20 kg) were washed under running tap water and deboned. Breast and leg muscles were cut into cubes of 2-3 cm and were marinated with curd for 1-2 hours. This was cooked for 10-15 min at 95-100°C and cooled to 30-40°C. The ingredient composition for the preparation of RTE pineapple chicken curry is given in Table.12. Cooking oil was heated in a stainless steel vessel to 110-120°C, added clove, cinnamon and cardamom and roasted for 1-2 minutes. Sliced onion, ginger, garlic, coriander leaves and green chillies were added and sautéed till light golden colour. Then added tomato puree, red chilli powder, coriander powder, turmeric powder, salt and pineapple cubes and cooked for 4 min on low flame. To this gravy mix, added cooked chicken cubes along with cooked out juice and mixed well. This was further heated for 5 min at 85-90°C. The product cooled to 30-40°C for 40-50 min. The product is exactly divided into two portions and to the second portion 0.5% glucose as cryoprotectant is added and separated the meat chunks from gravy.
The product was packaged in polythene pouches (300 gauges) with 100 g meat and 250 g gravy in each pouch for cryoprotectant treated and untreated samples. The sealed packets placed in wax coated carton and frozen in a plate freezer until the product temperature reached -45 to -60°C (115-125 min). A digital temperature recorder with metallic probes (Aptec, Chennai, India) was used to ensure adequate temperature decline of the product. The frozen product was then stored at a freezer maintained at -18±2°C.

Table.12. Ingredients composition of pineapple chicken curry.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken Cubes</td>
<td>2.22Kg</td>
</tr>
<tr>
<td>Pine apple</td>
<td>300 g</td>
</tr>
<tr>
<td>Onion</td>
<td>200 g</td>
</tr>
<tr>
<td>Green Chilli</td>
<td>32.0g</td>
</tr>
<tr>
<td>Salt</td>
<td>22.0g</td>
</tr>
<tr>
<td>Garlic</td>
<td>23.0g</td>
</tr>
<tr>
<td>Corn Flour</td>
<td>10.0g</td>
</tr>
<tr>
<td>Ginger</td>
<td>23.0g</td>
</tr>
<tr>
<td>Pepper</td>
<td>2.00g</td>
</tr>
<tr>
<td>Vegetable Oil</td>
<td>75.0ml</td>
</tr>
<tr>
<td>Vinegar</td>
<td>6 tsp</td>
</tr>
<tr>
<td>Soy sauce</td>
<td>3 tsp</td>
</tr>
<tr>
<td>Tomato sauce</td>
<td>100 g</td>
</tr>
<tr>
<td>Chili sauce</td>
<td>20.0g</td>
</tr>
<tr>
<td>Jaggery</td>
<td>20.0g</td>
</tr>
</tbody>
</table>

3.5.1. Quality Evaluation

Physical (pH, shear force) chemical (FFA, TBARs, Fatty acid profile by GC), microbiological studies (SPC, Yeast and mold, *Staphylococcus aureus*, *Salmonella* and *Escherichia coli*) and sensory analysis were conducted for 6 months of frozen storage. The product was thawed at 26±2°C for 30 min and was subjected to the analyses mentioned.
3.5.1.1. Physical and Chemical parameters

Separated the meat chunks and gravy and homogenized in a mixer for sampling. Proximate composition and NaCl content in chicken chunks and gravy were determined (AOAC, 2005). FFA, pH and TBA of both chicken chunks and gravy were determined periodically during the storage period. Free fatty acid (FFA) (Modi, et al., 2004) and thiobarbituric acid (TBA) were determined by aqueous extraction procedure (Pikul, et al., 1989) and pH by immersing a glass calomel electrode directly into the sample using a pH meter (Cyberscan 1000, Eutech Instruments, Singapore). L* (lightness), a* (redness) and b* ( yellowness) were recorded by Hunter colour measuring system (Labscan XE, Hunter Associates Laboratory Inc., Virginia, USA). Measured the viscosity of the gravy at ambient temperature (27±2°C) using Rheology International Viscosimeter (Model R1:2: L, Rheology International Shannon Ltd., Shannon, Ireland) using an ASTM No: 4 spindle at 400 rpm. Shear values were measured in a Lloyds Texturometer (LR5K, Lloyd Instruments Ltd., Hampshire, U.K.) in 100-kg load cell at a speed of 50mm/min with a 1mm thick blade using chicken chunks of 1×1×1.5 cm strips.

