# Chapter III

## EXPERIMENTAL METHODS AND MATERIALS

### 3.1 Preservation of Fresh Lime Fruit by Controlled-Atmosphere Storage

Preservation of fresh lime fruits with various concentrations of Benlate and at different percentages of carbondioxide ($CO_2$) and oxygen ($O_2$) were investigated. Firstly, the rate of respiration of fresh lime fruits in an enclosed system was studied, followed by the studies on quality changes during storage of limes fruits kept at different levels of $O_2$ and $CO_2$. Periodically, observation and analysis were conducted on weight of fruit, quantity of juice, density, pH, total soluble solid ($^{o}$Brix), percentage of acidity, ascorbic acid content, color, percentage of spoilage of lime and also acceptability test.

#### 3.1.1 Materials

3.1.1.1 Fresh green lime fruits with more or less same maturity were obtained from the same orchard whenever available. During off season, green lime fruits of good quality and same maturity were obtained from the market.

3.1.1.2 All chemicals used were laboratory grade.

3.1.1.3 $CO_2$, $O_2$ and $N_2$ gases were supplied in tubes from Department of Military Energy.

3.1.2 Instruments

3.1.2.1 Abbe Refractometer

3.1.2.2 pH Meter. Prolabo Paris

3.1.2.3 Orsat Measurement Apparatus

3.1.3 Methods

3.1.3.1 Preparation of fresh lime fruit sample

Fresh lime fruits were immersed under tap water for 10 minutes then washed and rinsed thoroughly to remove all dirt and contamination. The defect lime fruits were picked out and then the rest were left to dry in air. The lime fruits were treated with various concentration of Benlate for 10 minutes, left dried in air and placed these fruits in the apparatus which was shown in Figure 2.

3.1.3.2 Preparation atmosphere storage condition

The apparatus was consisted of a cylindrical glass jar 10" X 15" (d X h), with stainless steel shelves for placing the fruits. The glass jar can be covered hematically by an iron cover with screw knots. (Fig.2) There were three rotameters for measuring the rate of inlet gas. The composition of $O_2$, $CO_2$ and $N_2$ in the jar was measured by using Orsat measurement (Fig.3) and adjusted the flow rate of individual gas to obtain the desired gas composition. All the gases were passed through a bacteria filter before entering in the experimental jar. Saturated

Figure 2 Experimental Glass Jar with Stainless Steel Shelves and Iron Cover.

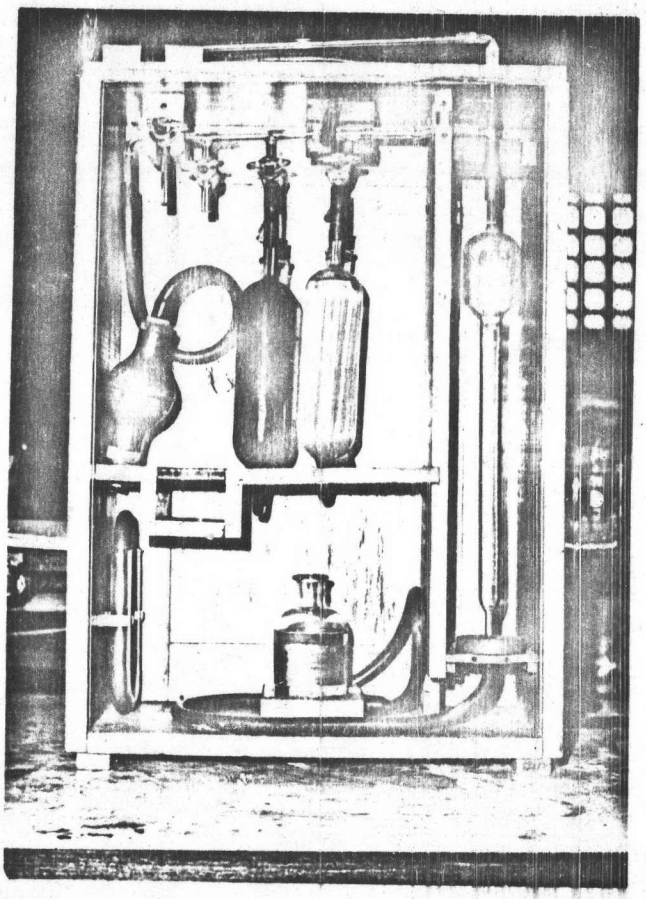


Figure 3 Orsat Measurement Apparatus

potassium chromate solution was contained in the bottom of glass jar to adjust the relative humidity in the jar to about 89% (30). The glass jar was kept in the refrigerator at temperature of 10°C.

