MATERIALS AND METHODS

3.1 INTRODUCTION

The present investigation entitled ‘Studies on minimal processing and preservation of fruits and vegetables by active packaging’ was carried out in the laboratories of the Department of Food Technology, Guru Jambheshwar University of Science and Technology and CCSHAU, Hisar during 2007-2011. This chapter contains relevant informations pertaining to the research design and methodological steps used in the present investigation.

3.2 MATERIALS

3.2.1 Fruits and Vegetables

Three major fruits (apple, Banana and orange) and vegetables (cauliflower, spinach and tomato) almost of similar maturity status, colour, size and shape were selected and obtained from the local fruit market, Hisar. The commodities were properly sorted out on the basis of uniform appearance, colour, size and absence of physical and pathological injuries apparently.

3.2.2 Packaging Material

Low density polyethylene bags (LDPE), gas permeable woven fabric sheet (for sachet preparation) were purchased from the local market, Hisar and Sadar Bazar, Delhi.

3.2.3 Chemicals

Most of the chemicals used in the present research work were of high purity analytical grade obtained from Sigma-aldrich, CDH, Qualigen or E.Merck, India. The media chemicals for microbial examination were of Hi-Media Company.

3.2.4 Apparatus

The following apparatus were used during the present investigation:

1. Refractometer (ATAGO, Japan)
2. Digital vernier caliper (Mitutoyo Corporation Model)
3. Digital weighing balance (Precesia)
4. Form, fill and seal machine (Manasi, India)
5. Gas chromatograph (Netel)
6. Gas analyzer (1902 D Dual Trak, Quantek, U.S.A.)
3.3 STANDARDIZATION OF THE STEPS FOR MINIMALLY PROCESSED FRUITS AND VEGETABLES

3.3.1 Selection of fruits and vegetables

Fresh fruits and vegetables were selected properly while purchasing and again sorted out in the laboratory on the basis of uniform appearance, colour, size, maturity status and absence of physical and pathological damages. Mature round shaped, red coloured apple fruits of length 57.81 mm and diameter 66.22 mm, angular shaped greenish yellow banana fruits were of diameter 36.67 mm and length 141.04 mm, oval shaped green orange fruits were of diameter 57.17 mm and length 64.81 mm, round shaped red tomato fruits were of length 42.46 mm and diameter 57.69 mm, round shaped creamish white cauliflower vegetable were of length 43.47 mm and diameter 113.35 mm and mature dark green spinach leaves, width 41.28 mm and length 138.23 mm were selected and their characteristics are presented in Table 3.1. Digital vernier calliper was used for measuring the diameter and length of fresh fruits and vegetables. Atleast 10-12 fruits/vegetables were chosen for taking average width and length of fresh samples.

Table 3.1: Selection of fruits and vegetables for minimal processing and active packaging

<table>
<thead>
<tr>
<th>Commodities</th>
<th>Visual Colour</th>
<th>Shape</th>
<th>Size (mm)</th>
<th>Maturity status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Length (mm)</td>
<td>Diameter (mm)</td>
</tr>
<tr>
<td>Apple</td>
<td>Red</td>
<td>Round</td>
<td>57.81</td>
<td>66.22</td>
</tr>
<tr>
<td>Banana</td>
<td>Greenish Yellow</td>
<td>Angular</td>
<td>141.04</td>
<td>36.67</td>
</tr>
<tr>
<td>Orange</td>
<td>Greenish Yellow</td>
<td>Oval</td>
<td>64.81</td>
<td>57.17</td>
</tr>
<tr>
<td>Tomato</td>
<td>Red</td>
<td>Round</td>
<td>42.46</td>
<td>57.69</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>Creamish White</td>
<td>Round</td>
<td>43.47</td>
<td>113.35</td>
</tr>
<tr>
<td>Spinach</td>
<td>Green</td>
<td>Leaf</td>
<td>138.23</td>
<td>41.28</td>
</tr>
</tbody>
</table>

3.3.2 Selection of disinfectants

Fruits and vegetables are covered with soil and dirt therefore they were carefully washed before processing. A second washing step was performed for reducing microbial
growth. Four different concentrations of NaOCl (100 ppm, 150 ppm, 200 ppm and 250 ppm) were used as disinfectant and the best concentration was selected by undertaking microbial analysis. No differences in microbial counts were observed between the 200 and 250 ppm concentration of NaOCl used so, that is why 200 ppm concentration was selected. Therefore, selected raw material was pre-washed in running water to remove impurities, again dipped in 200 ppm sodium hypochlorite solution (NaOCl) for 5 min., and rinsed with sterile water to remove the excess of chlorine of the solution.

