CHAPTER - III
ANALYTICAL STUDIES OF RAW MATERIALS
3. ANALYTICAL STUDIES OF RAW MATERIALS

3. INTRODUCTION:
The selection of raw material is very important in any microbial fermentation and involves carbon sources that produce carbon skeleton for the amino acids and energy source for fermentable microorganisms. The objective of this research is to identify novel cheap ‘C’ sources for the production of value added products like glutamic acid. The raw materials used were classified into two types:

Types of raw materials:

1. Fruits
   - Mimusops elengi
   - Muntingia calabura
   - Cashew apple pulp
   - Pine apple peel

2. Tubers
   - Mirabilis jalapa root tuber
   - Kedrostis foetidissima root tuber
   - Cissus trifoliata root tuber
   - Alocasia macrorrhiza stem tuber
   - Dioscorea bulbifera aerial tuber

Collection of Raw materials:
The raw materials used in the present study were collected in and around Visakhapatnam, India.

- Ripened fruits of Muntingia calaburaL. and Mimusops elengiL. were collected from the plants of parking areas.
- Cashew apples were collected from the Simhachalam area.
- Pine apple waste was collected from the fruit market.
- The tubers of Mirabilis jalapa were obtained from Visalakshi nagar.
- The tubers of Kedrostis foetidissima were collected from the Aruku hill areas.
• In very large numbers, huge tubers of *Cissus trifoliata* were found and collected from the sea shore areas of Rushikonda.
• Large tubers of *Alocasia macrorrhiza* were collected from the road-side plants.
• Yams or air potato tubers were collected from the plants grown in Punyagiri hill areas.

3.1 ANALYTICAL STUDIES OF FRUITS:

3.1.1 Qualitative analysis of fruits:

3.1.1a Test for carbohydrates:

**Materials and methods:**

**Chemicals:** All the chemicals were procured from Qualigens Fine chemicals Pvt.Ltd, Mumbai.

**Procedure:**

The fruits were thoroughly washed with tap water to remove dust. 10g of four fruit samples *M.elengi, M.calabura, cashew apple* pulp and *pine apple* peel were weighed, crushed using fruit juice processor with ratio of samples to purified water 1:1. The extracts were filtered through a muslin cloth. Then, the samples were centrifuged at 360xg for 10min. The clear supernatants were collected and subjected to qualitative analysis for the identification of various sugars present in the samples. The protocol was followed according to Sadasivam and Manickam, (1996).

- Molisch test
- Benedict’s test
- Fehling’s test
- Barfoed’s test
- Seliwanoff’s test
- Osazone test
Results: The samples showed positive to the above coloured reactions of carbohydrates. Since the samples contain multiple sugars these tests were not effective for their identification hence a confirmatory test was needed for their identification.

3.1.1b Confirmatory test for the sugars using paper chromatography: (Sadasivam and Manickam, 1996). The test is used to separate the mixture of sugars present in the samples and to identify the unknown compounds by comparing the Rf values of known compounds. The principle lies on the partition coefficient or distribution coefficient of molecules between two immiscible phases.

Materials and methods:

Chemicals and analytical instruments:
- Solvent system: Butan-1-ol/acetone/phosphate buffer pH 5 (40/50/10)
- 0.5% solution of 3,5 Dinitrosalicylic acid in 4% NaOH.
- Standard sugar solutions (1mg/ml)
- Chromatographic Chamber, Hot-air Oven, Sprayer etc.

