Chapter 2

Materials and experimental techniques

2.1 MATERIALS

*H. sabdariffa* fibers were collected from a local farming community at Chittoor, Andhra Pradesh, India. EVA was obtained from Polyolefin Industries Limited, Chennai, India. NaOH, acetic acid, oxalic acid (SD Fine Chemicals Ltd.), sodium hypochlorite (Rankem Ltd.), potassium permanganate (Qualigen Fine Chemicals.), ferrous ammonium sulphate (RFCL Ltd.) were of reagent grade. Nutrient agar was procured from Difco laboratories, Detroit, USA. Curd and pasteurized milk (Aavin, Tamil Nadu Co-operative Milk Producers Federation Ltd) was purchased from the local market and transported immediately to the laboratory. Orange juice was obtained by grinding peeled fresh oranges followed by filtering the pulped juice through linen cloth.

2.1.1 EVA
EVA used in the present experiment is of food grade and contains 18% vinyl acetate content. The basic characteristics of EVA used is given in Table. 2.2.

Table. 2.2: Basic characteristics of EVA

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vinyl acetate content (%)</td>
<td>18.000</td>
</tr>
<tr>
<td>Melt flow index (kg/10 minutes)</td>
<td>00.002</td>
</tr>
<tr>
<td>Vicat softening point (°C)</td>
<td>59.000</td>
</tr>
<tr>
<td>Density (kg/m³)</td>
<td>940.000</td>
</tr>
</tbody>
</table>

2.1.2 *H. sabdariffa* FIBERS

*H. sabdariffa* is an annual dicotyledonous herbaceous shrub. It is a warm seasonal plant and grows in six month with a single and straight stem. It has been used as a cordage crop to produce twine, rope and sackcloth and is extensively cultivated in Andhra Pradesh, India. They have broad leaves and become soft on boiling and the fibers thus obtained are generally used for making ropes. The long thread like fibers when soaked and washed with water becomes soft and has high mechanical properties. The physical properties such as the density and tensile strength of *H. sabdariffa* fiber were found to be 0.75g/m and 400-550 MPa (Aziz et al (1984)).

2.2 EXPERIMENTAL TECHNIQUES

2.2.1 ISOLATION OF CELLULOSES MICROFIBERS FROM *H. sabdariffa* FIBERS

*H. sabdariffa* fibers were used for the extraction of microfibers after combing and removing the external dust particles from the fibers. The fibers were washed and cleaned with distilled water and then dried under sun. They were chopped to 5 cm
length and subjected to a series of treatments as detailed below. The schematic representation of the isolation of CMF is given in Scheme. 2.3.1.

2.2.1.1 STEAM EXPLOSION IN ALKALINE ENVIRONMENT

The fibers were treated with 2% NaOH in an autoclave at a pressure of 15 lbs at a temperature of 120 °C for a period of 1 hour. Pressure was then released immediately. The fibers were removed from the autoclave and continuously washed with water until the solution obtains neutral pH. The fibers were then dried in a hot air oven at 70 °C for 2 hours.

2.2.1.2 BLEACHING PROCESS

The steam exploded fibers were bleached using a mixture of NaOH and acetic acid followed by a mixture of 1:3 volume of sodium hypochlorite solution. Each bleaching process took 1 hour and the process was repeated six times. After bleaching, the fibers were thoroughly washed in distilled water until the smell of the bleaching agent was gone.

2.2.1.3 STEAM EXPLOSION IN ACID ENVIRONMENT

The steam exploded bleached fibers were treated with oxalic acid for 3 hours in an autoclave after attaining a pressure of 20 lbs. The pressure was released immediately. The autoclave was again reset to reach a pressure of 20 lbs and the fibers were kept under pressure for 15 minutes. The fibers were taken out and then washed until the solution obtains neutral pH.

2.2.1.4 MECHANICAL TREATMENT OF FIBERS

The acid hydrolyzed fibers were suspended in water and kept stirring with a mechanical stirrer of type IKA RW 20 Digital Dual at 8000 rpm for 4 hours. The pH of the suspension was observed to be around 6.1. The suspension was then dried at room temperature.  

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H. sabdariffa
2% NaOH, 120 °C, 1 hour, 15 lbs
Residue
NaOCl, room temperature, 6 hours
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2.3 PREPARATION OF CMF/EVA COMPOSITES

The mixing and extrusion of EVA with CMF (1, 2.5, 5, 7.5, 10, 12.5 and 15 weight %) was carried out in a twin screw extruder at 150 °C for 15 minutes at a mixing speed of 60 rpm. The mixes were passed through rollers to obtain a uniform film of thickness 0.5 (±0.02mm).