3.3.3 Peeling, cutting, slicing or shredding

After washing and disinfecting the raw material (fruits and vegetables), a sterile as well as sharp knife was used for cutting the fruits and vegetables, discarding the waste i.e. seed core from apple, peel from banana and orange, all parts except the curd from cauliflower and undersized, insect infested, greenish yellow leaves along with the stalks were discarded from spinach. Forms and size specifications for minimal processing of fruits and vegetables were determined as per the results of hedonic scale rating test for sensory evaluation at 0 day and most preferred samples were determined for the study. With peel vertical slices of apple (thickness 18.12 mm and length 55.96 mm), without peel horizontal slices of banana (thickness 14.11 mm and length 34.36 mm), without peel orange fragments (thickness 13.54 mm and length 57.20 mm), horizontal slices of tomato (thickness 9.04 mm and length 45.81 mm), florets of cauliflower (thickness 39.28 and length 50.71 mm) and trimmed spinach (thickness 8.07 mm and length 39.34 mm) were selected for studying the effect of active packaging treatments on minimal processing of fruits and vegetables and the selected forms with their dimensions are given in Table 3.2. The cut fruits and vegetables especially apple, banana and cauliflower would turn brown rapidly after cutting, prior to active packaging treatments. To overcome this problem, approximately 150g of fruit/vegetable was used to cut at the same time.

Table 3.2: Selected forms of fruits and vegetables

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Forms of MP</th>
<th>Size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Length (mm)</td>
</tr>
<tr>
<td>Apple</td>
<td>Vertical slices (with Peel)</td>
<td>55.96±0.44,</td>
</tr>
<tr>
<td>Banana</td>
<td>Horizontal slices (without Peel)</td>
<td>34.36±2.23,</td>
</tr>
<tr>
<td>Orange</td>
<td>Fragments</td>
<td>57.20±0.04,</td>
</tr>
<tr>
<td>Tomato</td>
<td>Horizontal slices</td>
<td>45.81±0.78,</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>Florets</td>
<td>50.71±1.54,</td>
</tr>
<tr>
<td>Spinach</td>
<td>Trimmed</td>
<td>39.34±0.87,</td>
</tr>
</tbody>
</table>
Selection of Fruits and Vegetables
↓
Washing with Tap water
↓
Disinfecting with NaOCl (200 ppm)
↓
Rinsing with sterile water
↓
Keeping aside for surface drying
↓
Peeling, Cutting, Slicing or Shredding
↓
Packaging
↓
Storage

Fig. 3.1: Flow sheet for the preparation of Minimal Processing

3.4 ACTIVE PACKAGING TREATMENTS

Five treatments, oxygen scavenger, carbon dioxide scavenger, moisture scavenger, ethylene scavenger and chitosan based antimicrobial coating were selected under active packaging concept. Their formulations were standardized as per the results given in chapter 4, and best formulation was finalised for studying the effect of AP on MP and whole fruits and vegetables. These scavengers were prepared and filled into the sachet. The formulations/preparation of AP treatments as under:

3.4.1 Oxygen scavenger

100g Iron powder based oxygen scavenger was prepared by selecting the following chemical materials i.e. 40g Iron powder, 30g magnesium sulphate (MgSO₄), 20g sodium chloride (NaCl) and 10g silica gel and all the chemical ingredients were mixed well with spatula.

3.4.2 Carbon dioxide scavenger

100g activated charcoal based carbon dioxide scavenger was prepared by properly mixing of 80g activated charcoal and 20g silica gel with spatula by maintaining the ratio of 8:2.

3.4.3 Moisture scavenger/ absorber

For 100 g of moisture scavenger only silica gel was taken.
3.4.4 Ethylene scavenger

Potassium permanganate based ethylene scavenger was prepared by dipping 96g silica gel powder (AR grade) in 4 per cent KMnO₄ (100 ml) solution for the preparation of 100g ethylene scavenger. The homogenous mixture was kept at 28-30°C temperature for 48 hrs. in hot air oven. Dark purple coloured ethylene scavenger powder was prepared and was packed in LDPE pouches for further use.