Procedure:
- Whatman No.1 filter paper was cut into 20x20cm dimension with uniform edges.
- Along one edge of a paper a baseline was drawn about 2cm from the bottom.
- 2.5ml of standard sugar samples and test samples were spotted on the baseline.
- The spots were allowed to dry before keeping in the developing chamber.
- Chromatography paper was developed by keeping in free standard chamber for 4-5h.
- Saturation was done by solvent system.
- The paper was removed from the chamber after 5h.
• Solvent front was marked on the paper and the paper was allowed to dry to identify the sugars.
• On the chromatogram 0.5% solution of 3,5 Dinitrosalicylic acid in 4% NaOH was sprayed and kept in the hot-air oven which was adjusted to 90°C.
• The spots were circled and $R_f$ values were calculated for each individual sugars.
• The distance travelled by the solvent front was also measured.
• $R_f$ is the distance travelled by the solute divided by the distance travelled by the solvent front.
• By comparing the $R_f$ values of the standard samples with the unknown samples, the unknown sugars present in the fruit samples were identified.

Results: The qualitative analysis on four fruit substrates of *Muntingia calabura*, *Mimusops elengi*, *cashew apple* pulp and *pine apple* peel reveals that, the fruit samples contain various types of sugars (Table 3.1). These sugars are identified based on their $R_f$ values (Table 3.2) similar to that of authentic standard sugar samples.

<table>
<thead>
<tr>
<th>Fruit substrates</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Muntingia calabura</em></td>
<td>Glucose, fructose, sucrose</td>
</tr>
<tr>
<td><em>Mimusops elengi</em></td>
<td>Glucose, fructose, sucrose, lactose</td>
</tr>
<tr>
<td><em>Cashew apple</em></td>
<td>Glucose, fructose, maltose, sucrose</td>
</tr>
<tr>
<td><em>Pine apple</em></td>
<td>Glucose, fructose, sucrose</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sugars</th>
<th>$R_f$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.18</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.22</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.15</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.11</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.23</td>
</tr>
</tbody>
</table>
3.1.2 Quantitative analysis of fruits:
The fruits were thoroughly washed with tap water to remove dust. 100g of four fruit samples *M.elengi*, *M.calabura*, *cashew apple* pulp and *pine apple* peel were weighed, mashed and subjected to acid hydrolysis with 500ml of 2.5N HCl for 3h. Later, the cooled hydrolysates were neutralized with Na₂CO₃ until the effervescence completely ceases and were subjected to centrifugation by adjusting their volume to 1L. Subsequently the resultant supernatants were separated and subjected to quantitative test for the determination of amount of sugars. Phenol sulphuric acid method Dubois et al. (1956) was used for the quantitative determination of sugars in the fruit samples.

**Phenol-sulphuric acid method:** The method is used to estimate the total carbohydrates present in the samples.

**Materials and methods:**

**Chemicals:** All the chemicals were procured from Qualigens Fine chemicals Pvt.Ltd. Mumbai.

- 5% Phenol: 50g of phenol was dissolved in water and diluted to 1L.
- 96% Sulphuric acid: 96ml of sulphuric acid was dissolved in 4ml of water.
- Glucose standard: 1mg/1ml of glucose solution standard was prepared with water.

**Procedure:**

- For standard graph initially, 0.2, 0.4, 0.6, 0.8 and 1ml of standard glucose solution was pipette out into 5 test tubes and then water was added to make the volume to 1ml in each test tube.
- A blank was set up with 1ml of water and the 4 test samples were prepared by pipette out 0.1ml of supernatants in 4 different test tubes.
- 1ml of phenol solution was added to each test tube followed by the addition 5ml of 96% sulphuric acid to each test tube along with the blank and tests and mixed well.
- After 10 min the tubes were kept in boiling water bath for 20 min at 25-30°C.
After boiling the tubes were cooled to room temperature and optical density was measured at 490nm in a U.V-Visible spectrophotometer (Systronics instruments, Ltd).

**Results:**
The total amount of carbohydrates present in the raw materials was calculated using a standard graph (Figure 3.1) produced by Phenol-sulphuric acid method. The results were expressed as g of sugars present per 100g of substrate (Table 3.3). The results were also confirmed by Vimta labs, Hyderabad.