3.1.3.3 Analytical method for studying the changes the stored lime fruits.

3.1.3.3.1 Determination of weight of fruit. A set number of fruits were marked with number before experiment. These fruits were weighed each month to calculate the weight loss of the fruits due to evaporation.

3.1.3.3.2 Determination of quantity of juice. The average weight and volume of juice of three fruits were measured at each month and calculated as percentage of the initial fruit weight.

3.1.3.3.3 Density measurement. The density of lime juice was calculated by dividing the average weight of juice by volume of juice.

3.1.3.3.4 pH measurement. The pH of the lime juice was measured by using pH meter.

3.1.3.3.5 Total soluble solid. (°Brix) The total soluble solid was measured by using Abbe Refractometer.

3.1.3.3.6 Color change. The skin color of lime fruits was observed by visual inspection. Each fruit sample was classified according to the following colors:-

G - Green

YG - Yellowish - green

GY - Greenish - yellow

Y - Yellow

B - Brown

The number of lime fruits with different colors were counted and the percentage of each color is calculated on the basis of the total number of fruits.

3.1.3.3.7 Titratable acidity. Two ml. of lime juice was titrated against 0.1N NaOH using phenolphthalein as an indicator and calculated as percentage of anhydrous citric acid, the predominant acid in the lime juice.

$$\text{citric acid mg/100 ml} = \frac{64}{1000} \times \frac{\text{ml NaOH}}{\text{ml sample}} \times 0.1\text{N NaOH} \times 100$$

3.1.3.3.8 Determination of ascorbic acid content. The volumetric determination using 2,6-dichlorophenol indophenal, as described by Cox and Pearson (31) was used and the ascorbic and content in the sample was calculated as mg ascorbic acid per 100 ml of lime juice.

3.1.3.3.9 Sensory evaluation test. To evaluate the acceptability of the treated fresh lime juice; scores were allotted to different liking on the Hedonic scale from 1 at 'dislike extremely' to 9 at 'like extremely'. The scores of each characteristic were analysed. The teste panel form used is shown in Appendix I.

3.1.3.4 Rate of $O_2$ up-take and $CO_2$ released

To study the rate of respiration as measured by rate of $O_2$ uptake and $CO_2$ released of lime fruits kept at 10°C and 89% R.H., 47 fresh green lime fruits were weighed and dipped in water for 10 minutes and then left dried in air. Then all the lime fruit samples were placed in the experimental glass jar under ordinary atmospheric contition. Analysis of % $O_2$ and % $CO_2$ was made for 15 consecutive days.

3.1.3.5 Effect of 10% $O_2$, 5% $CO_2$ at 10°C and 89% R.H. on the fresh lime fruits treated with 1000ppm Benlate solution

To study the effect of 10% $O_2$, 5% $CO_2$ at 10°C and 89% R.H. with 1000 ppm Benlate on the fresh lime fruits, the same procedure as in 3.1.3.2 was followed using 72 lime fruit samples. Adjustment of the gas mixture in the experimental jar was made almost everyday to control the exact composition.

3.1.3.6 Effect of 10% $O_2$, 5% $CO_2$ at 10°C and 89% R.H. on the fresh lime fruits treated with 2000ppm Benlate solution

To study the effect of 10% $O_2$, 5% $CO_2$ at 10°C and 89% R.H. on the fresh lime fruits treated with 2000 ppm Benlate, the procedure as in 3.1.3.5 was followed using 72 lime fruit samples.

3.1.3.7 Effect of 10% $O_2$, 30% $CO_2$ at 10°C and 89% R.H. on the fresh lime fruits treated with 1000 ppm Benlate solution.

80 fresh lime fruits treated with 1000 ppm Benlate were used to study the effect of 10% $O_2$, 30% $CO_2$ at 10°C and 89% R.H. The procedure in 3.1.3.5 was followed.

3.1.3.8 Effect of 10% $O_2$, 15% $CO_2$ at 10°C and 89% R.H. on the fresh lime fruits treated with 1000 ppm Benlate solution

The procedure in 3.1.3.5 was followed using 70 fresh lime fruits.

3.1.3.9 Effect of 5% $O_2$, 5% $CO_2$ at 10°C and 89%R.H. on the fresh lime fruits treated with 1000ppm Benlate solution

The procedure as in 3.1.3.5 was followed using 72 fresh lime fruit samples.