(i) Sachet Preparation: Sachet was prepared by selecting high density woven fabric (100 gauge) which was permeable to gases but impermeable to active packaging ingredients, it was cut into 8 x 4 cm with scissors and 2 sides were sealed by using an electronic form, fill and seal machine. 4 x 4 cm size of each sachet was prepared with one side remained open for incorporating the prepared scavengers and packed in LDPE pouches for further use.

(ii) Filling of prepared scavengers inside sachet: 5g dried scavenger granules of O₂, CO₂, moisture and ethylene scavengers were weighed and filled in sachet (4 x 4 cm) prepared from gas permeable high density woven fabric but impermeable to sachet ingredients and heat-sealed by using an electronic form, fill and seal machine and packed in LDPE pouches for further use.

(iii) Characterization of O₂ and CO₂ scavengers: For the preparation of O₂ and CO₂ scavengers, four different types of formulations were tried as given in chapter 4 and the best one was selected on the basis of absorption of O₂ and CO₂ gases, respectively. O₂ and CO₂ concentrations were checked by placing 5g of sachet of these scavengers in two 100 ml air tight glass jars fitted with a rubber septum. O₂ and CO₂ concentrations were periodically analyzed by using headspace analyzer.

3.4.5 Chitosan based antimicrobial Film forming solution

Chitosan (Sigma Chemical Co.) coating solution was prepared by dissolving 2g chitosan powder (correspond to 2 per cent) and volume made to 100 ml by 1 per cent acetic
acid solution in which 0.4 ml glycerol was added as a plasticizer with continuous hand stirring from glass rod for approximately 30 minutes, until the whole chitosan powder was dissolved. The prepared solution was then de-gassed by using vacuum pump to avoid bubbling in the solution and before application the solution was kept undisturbed at 5±1°C for 24 hours.

![Flowchart of Film Forming Solution Preparation](image)

Fig. 3.3: Preparation of Film Forming Solution

3.4.5.1 Preparation and characterization of chitosan based film

(i) **Preparation:** For the preparation of film, 20 ml film forming solution was pipetted out, spread on petriplate and placed in refrigerated conditions at (6±1°C) for 48 hours. The dried thin film stick to the surface of petriplate was scrapped out and stored in LDPE bags.

(ii) **Film Thickness:** Film thickness was measured with the help of digital vernier calliper and ranged from 0.06-0.10 mm. The values are the mean of six measurements.

(iii) **Water Vapour Transmission Rate:** Water vapour transmission rate (WVTR) was determined by a static method. Calcium chloride was taken in dried beakers and the prepared films were tightly fixed over the brim of the beakers with the aid of an adhesive. The vials were accurately weighed and kept in a desiccator containing a saturated solution of potassium chloride at room temperature. The beakers were removed and weighed daily for 7 days. Water vapour diffusion rate or water vapour transmission rate (WVTR) for chitosan based film was calculated by

\[
WVTR = \frac{W}{LS}
\]

where \(W\) is the weight of water transmitted, \(L\) the thickness of the film and \(S\) the film surface area. The tests were performed in triplicate. WVTR was expressed as g.cm\(^2\)/d by observing the change in weight per unit time.
3.5 Packaging and Storage of Fruits and Vegetables Under AP Technology

(i) MP Fruits and Vegetables: After the fruits and vegetables were made into uniform size pieces with approximately similar thickness about 150±5 g MP products was weighed, packed in (8 x 6.5) inches LDPE packages (100 gauge) and sealing was done after inserting the prepared sachets (4 x 4 cm) of \(O_2\), \(CO_2\), moisture and ethylene scavengers separately in the main packages. For preparation of chitosan coated samples, small size brush was used for uniform coating to the samples and then fruit and vegetable pieces were inserted in the LDPE packages and then stored at (5±1°C) refrigeration temperature (RT).

(ii) Whole Fruits and Vegetables: In case of cauliflower small sized curds were selected while purchasing from the local market, after washing and disinfecting the whole fruits and vegetables, 150-250 g of samples were taken in LDPE bags. Further, sealing was done after incorporating the prepared sachets of particular scavengers. Chitosan coating solution was applied with brush and left for 5-10 min for surface drying and treated samples were stored at (5±1°C) refrigeration (RT) and (30±2°C) ambient temperature (AT).