**Figure 3.1: Standard graph of Phenol-sulphuric acid method**

![Graph showing concentration of standard glucose vs O.D at 490nm](image)

**Table 3.3: Estimation of sugars present in fruit substrates**

<table>
<thead>
<tr>
<th>Test samples</th>
<th>Conc. of sugars (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. calabura</em> fruits</td>
<td>1.70</td>
</tr>
<tr>
<td><em>M.elengi</em> fruits</td>
<td>1.69</td>
</tr>
<tr>
<td>Cashew apple pulp</td>
<td>0.45</td>
</tr>
<tr>
<td>Pine apple peel</td>
<td>0.59</td>
</tr>
</tbody>
</table>
Calculations:

100g of *M. calabura* fruit substrate contains 17g of sugars
100g of *M. elengi* fruit substrate contains 16.9g of sugars
100g of *cashewapple* fruit pulp contains 4.5g of sugars
100g of *pine apple* peel contains 5.9g of sugars

3.2 ANALYTICAL STUDIES OF TUBERS:

3.2.1 Extraction and Determination of starch content: (Lodha and Nemade, 2012)

**Materials and methods:**
The collected *Mirabilis jalapa* tubers, *Kedrostis foetidissima* tubers, *Cissus trifoliata* tubers, *Alocasia macrorrhiza* stem tubers and *Dioscorea bulbifera* bulbs or aerial tubers were washed thoroughly with water to remove soil particles and then peeled, the peeled tubers were cut and grated. However for *Alocasia macrorrhiza* young tubers were collected as they contain low amount of oxalate crystals cause only mild irritation. The grated portions were dissolved in water and sieved through a nylon membrane. This makes sure that the unwanted debris was removed. The starch slurries were collected in large basins and allowed to settle for 10h. The supernatants were decanted. The resultant starch slurries were severally washed with distilled water while passing through a 100 mesh sieve. The final starch slurries were allowed to settle for 10h, decanted and dried to a constant weight in a hot air oven at 60°C for 2h. The dried starch contents were weighed and determined.

**Results:**
After extraction of starch in the powdered form (Plate 3.1) from the tubers their quantity was estimated in a weighing balance. The results of the starch content were given per 100g of tuber (Table 3.4).
Table 3.4: Starch content of tuber substrates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Starch content/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mirabilis jalapa</em> tuber</td>
<td>3.4g</td>
</tr>
<tr>
<td><em>Kedrostis foetidissima</em> tuber</td>
<td>17g</td>
</tr>
<tr>
<td><em>Cissus trifoliata</em> tuber</td>
<td>8.17g</td>
</tr>
<tr>
<td><em>Alocasia macrorrhiza</em> tuber</td>
<td>11.6g</td>
</tr>
<tr>
<td><em>Dioscorea bulbifera</em> bulb</td>
<td>14.7g</td>
</tr>
</tbody>
</table>

Plate 3.1: Starch powder extracted from tuber

3.2.2 Isolation and identification of *Bacillus subtilis* culture for α-amylase production: (Dubey and Maheswari, 2002), (Cappuccino, 2005)

In the present research, all the five tuber substrates were subjected to starch hydrolysis by the enzyme amylase extracted from the bacterial culture *B. subtilis*. A serial dilution was used to isolate *B. subtilis* from soil sample. The plating was done in nutrient agar plates. For identification, confirmation and pure culture preparation three procedures were followed in the present research.

3.2.2a. Microscopic examination
3.2.2b. Culture method
3.2.2c. Biochemical tests
3.2.2a Microscopic examination:

Gram staining:

Materials and methods:
A small amount of culture from nutrient agar plate was placed on a drop of water and a thin preparation was prepared with the help of inoculating loop and was heat fixed on the glass slide. Now the smear was subjected to Gram staining procedure according to Dubey and Maheswari (2002).

Basing on Gram staining, shape and arrangement of organisms were noted. Pink colour indicates gram negative bacteria and Violet colour indicates gram positive bacteria.