3.5.1.1.1. Total protein in meat samples (AOAC, 2005)

One gram of meat sample was weighed and transferred to a 250ml Kjeldahl flask taking care to see that no portion of the sample clinged to the neck of the flask. Approximately 1g of catalyst mixture (2.5g SeO2, 100g K2So4 and 20g CuSo4 5H2O) and 25ml of concentrated H2So4 were added and placed the flask on the stand in the digestion chamber. The flask was heated gently over a flame until the initial frothing ceased and the mixture boiled briskly at a moderate rate. The digestion continued until the digest becomes clear. The digest was cooled and made up to 100ml in a volumetric flask with distilled water. A blank digestion was also carried out without the sample and made up the digest to 100ml.

1ml aliquot was distilled with the addition of 2ml of 30% NaOH solution. The ammonia liberated was absorbed into 5ml of a 2% solution of boric acid containing a mixed indicator of bromocresol green and methyl red. The contents of the flask were titrated against 0.01N HCl to bluish green to pink colour. The blank distillation and titration were carried out as in the case of the sample.
Nitrogen % = \frac{(\text{Sample titre – blank titre}) \times N \text{ of HCl} \times \text{Volume made up of the digest} \times 100}{\text{Aliquot of the digest taken} \times \text{wt. of the sample taken} \times 1000.}

Protein % = \text{nitrogen %} \times 6.25.

3.5.1.1.2. Determination of fat content (Soxhlet method)

Fat content of dehydrated meat and poultry samples was determined as per AOAC (1990). The fat was determined by extracting 5 – 10g of moisture free sample with petroleum ether for 6 – 8 h in a Soxhlet apparatus. The solvent was evaporated by heating at 60\(^\circ\)C using flash evaporator and the fat content was determined. The weighing was repeated until a constant weight was obtained. Increase in the weight of tarred flask after complete evaporation of petroleum ether indicates the weight of fat.

\[
\% \text{ of fat} = \frac{\text{Increase in weight of the flask} \times 100}{\text{weight of sample}}
\]

3.5.1.1.3. Total ash

The tare weight of silica dishes (7–8 cm diameter) was noted. Five g of the sample was weighed out into each dish and ignited the contents on a Bunsen burner. The material was ashed not more than 525\(^\circ\)C for 6 h in a muffle furnace, cooled the dishes and weighed. The difference in weight gave the total ash content and was expressed as percentage (Ranganna, 2004).

3.5.1.1.4. Microbiological Quality

Chicken chunks in the curry were cut using a sterile knife and mixed with the gravy. Placed a 50g sample of the mixture in a sterile stomacher bag containing 450 ml sterile saline (0.85% NaCl) solution and blended in a stomacher (Seward Stomacher400, Seward Medical, London, U.K.). The blended samples were tested for standard plate counts (SPC), coliforms, yeast and mould (Y&M), staphylococci, salmonella and E. coli by pour plate method (APHA, 2001).
3.5.1.1.5. Sensory Quality

Packets of RTE chicken curry was thawed by holding them at 26±2°C and warmed the product in a hot pan maintained at 80-90°C for 3-4 min. The coded samples were subjected to sensory evaluation by in-house trained panelists using a 9-point hedonic scale (ASTM, 1996: Modi, et al., 2003) and recorded the mean score of each attribute (colour, flavor, mouthfeel, consistency of the gravy, meat texture and overall acceptability).

3.5.1.1.6. Thiobarbituric acid Reactive substance (TBARS) determination

TBARS values in meat/poultry samples were determined as per Taraldgis method (1960).

0.02 M 2-Thiobarbituric acid in 90 % glacial acetic acid was prepared by warming on a boiling water bath.

3.5.1.1.6.1. TEP standard preparation: 1 X 10^{-3} M solution of 1, 1, 3, 3 Tetraethoxy propane (TEP) was prepared in distilled water. Appropriate concentration of TEP was prepared from the main stock solution. Color development was carried out using TBA reagent at various concentrations of the standard to draw the standard TEP curve as shown in figure.9.