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**Minimally Processed F &V**

- Selecting Fruits & Vegetables
- Washing with tap water
- Disinfecting with NaOCl (200 ppm)
- Keeping aside for Surface Drying (10-15 min)
- Peeling, Cutting, Slicing or Shredding
- Weighing 150±5 gm
- Applying Active Packing Treatments
- Inserting Scavenger (\(O_2/CO_2/Moisture/Ethylene\)) Sachet
- Applying Chitosan Coating with brush
- Refrigeration Storage (5±1°C)

**Whole F &V**

- Selecting Fruits & Vegetables
- Washing with tap water
- Disinfecting with NaOCl (200 ppm)
- Keeping aside for Surface Drying (10-15 min)
- Weighing 150±5 gm
- Applying Active Packing Treatments
- Inserting Scavenger (\(O_2/CO_2/Moisture/Ethylene\)) Sachet
- Applying Chitosan Coating with brush
- Refrigeration Temperature (5±1°C)
- Ambient Temperature (30±2°C)

**Fig. 3.4: Application of active packaging in MP and whole fruits and vegetables**
It is to be noted that no harmful chemical preservatives were added during minimal processing but the effect of AP treatments were judged on MP at RT, as well as on whole fruits and vegetables at RT and AT. Samples without treatment were considered as control and the values given in the tables are the mean of three replications.

For the study various treatments and combinations were selected:

1. MP fruits and vegetables treated with O$_2$ scavenger
2. MP fruits and vegetables treated with CO$_2$ scavenger
3. MP fruits and vegetables treated with moisture scavenger
4. MP fruits and vegetables treated with ethylene scavenger
5. MP fruits and vegetables treated with chitosan coating
6. Whole fruits and vegetables treated with O$_2$ scavenger at RT and AT
7. Whole fruits and vegetables treated with CO$_2$ scavenger at RT and AT
8. Whole fruits and vegetables treated with moisture scavenger at RT and AT
9. Whole fruits and vegetables treated with ethylene scavenger at RT and AT
10. Whole fruits and vegetables treated with chitosan coating at RT and AT

3.6 PHYSICAL AND PHYSIOLOGICAL PARAMETERS

3.6.1 Physiological Loss in weight (PLW)

Procedure: For estimation of PLW in all samples initial and final weight readings were taken along with polythene bags in case of MP products whereas, samples were withdrawn and weighed separately for AP treatments in case of whole fruits and vegetables. PLW was calculated by the following formula.

\[ PLW(\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \]

3.6.2 Texture and Firmness

Procedure: Shear force of samples prepared was determined by Texture analyzer. The texture analyzer was switched ‘ON’ and then settings were adjusted as per directions mentioned in the application guide of texture analyzer. The probes Krammer blade for texture, needle shaped for firmness analysis were selected and the instrument was calibrated. The observations were recorded by placing samples on the platform of the texture analyzer. The maximum amount of force needed to puncture the sample was recorded.
For assessment of texture and firmness, all samples were subjected to instrumental analysis by Texture analyzer (TA-XT2). Probes were selected by help of application guide of software. Amount of applied force was recorded automatically by load cell (5 kg) and inbuilt software. The force applied for measuring texture was measured in g.

3.6.3 Oxygen and Carbon dioxide concentration

*Procedure:* The head space gas concentration was observed using Gas analyzer fitted with chromosorb column and thermal conductivity detector (TCD). The sample packages were pierced by the piercing needle and subjected to the Gas analyzer and readings were noted. For all the samples headspace Gas analyzer was used in similar manner. The rate of carrier gas (nitrogen) was 18 ml/min, oven temperature 100°C and detector temperature 120°C was maintained for O₂ and CO₂ concentration. The rate was expressed as per cent (%).

3.6.4 Ethylene

Ethylene evolution was determined by the procedure adopted by Banks (1984).