Results: Violet coloured rods were observed. It indicates that, the organism was a Gram positive bacterium (Plate 3.2).

Plate 3.2: Gram staining

Hanging drop method:

Materials and methods:
The culture was taken and placed on a cover slip. The concave side of the slide was inverted over the cover slip which was sealed around the edges with gel. The slide was turned around quickly and hanging drop was observed. Organisms were focused and motility was observed.
**Results:** Highly motile bacteria were observed under the microscope. It indicates that, the organism was a flagellated bacterium.

### 3.2.2b Culture methods:

**Starch nutrient agar:**

**Materials and methods:**

- The medium for this test was a nutrient agar containing starch, prepared in a petriplate.
- The organism to be tested was streaked on the plate.
- When the culture had grown, the plate was flooded with Gram Iodine solution.
- The medium turns blue in all the areas where the starch remains intact.
- The areas of starch medium surrounding the organism was hydrolyzed remain clear and colourless.
- Zone of clearing around the colony was formed.
- The zone represents the area where the starch has been hydrolyzed so that it is no longer available to react with the Iodine solution.

**Starch nutrient agar medium composition:**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>10g</td>
</tr>
<tr>
<td>K$_2$HPO$_4$</td>
<td>5g</td>
</tr>
<tr>
<td>Soluble starch</td>
<td>3g</td>
</tr>
<tr>
<td>Agar</td>
<td>20g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1L</td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
</tr>
</tbody>
</table>

**Results:** Zone of clearing was seen around the colony (Plate 3.3). It represents that, the starch was hydrolyzed by the isolated bacterium. So, it is no longer available to react with the iodine solution.
Plate 3.3: Growth on Starch nutrient agar

Bile esculin medium:

Materials and methods:
The bile esculin agar plates were prepared and the test organism was inoculated and the plates were incubated at 37°C and observed. The change in the medium colour brown or black indicates positive test. The colour change was due to the reaction of iron salts in the medium with 6,7-Dihydroxy coumarin liberated as a result of hydrolysis of glycoside esculin in presence of bile.

Bile esculin medium composition:

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue</td>
<td>5g</td>
</tr>
<tr>
<td>Beef extract</td>
<td>3g</td>
</tr>
<tr>
<td>Ox gall</td>
<td>40g</td>
</tr>
<tr>
<td>Esculin</td>
<td>19g</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td>0.5g</td>
</tr>
<tr>
<td>Agar</td>
<td>15g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1L</td>
</tr>
<tr>
<td>pH</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Results: The colour of the medium was changed to black colour after 24h of incubation.
3.2.2c Biochemical tests:

Catalase test:

Materials and methods:

- A culture was directly added to a few drops of hydrogen peroxide on a glass slide and vigorously mixed with the help of a loop, this was called slide method.
- A small amount of culture was taken in a test tube, to this, few drops of hydrogen peroxide was added and mixed vigorously, this was called tube method.
- Formation of air bubbles indicates a positive test and if there was no bubble formation, it is a negative reaction.

Results: Tiny bubbles were observed by the addition of hydrogen peroxide. The isolated organism was catalase positive.

Citrate utilization test:

Materials and methods:

- The test organism was streaked onto a Simmon's citrate agar slant and incubated for 24h in an incubator.
- Following incubation, citrate positive culture was identified by the presence of growth on the surface of the slant, which was accompanied by blue coloration.
- Citrate negative culture will show no growth and the medium will remain green.

Simmon’s citrate agar medium composition:

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium sulphate</td>
<td>0.24</td>
</tr>
<tr>
<td>Ammonium dihydrogen sulphate</td>
<td>1.00</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>1.00</td>
</tr>
<tr>
<td>Sodium Citrate</td>
<td>2.00</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.00</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.08</td>
</tr>
<tr>
<td>Agar</td>
<td>15.00</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
<tr>
<td>pH</td>
<td>6.8</td>
</tr>
</tbody>
</table>
The above ingredients were weighed and mixed in the conical flask and then sterilized in an autoclave at 121°C, 15 lb pressure for 15-30 minutes. This medium was poured into the test tubes and prepared agar slants. After the medium solidifies, slants were kept in a refrigerator till it was used.