![Figure 9. TEP standard curve for TBARS estimation](image-url)
3.5.1.1.6.2. Sample distillation and Estimation

Taraldgis method (1960) is one of the most widely used tests to evaluate the extent of lipid oxidation in meats. This is based on the reaction between important oxidation product malonaldehyde with TBA reagent to produce a colour complex. The chromogen results from the condensation of 2 molecules of TBA with 1 molecule of malonaldehyde. This reaction is indicated in Figure.10.

20 g of blended sample was accurately weighed and transferred into a RB flask. To that 2.5 ml of con. HCl was added along with 97.5 ml of distilled water. pH was adjusted to 1.5. Mixture was steam distilled and 50ml distillate was collected in 10 min. From this 25 ml of distillate was transferred into stoppered glass tubes and 5ml of TBA reagent was added. The test tubes were kept in boiling water bath for 35 min and it was cooled and OD was measured at 538nm. The blank was prepared as the same procedure, but without the sample. The TBARS values were calculated using the standard curve.

![Figure 10. Chemistry of TBA reaction](image-url)
3.5.1.1.7. Fatty Acid Analysis by Gas Chromatography

3.5.1.1.7.1. Esterification of fatty acids

The samples were esterified as per the procedure of Metcalfe et al. (1966) with slight modifications.

About 150 mg of lipid was accurately weighed into a clean and dry stoppered test tube. 4 ml of 0.5 N alcoholic sodium hydroxide solutions was added and heated for 5 min over a water bath at 90\(^0\) C. On cooling 5 ml of Boron trifluoride-methanol reagent (14%) was added and heated for 5 min at 90\(^0\) C over a water bath, followed by addition of 10 ml of saturated sodium chloride solution. The samples were thoroughly cooled to room temperature and 5 ml of hexane was added to each tube. It was shaken well and kept undisturbed. The upper hexane layer was drawn out into clean dry conical flask and dried over anhydrous sodium sulphate to remove the traces of moisture, if present. The samples were filtered and transferred to stoppered clean dry tubes for gas chromatographic analysis.

3.5.1.1.7.2. Quantification of fatty acid analysis by Gas chromatography

Analysis of total fatty acids was carried out by ceres – 800, Chemito model Gas chromatograph fitted with BPX 70 column (25 mt, 0.32mm ID) and flame ionisation detector. Temperature gradient programming was employed from 150 to 220\(^0\) C. Split ratio was adjusted to 1:25 and capillary flow of carrier 2 ml/min. Injector and detector port temperatures were adjusted as 230 and 240 respectively. For FID, Hydrogen and Oxygen was used and the flow was adjusted as 45 ml/min and 45 ml/min respectively. Along with samples standard esters of fatty acids were also injected and the fatty acids were detected by comparing the retention time of the standard esters of fatty acids. The quantification of the fatty acids was carried out by evaluating with the standard fatty acid esters area corresponding to each peak in the chromatogram. Iris 32 software is used to integrate and evaluate the chromatogram in the analysis.

3.5.1.1.8. Estimation of Sodium Chloride (Volhard method)

The sodium chloride content is determined by the Volhard method (AOAC, 2000). The sample is treated with AgNO\(_3\) then wet-ashed, and the excess AgNO\(_3\) is
back titrated with KSCN. The AgNO₃ solution must be added first, followed by the concentrated HNO₃. This order of addition is critical to ensure complete precipitation of the chlorides. If HNO₃ is added first, loss of chloride by volatilization as HCl could occur because HCl has a higher vapor pressure than HNO₃. The volume of AgNO₃ solution added must be in excess of that required to react with the chlorides in the sample. The concentrated solution of KMnO₄ is added to oxidize any organic matter not disposed of by the HNO₃. Should too much KMnO₄ be accidentally added, the addition of small quantities of sugar or a small piece of filter paper will cause color removal.

Following boiling, cooling, and dilution, back-titrate the excess AgNO₃ with KSCN solution, employing ferric ammonium sulfate solution as an indicator. The FeNH₄(SO₄)₂ reacts with an excess of thiocyanate, forming the salmon colored complex, ferric thiocyanate FeSCN²⁺, indicating the end point.