*Procedure:* Weighed fruits (150±5g) from each replicate (three replicates per treatment) were sealed in 500 ml glass jars for 2 hrs. at 25°C, then 0.5 ml of the head space gas was
withdrawn from the headspace with the help of 1ml syringe and fed into the gas chromatograph (Netel) fitted with chromosorb101 column and FID. The flow rate of carrier gas (nitrogen) was 29 ml/min, oven temperature 180°C, injector and detector temperature 200°C. Ethylene was identified by comparison of the retention times with standards. The peak height were recorded and compared with that of given concentration of ethylene standard. The standard ethylene 110 ppm was used for calibration. Results were expressed as µl C₂H₄/Kg/hr fruit.

Fig. 3.7 Gas chromatograph

3.7 BIO-CHEMICAL PARAMETERS

3.7.1 Moisture content

Moisture content was determined by following the standard method of AOAC, 2005.

*Procedure:* 20g of fruit/vegetable sample was weighed in a petridish, dried in an oven at 60±2°C for 7-8 hrs. or till a contant weight was obtained. The weight of the sample was taken after cooling it in a dessicator.

*Calculation:*

\[
\text{Total solids (％)} = \frac{(\text{Weight of dried sample})}{(\text{Weight of petridish})} \times 100 - \frac{(\text{Weight of petridish})}{(\text{Weight of fresh sample})}
\]

\[
\text{Moisture (％)} = 100 - \text{Total solids (％)}
\]

3.7.2 Total soluble solids (TSS)

*Procedure:* Total soluble solids (TSS) of fruits and vegetables were determined at ambient temperature by using hand and Abbe's refractometer (0-32°Brix).

3.7.3 Titratable acidity

Total acids were estimated by the method of Ranganna (1986).
**Preparation of sample:** Sample was pulped in a blender or in mortar and mixed thoroughly. To the weighed pulped material, water was added and boiled for 1 hr. replacing the water lost by evaporation. It was cooled, filtered and transferred to a 250 ml volumetric flask and made volume.

**Procedure:** An aliquot of sample prepared as above was diluted with recently boiled distilled water. It was then titrated with 0.1 N NaOH using a few drops of 1 per cent phenolphthalein solution as indicator. Titre value was noted and results were calculated as per predominant acid present in the samples.

**Calculation:**

\[
\% \text{ Titratable acidity} = \frac{\text{Titre} \times \text{Normality of alkali} \times \text{Vol. made up} \times \text{Eq. Wt. of acid} \times 100}{\text{Vol. of sample taken} \times \text{Wt. or vol. of sample taken} \times 1000}
\]

3.7.4 Ascorbic acid (vitamin C)

Ascorbic acid was determined by procedure detailed by AOAC, 1990.

**Reagents**

1) Meta phosphoric acid-acetic acid (HPO₃) solution: It was prepared by dissolving 15g HPO₃ pellet in 40 ml acetic acid and 200 ml distilled water and diluted to 500 ml by distilled water in 500 ml capacity volumetric flask. It was filtered rapidly into a coloured bottle.

2) Ascorbic acid standard solution: It was prepared by dissolving 50 ml ascorbic acid standard in 50 ml of meta-phosphoric acid in 50 ml capacity volumetric flask.

3) Indophenol standard solution: It was prepared by dissolving 50 mg sodium salt of 2, 6-dichlorophenol indophenol that had been stored in a dark coloured reagent bottle away from sunlight. The solution was then added 50 ml water containing 42 mg of sodium bicarbonate. It was shaken vigorously and diluted to 200 ml with water. Filtered and stored in an amber glass bottle.

**Preparation of sample:** 10g of sample was taken and blended with 100 ml of meta-phosphoric acid to make up the volume to 100 ml and it was designated as V.

**Procedure:** Three 2.0 ml aliquots of ascorbic acid standard were transferred to each of 50 ml conical flask containing 5 ml meta-phosphoric acid solution. It was titrated rapidly with indophenol solution until light but distinct rose pink colour persisted for more than 5 seconds. Similarly 7 ml of meta-phosphoric acid solution were taken in a conical flask. Equal volume of water was added equal to volume of indophenol used against standard and titrated for blank. Three readings were taken. Then 2 ml of sample aliquots were taken in a conical flask containing 5 ml metaphosphoric acid solution. It was then titrated against the dye solution and three readings were taken.
Calculation:

Ascorbic acid (mg/100g) = \[ \frac{Y - B}{X - B} \times \frac{V}{W} \]

Where,  
W= Weight of sample  
V= Volume of aliquot made  
X= Volume of dye solution used against standard  
Y= Volume of dye solution used against sample aliquot  
B= Volume of dye solution used against blank

3.7.5 Pectin

Pectin content was determined according to the method of Ranganna (1986).