**Results:** The change in the citrate medium from green to deep Prussian blue colour was observed. It indicates that the organism was capable of utilizing citrate as a substrate.

As the isolated bacterium from soil shows positive to the above morphological and biochemical tests the organism was confirmed as *Bacillus subtilis*. The isolated culture was used for the production of α-amylase.

### 3.2.3 Production and Extraction of α-amylase enzyme: (Dubey and Maheswari, 2002)

**Materials and methods:**

- Initially Tendler’s nonsynthetic medium (TNS) was prepared and sterilized. The sterilized medium was used to make TNS slants.
- The isolated *B. subtilis* was streaked on TNS slants and incubated at 30°C for 72h.
- A scrap of bacterial growth was taken from the slant and transfer into other sterile test tube containing some volume of distilled water.
- The volume of the suspension was so adjusted aseptically by diluting it so as to get 1 O.D at 540nm.
- 1ml of the suspension was transferred into the flasks containing 50ml sterilized medium.
- The flasks were incubated at 30°C for 24, 48 and 72h on orbital shaker at 200 rpm.
- The medium was centrifuged at 4000 rpm and supernatant was collected and used as an active enzyme.
TNS medium composition:

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>10g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>4g</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>0.5g</td>
</tr>
<tr>
<td>Ammonium nitrate</td>
<td>1g</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>0.3g</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.5g</td>
</tr>
<tr>
<td>Starch</td>
<td>2.0g</td>
</tr>
<tr>
<td>Water</td>
<td>1L</td>
</tr>
<tr>
<td>pH</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Results: A clear supernatant was produced containing an amylase enzyme. This solution was used for the starch digestibility of the powdered samples of tubers.

3.2.4 Starch digestibility and enzyme assay:

Materials and methods:

- For analyzing the starch digestibility the in vitro procedure which was used at present was illustrated by Padmaja et al. (2001).
- Initially 100g of each powdered samples of tubers were weighed and to this 900ml of 0.02M sodium phosphate buffer pH 6.9 (1.4g of NaH₂PO₄·2H₂O and 1.56g Na₂HPO₄ and 4.5g NaCl dissolved in 900ml of water) was added and the contents were thoroughly mixed.
- The flasks were kept in a boiling water bath to gelatinize starch present in the sample by stirring continuously.
- Lump formation should be prevented.
- The isolated α-amylase solution 100ml from B. subtilis culture was added to the above solution and incubated at 30°C till starch hydrolysis will take place. The total contents of the flask were 1L.
- To nullify the effect of free reducing sugars, control flasks were also set up.
- Control flask was prepared to contain sample as above and no enzyme was added and incubated for 30°C.
- Commercial soluble starch was used as standard. Procedure for the standard sample preparation was same as above.
- Now all the conical flasks were kept in a boiling water bath to inactivate the enzyme activity.
- Upon cooling, 1ml aliquots from each of the test, standard as well as control were pipette out into separate test tubes. The reducing sugars formed by the action of α-amylase were estimated by Dinitrosalicylic acid method.

**Estimation of sugars by Dinitrosalicylic acid method:** (Sadasivam and Manickam, 1996)

**Materials and methods:**

**Reagents:**
Glucose standard: 1mg/1ml of glucose solution standard was prepared with water.
Dinitrosalicylic acid reagent: Dissolve by stirring 1g dinitrosalicylic acid, 200mg crystalline phenol and 50 mg sodium sulphite in 100ml 1%NaOH. Store at 4°C. Since the reagent deteriorates due to sodium sulphite, if long storage was required, sodium sulphite may be added at the time of use.
40% Rochelle salt solution (40% potassium sodium tartarate).