## 3.2 Lime Juice Concentrate Processing

Firstly, the effect of vacuum concentration on physical and chemical properties of lime juice without additive was studied. The next part of research experiment involved the study of the effect of storage time and storage temperature on concentrated lime juice with addition of 0 and 300 ppm of potassium metabisulfite as preservative. Quality changes of concentrated

lime juice was followed periodically in term of ascorbic acid content, pH, °Brix, acidity, optical density, flavor and color.

### 3.2.1 Instruments

Abbe Refractometer

pH Meter Prolabo Paris

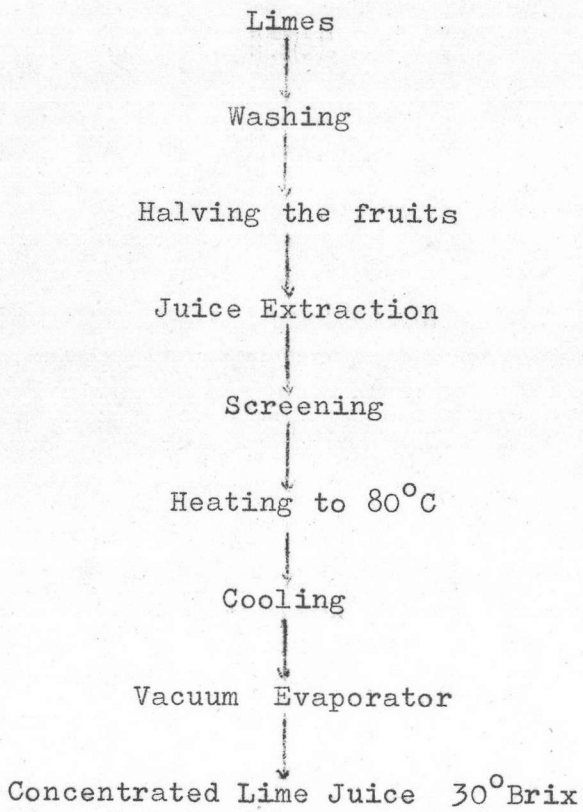
Prolabo Spectrophotometer

Vacuum Evaporator (Centri-Therm CT-1B of Alfa-Laval)

### 3.2.2 Preparation of Concentrated Lime Juice

The lime juice used in this study was prepared from lime fruits Citrus aurantifolia as follows:

The lime fruits were washed thoroughly under tap water to remove all dirt and contaminants. The defected limes were picked out and rejected. The limes were halved by a sharp stainless steel knife and the juice was extracted by hand-pressed extractor. The juice was passed through two-fold muslin cloth to remove rag and seeds; heated up to 80°C for pasteurization and inactivation of enzymes and cooled immediately. The lime juice at 6 °Brix was concentrated in a vacuum evaporator (Centri-Therm CT-1B of Alfa-Laval) at 0.9 kg/sq.cm steam pressure and at the temperature of 60-65°C. The evaporation temperature used was 45°C. The concentrated lime juice after evaporation was 30 Brix. The procedure described above is presented in the following scheme.




3.2.3 Storage Study

The concentrated lime juice (30°Brix) was cut back to 24°Brix by addition of 7°Brix fresh lime juice in order to improve the aroma of lime juice. The concentrate (24°Brix) was separated into 2 lots as follows:

1. Heated to 80-85°C for pasteurization, filled hot into 60 ml. sterilized brown bottles. The bottles were stoppered tightly, leaving 1 cm. head space and cooled. One batch of bottles was stored at room temperature, while the other was stored at refrigerator temperature of 10°C.

2. To the concentrated juice, 300 ppm of potassium metabisulfite was added and proceeded as 1.

3.2.4 Analytical Method

3.2.4.1 The quality of the juice was checked periodically in terms of ascorbic acid content, pH, °Brix, and total acidity by following the procedure in 3.1.3.3.

3.2.4.2 Determination of the optical density. The optical density of the juice was determined by using Prolabo Spectrophotometer. The lime juice sample was prepared by diluting with ethyl alcohol in ratio 1:2, filtered, and the optical density of the supernatant was measured against water at 410 mμ. The increase in the optical density indicates the increase in degree of browning of the juice.

3.2.4.3 Flavor evaluation. The flavor was evaluated by a panel of judges using Hedonic scaling. The taste panel form used is shown in Appendix 2. The lime juice samples were prepared by diluting with drinking water in the ratio of 1 : 2.

3.2.4.4 Color measurement. The color of the lime juice was observed by visual inspection. The increase in the number of plus signs indicates the increase in degree of browning of the juice.