Reagents

1) 1 N Acetic acid: It was prepared by adding 30 ml of glacial acetic acid to 500 ml of water.
2) 1 N CaCl\(_2\): It was prepared by dissolving 27.5 g of anhydrous CaCl\(_2\) in water and diluting to 500 ml.
3) 1% Silver nitrate: It was prepared by dissolving 1 g of AgNO\(_3\) in 100 ml of water.

Procedure:

(a) Preparation of sample: About 50 g of blended sample was taken into a 1000 ml beaker, with 400 ml of 0.05 N HCl. It was then placed in water bath at 80-90°C for 2 hrs. replacing the water lost by evaporation. It was then cooled, transferred to a 500 ml volumetric flask and the volume made up with water and filtered.

(b) Estimation: To 50 ml of the filtrate, 50 ml of distilled water and 5 ml of 1N NaOH was added and kept overnight. Next day, 25 ml of acetic acid and 12.5 ml 1N calcium chloride solution were added with stirring. After an hour it was boiled for a minute and filtered through oven dried, previously weighed whatman filter paper no. 41. The precipitates were washed with distilled water until they were free from chloride, (tested with 1 per cent silver nitrate solution). The precipitates were then dried at 100°C overnight, cooled in a desiccator, weighed and per cent calcium pectate was calculated.

Calculation:

% calcium pectate = \[ \frac{\text{Wt. of calcium pectate} \times \text{Vol. made up} \times 100}{\text{Vol. of filtrate taken} \times \text{Wt. of sample taken}} \]

3.7.6 Total sugars

Total sugars were estimated in the samples as explained by Hulme and Narain (1931).
Reagents

1) Potassium ferricyanide solution: 8.25g potassium ferricyanide and 10.6g anhydrous sodium carbonate were dissolved in water and volume was made to one litre with distilled water.

2) Potassium iodide solution: 12.5g potassium iodide, 25g zinc sulphate and 125g sodium chloride were dissolved in water and volume was made to 500 ml.

3) Sodium thiosulphate solution: 2.482g sodium thiosulphate was dissolved and volume was made 1 litre with distilled water.

4) 5 per cent Acetic acid solution (V/V): It was prepared by taking 50 ml glacial acetic acid and finally making 1 litre volume with distilled water.

5) Starch solution (indicator): It was prepared by dissolving 1g soluble starch and 20g sodium chloride and finally making the volume to 100 ml with distilled water.

Preparation of sample: 10g of the sample was weighed and transferred to a 250 ml beaker. 50 ml water was added, heated to boil, cooled and then diluted to appropriate concentration for estimation.

Procedure:

(a) Reducing sugars: 5 ml of solution was taken in a test tube and 5 ml of potassium ferricyanide solution was added. The test tube was cooled under running tap water and 5 ml of iodide-zinc solution was added followed by 3 ml of acetic acid solution (5% V/V). It was then titrated with sodium thiosulphate (0.01N) using starch as an indicator. The end-point was the disappearance of blue colour and appearance of milky white colour. A blank with 5 ml of distilled water was also run simultaneously.

(b) Total sugars: To 25 ml of filtrate in a 50 ml volumetric flask 4 ml of concentrated HCl was added and kept it on water bath for 15 min at 68°C. It was cooled and neutralized by using anhydrous sodium carbonate till no effervescence was seen and made up to the volume with water. An aliquot (5 ml) was taken and total sugars were determined as in reducing sugars.

3.7.7 Total chlorophyll

Total chlorophyll content was estimated by using the method of Amar Singh (1977).

Procedure: A small amount of CaCO₃ was added to 1g of sample and blended with 80 per cent acetone (pre-chilled at 4°C) for 3 minutes. The mixture was filtered and washed with 80 per cent acetone until colourless. The extract was transferred to a 100 ml volumetric flask and made up to volume with 80 per cent acetone. Absorbance reading was recorded at 645 nm and 663 nm using spectrophotometer. 80 per cent acetone was used as blank before absorbance reading of sample taken.
Calculation:

\[ C_t = \frac{(20.2 \times \text{OD at 645 nm} + 8.02 \times \text{OD at 663 nm}) \times \text{Vol. made up} \times \text{weight of sample}}{100} \]

\( C_t = \text{Total chlorophyll} \)

3.7.8 Tannins

Method of AOAC (2005) was followed for determining the Tannin content of sample.