**Procedure:**
For standard graph initially, 0.2, 0.4, 0.6, 0.8 and 1ml of standard glucose solution was pipette out into 5 test tubes and then water was added to make the volume to 1ml in each test tube.
- 1ml of DNS reagent was added to each tube along with the test samples and control and kept in a boiling water bath for 10 min.
- The tubes were cooled and 10 ml of distilled water was added in each tube orange red colour which was developed was measured at 520nm in a spectrophotometer.
- The amount of reducing sugars released was determined by using this standard graph.

**Units of amylase enzyme activity:** Amylase activity may be defined as mg of D-glucose liberated per ml of enzyme per unit period of time.
Results:
The amount of sugars released by the starch hydrolysis of tubers by the α-amylase was estimated using a standard graph (Figure 3.2) of DNS method. The enzyme activity for the standard and 5 test samples was calculated in mg/ml (Table 3.5). It was determined that amylase activity was higher for standard commercial soluble starch i.e 7.7mg/ml of sugars were liberated from 1ml of starch sample. The amylase activity for test samples was low while comparing with standard soluble starch (Table 3.5).

Table 3.5: Enzyme activity of tuber samples

<table>
<thead>
<tr>
<th>Test samples</th>
<th>Incubation time</th>
<th>Enzyme activity (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Standard soluble starch</em></td>
<td>2h</td>
<td>7.7</td>
</tr>
<tr>
<td><em>M. jalapa</em> tuber</td>
<td>7h</td>
<td>1.2</td>
</tr>
<tr>
<td><em>K. foetidissima</em> tuber</td>
<td>4h</td>
<td>5.9</td>
</tr>
<tr>
<td><em>C. trifoliata</em> tuber</td>
<td>5h</td>
<td>2.1</td>
</tr>
<tr>
<td>A. macrorrhiza* tuber</td>
<td>2h</td>
<td>4.1</td>
</tr>
<tr>
<td><em>D. bulbifera</em> bulb</td>
<td>5h</td>
<td>4.9</td>
</tr>
</tbody>
</table>
Calculations:

100g of *M. jalapa* tuber nearly liberates 12g of sugars
100g of *K. foetidissima* tuber liberates 59g of sugars
100g of *C. trifoliata* tuber liberates 21g of sugars
100g of *A. macrorrhiza* tuber liberates 41g of sugars
100g of *D. bulbifera* bulb liberates 49g of sugars

Discussion: From the results, it was determined that, the incubation time required for α-amylase susceptibility of tubers to starch hydrolysis was different for all the tubers (Table 3.5). Of all the 5 different tuber starches tested, *A. macrorrhiza* starch susceptible to starch hydrolysis with in 2h of incubation time where as *M. jalapa* tuber starch required more time 7h for starch hydrolysis.

The extent of starch digestibility was based on the crystalline polymorphic forms of starch granules and generally ‘B’ type crystallites were hydrolysed more slowly than the ‘A’ type ones Williamson et al. (1992). Therefore in the present research all the 5 starch samples were subjected to X-ray diffraction studies and it was observed that the tuber starch of *A. macrorrhiza* showed ‘A’ type X-ray diffraction pattern hence digested easily while the other 4 tuber starches of *M. jalapa*, *K. foetidissima*, *C. trifoliata* and *D. bulbifera* showed ‘B’ type X-ray diffraction pattern thus required more incubation time for digestion.

The X-ray diffraction spectra of ‘A’ type exhibited characteristic strong peaks at 2θ values of 9.9°, 15°, 17° and 23° (Figure 3.3). Where as the ‘B’ type X-ray diffraction spectra exhibited characteristic strong peaks at 2θ values of 15°, 17°, 22° and 24° Delpeuch et al. (1978) (Figure 3.4).
Figure 3.3: ‘A’ type X-ray diffraction Spectra

Figure 3.4: ‘B’ type X-ray diffraction Spectra