**Procedure:**

Weighed 2.5-3.0 g of finely comminuted meat and thoroughly mixed sample into a 300 ml Erlenmeyer flask. Run a reagent blank and a previously analyzed sample as a recovery with each set of samples. Add 25.0 ml of 0.1000 ± 0.0005 N AgNO₃ solution, swirl flask until sample and solution are in intimate contact, and then add 15 ml of conc. HNO₃ and sufficient boiling chips and boil until meat digests. (Solution will turn from a cloudy white color to yellow). To this, add KMnO₄ solution in small portions while boiling to turn solution dark brown. Continue boiling until color disappears. Continue adding small portions of KMnO₄ until solution retains dark color for several minutes before clearing. Wash sides of flask with water. (NOTE: If solution retains color and will not become colorless, add a small amount of lactose until color disappears). Added approximately 25 ml of water; boil for approximately 5 min, cool to room temperature in the fume hood, rinse the neck of the flask and dilute to approximately 150 ml with water. To this add approximately 5 ml of diethyl ether (optional), approximately 2 ml of the ferric alum indicator, and swirl to coagulate the precipitated AgCl. (If results are rounded to 0.1 %, the diethyl ether need not be added.) Titrate the excess AgNO₃ with KSCN solution to a permanent, salmon colored, end point.

NOTE: If titration with KSCN is less than 2 ml, repeat with a smaller sample weight.
3.6. Preparation of RTE mutton curry

Mutton was procured from the local market, and was brought to the FD&APT division lab, Mysore, India. On the day of preparation, the carcasses (3 kg) were washed under running tap water and deboned. The meat samples were cut into cubes of 2-4 cm and were marinated in curd for 1-2 hours. This was cooked for 20-25 min at 95-100°C and cooled to 30-40°C. The ingredient composition for the preparation of RTE mutton curry is given in Table.13. Cooking oil was heated in a stainless steel vessel to 110-120°C, added clove, cinnamon and cardamom and roasted for 1-2 minutes. Sliced onion, ginger, garlic, coriander leaves and green chillies were added and sautéed till light golden colour. Then added tomato puree, red chilli powder, coriander powder, turmeric powder, salt and cooked for 6 min on low flame. To this gravy mix, added cooked mutton cubes along with cooked out juice and mixed well. This was further heated for 5 min at 85-90°C. The product cooled to 30-40°C for 40-50 min. The product is exactly divided into two portion and to the second portion 0.5% glucose as cryoprotectant is added and separated the meat chunks from gravy.

The product was packaged in polythene pouches (300 gauge) with 110 g meat and 240 g gravy in each pouch. The sealed packets placed in wax coated carton and frozen in a plate freezer until the product temperature reached -45 to -60°C (115-125 min). A digital temperature recorder with metallic probes (Aptec, Chennai, India) was used to ensure adequate temperature decline of the product. The frozen product was then stored at a freezer maintained at -18±2°C.
Table 13. Ingredients composition of RTE mutton curry

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutton</td>
<td>3 Kg</td>
</tr>
<tr>
<td>Onion</td>
<td>750 g</td>
</tr>
<tr>
<td>Tomato</td>
<td>750 g</td>
</tr>
<tr>
<td>Green Chilly</td>
<td>95 g</td>
</tr>
<tr>
<td>Ginger</td>
<td>75 g</td>
</tr>
<tr>
<td>Garlic</td>
<td>75 g</td>
</tr>
<tr>
<td>Kashmiri Chilly</td>
<td>40 g</td>
</tr>
<tr>
<td>Salt</td>
<td>75 g</td>
</tr>
<tr>
<td>Garam Masala</td>
<td>25 g</td>
</tr>
<tr>
<td>Coriander Powder</td>
<td>20 g</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>4 g</td>
</tr>
<tr>
<td>Anise</td>
<td>2 g</td>
</tr>
<tr>
<td>Clove</td>
<td>2 g</td>
</tr>
</tbody>
</table>

3.7. Statistical analysis

Statistical analysis was performed with SPSS Software (SPSS Inc., 2010). Completely random block design with three replicates was used to analyze the data. ANOVA (one-way analysis of variance) was performed to test significance among different treatments. Duncan’s Multiple Range test was employed to determine the statistical significance (P $\leq$ 0.05) of differences between the means to test the significant effect of various parameters at 5% level of significance.