**Reagents**

1) Folin Denis Reagent 200 ml (1:1): 100 ml
2) Sodium carbonate: 35g in 100 ml
3) Tannic acid: 100 mg in 1 lt.

**Preparation of sample:** 10 g of sample was taken and 40 ml of distilled water was added. It was then heated in water bath for about 90 min. Sample was cooled and volume was made to 100 ml with water.

**Preparation of standard curve:** For the preparation of Standard curve 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of aliquots of standard tannic acid solution were pipetted in test tubes containing 7.5 ml of water. 0.5 ml of Folin Denis Reagent and 1 ml of sodium carbonate was added and volume made to 10 ml with distilled water. The test tubes were allowed to stand for 30 min and the colour absorbance was measured at 760 nm using UV-Vis spectrophotometer against blank. Tannic acid was used as blank. Tannin values were expressed in terms of the standard reference compound as tannic acid equivalent (mg/100g, fresh weight) of the sample.

Tannins as (%) tannic acid = \[ \frac{\text{mg of tannic acid} \times \text{dilution} \times 100}{\text{ml of sample} \times \text{wt of sample taken} \times 1000} \]

Fig. 3.4: UV-Vis spectrophotometer

3.7.9 Total phenols

The concentration of total phenols was determined by the method described by McDonald et al. (2001).

**Procedure:** 1g of fresh sample was extracted with 10 ml of methanol: water (50:50, v/v). Also, 0.5 ml of the diluted (1:10) extract or the standard phenolic compound (gallic acid) was
mixed with 5 ml of Folin-Denis reagent (1:10 diluted with distilled water) and 4 ml of aqueous Na₂CO₃ (1M). The mixtures were allowed to stand for 15 min and the optical density of the mixtures was measured against the blank at 765 nm using a UV-Vis spectrophotometer. Standard curve was prepared using 0, 50, 100, 150, 200, and 250 µg gallic acid/ml in methanol: water (50:50, v/v). Total phenol values were expressed in terms of the standard reference compound as gallic acid equivalent (mg/100g, fresh weight) of the sample.

3.7.10 Poly phenol oxidase (PPO) activity

Poly phenol oxidase was assayed by the method by Kaul and Farooq (1994).

Reagents
1) Substrate: 0.05 M catechol in 0.03M phosphate buffer (pH= 6.0)
2) 10 per cent tri chloro acetic acid (W/V): It was prepared by dissolving 10g TCA in 100 ml water.

Preparation of sample: 4g of fruit pulp was homogenized in 10 ml of cold 0.2 M tris-HCl buffer (pH=7.5) containing 0.1M each of cystein and EDTA. The homogenate was centrifuged at 15000 rpm for 20 min at 4°C in a refrigerated centrifuge. The supernatant was used for enzyme essay.

Enzyme Assay

0.5 ml of enzyme extract was incubated with 4 ml of 0.05M catechol in 0.03 M phosphate buffer (pH 6) for 20 min at 30°C. The reaction was terminated by adding 1 ml of chilled 10 per cent tri chloro acetic acid and the optical density was read at 430 nm against the reagent blank. The enzyme total activity was measured in units/g fresh wt. /h. 1 unit represents increase in O.D by 1.0 under the standard condition.

3.8 MICROBIOLOGICAL EVALUATION

Microbiological condition of the minimally processed product, stored at 5±1° C and whole at room as well as refrigeration temperature. Total bacterial and Yeast and Mould counts were done according to the method prescribed by Sharf (1966).

The total counts in terms of yeasts and moulds and bacteria were recorded separately based on colony characteristics. The sterilization of media was done at 15 lbs pressure for 20 min. 1g sample was taken and put in test tube containing 9 ml of sterilized distilled water. It was macerated and shaken for half an hour from this, 1 ml of homogenous suspension from each dilution was transferred to different sterile petridishes with the help of sterile pipettes. About 20 ml molten and cooled respective media were poured on each labeled petridish. They were rotated in a manner to facilitate uniform distribution of material. Medium was allowed to solidify. Plates containing 30-300 colonies were selected for counting. Microbes
per gram of samples were calculated by multiplying the number of colonies with respective dilution factor. Microbiological data are the average of two replicates.

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<thead>
<tr>
<th>Organism</th>
<th>Media</th>
<th>Incubation</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bacterial count</td>
<td>NA</td>
<td></td>
<td>37±1°C</td>
<td>24-48 hrs.</td>
</tr>
<tr>
<td>Yeast and Mold count</td>
<td>PDA</td>
<td></td>
<td>24±1°C</td>
<td>24-72 hrs.</td>
</tr>
</tbody>
</table>

### Table 3.3 Types of media, incubation temperature and time

### 3.9 ORGANOLEPTIC EVALUATION

All samples of whole and minimally processed products of fruits and vegetables were subjected to sensory evaluation. The organoleptic evaluation was conducted by a semi-trained panel for colour, flavour, texture, taste, appearance and overall acceptability. The judges scored quality characteristics of each sample on 9 point hedonic rating scale (Annexure-3). Score of 9 indicated “very desirable” trait, which gradually decreases with reduction in acceptability. The score of five shows “neither desirable nor undesirable trait. The characteristics with mean score of 6 and above were considerable acceptable. The mean scores obtained after evaluation of different samples for each trait were calculated (Ranganna, 1986).

#### 3.9.1 Shelf-life

The shelf-life of fruits and vegetables was decided on the basis of organoleptic evaluation scores. When fruits and vegetable showed scores less than 5 then it was considered to have reached end of shelf-life.

### 3.10 STATISTICAL ANALYSIS

**ANOVA:** The data was subjected to statistical analysis of variance using SAS (version 9.1). A significance level of 0.5 was chosen. Factors namely ethylene scavenger, O₂ scavenger, CO₂ scavenger, moisture scavenger, antimicrobial film and storage duration were selected or the study as factorial arrangements in Completely Randomized Design (CRD). Each mean value presented in the tables is the average of three replications.

**Principal Component Analysis (PCA):** The data was subjected to principal component analysis using SPSS software version 16.0. Principal component analysis is normally conducted in a sequence of steps, with somewhat subjective decisions being made at many of these steps.

1. **Initial extraction of the components**- In principal component analysis, the number of components extracted is equal to the number of variables being analyzed. The first component can be expected to account for a fairly large amount of the total variance.
Each succeeding component will account for progressively smaller amounts of variance. Although a large number of components may be extracted in this way, only the first few components will be important enough to be retained for interpretation.

2. **Determining the number of “meaningful” components to retain** - The next step of the analysis, is to determine how many meaningful components should be retained for interpretation, one of the most commonly used criteria for solving the number-of-components problem is the eigenvalue-one criterion, also known as the Kaiser criterion (Kaiser, 1960). With this approach, any component with an eigenvalue greater than 1.00 retained and thereby interpreted.

3. **Rotation to a final solution** - After extracting the initial components, an unrotated factor pattern matrix will be created. The rows of this matrix represent the variables being analyzed, and the columns represent the retained components (these components are referred to as FACTOR1, FACTOR2 and so forth in the output). Unfortunately, when more than one component has been retained in an analysis, the interpretation of an unrotated factor pattern is usually quite difficult. To make interpretation easier, you will normally perform an operation called a rotation. A rotation is a linear transformation that is performed on the factor solution for the purpose of making the solution easier to interpret.

   \[
   \text{ROTATE=VARIMAX}
   \]

   A varimax rotation is an orthogonal rotation, meaning that it results in uncorrelated components. Compared to some other types of rotations, a varimax rotation tends to maximize the variance of a column of the factor pattern matrix (as opposed to a row of the matrix).

4. **Interpreting the rotated factors** - Interpreting a rotated solution means determining just what is measured by each of the retained components. Briefly, this involves identifying the variables that demonstrate high loadings for a given component, and determining what these variables have in common. Usually, a brief name is assigned to each retained component that describes its content.

5. **Interpreting the component plot of factors** - When appropriate two components have been retained in an analysis, the graph will be plotted in 2D plane and for three components the 3D graph will be plotted.