2.4 CHARACTERISATION AND EVALUATION

2.4.1 EVALUATION OF CHEMICAL COMPOSITION OF CMF

Chemical compositions of fibers (α-cellulose, hemicellulose and holocelluloses) were estimated according to TAPPI procedures: α-cellulose – T203cm-99 and holocellulose – Tappi 249-75. The difference between the values of holocellulose and α-cellulose gives the hemicellulose content of the fibers. 5 gm of sample was mixed with 7.5 % NaOH and stirred at 25 °C for 1 hour. At the end of the 60-minutes period, the pulp suspension was filtered. The chemical composition was determined using the filtrate. 25 mL of the prepared filtrate and 10 mL of 0.5 N potassium dichromate were taken and 50 mL of concentrated sulphuric acid was added into it under stirring. 50 mL of water was added to the mixture, followed by 2 drops of ferroin indicator and then titrated against 0.1N ferrous ammonium sulphate solution till a purple color appears. 12.5 mL of 17.5% NaOH and 12.5 mL of water were used as blank.

The α-cellulose content was estimated using the following equation:
\[
\alpha - \text{Cellulose} = \frac{[6.85 \times (V_2 - V_1) \times N \times 20]}{[A \times W]} \times 10
\]

Where \( V_1 \) and \( V_2 \) are the titre volume of filtrate and blank, \( N \) is the normality of ferrous ammonium sulphate solution, \( A \) is the volume of pulp filtrate, \( W \) is the oven-dry weight of fiber.

2 gm of the sample was weighed and kept in hot air oven at 150 °C for 2 hours. The ash residue was weighed. From this, the holocellulose content was evaluated as follows

\[
\text{Holocellulose} = \frac{(A - B)}{C} \times 100
\]

Where \( A \) - oven dry weight of fibers, \( B \) - weight of ash formed while the fibers placed in oven, \( C \) - initial weight of fiber.

For the estimation of lignin, 75mL of 0.1M sodium hydroxide was added to 1.5 mg of the sample and kept under stirring in hot water bath. After one hour, the sample was removed and washed first with distilled water followed by 10% acetic acid. The samples were dried in a hot air oven. 10 mg of this sample was soaked in 0.2 mL of 72 % sulphuric acid for 2 hours and then added 10 mL of distilled water. The sample was filtered, washed and dried under high vacuum.

\[
\text{Lignin content} = \frac{(C - D)}{C} \times 100
\]

C - initial weight of fibers, \( D \) – final weight of fibers.

2.4.2 DETERMINATION OF MOISTURE CONTENT OF CMF

The moisture content of the fibers was estimated by T264om-88 procedure. The weighed amount (E) of sample was kept in an air oven for 2 hours at 105 ±3 °C followed by cooling in a desiccator, replaced the stopper and opened the stopper momentarily to equalize the air pressure and weighed. The bottle was returned to the oven for 1 hour. Again the bottle has been cooled and weighed as above for successive one hour periods until constant weight (F) was reached, that is, until successive
weight of the sample do not change by more than 0.002g. The moisture content was calculated as follows:

\[
\text{Moisture content (\%)} = \frac{(E - F)}{E} \times 100
\]

----- (2.4)

E – initial weight of the sample, F – final oven dry weight of the sample after the removal of the moisture.

2.4.3 MORPHOLOGICAL STUDIES

2.4.3.1 SCANNING ELECTRON MICROSCOPE (SEM)

The SEM photographs of fibers and CMF/EVA composites were taken using a SEM of model ZEISS EVO. The composite samples were sputter coated with gold and then photographed in different magnifications.

2.4.3.2 OPTICAL MICROSCOPY

The optical photographs of fibers and CMF/EVA composites were taken using a Carl Zeiss microscope. The samples were mounted on a clean glass slide and photographed. The dimensions were evaluated using Clemex software version 4.0.

2.4.3.3 X-RAY DIFFRACTION (XRD)

X-ray diffraction (XRD) profiles of fibers and CMF/EVA composites were taken using a Bruker D8 Advance X-Ray Diffractometer, Cu Kα (1.5406 Å), Ni filtered radiation with 40 kV voltage and 40 mA intensity in the range of 10-80° in the speed of 2°/minute. Crystallinity of cellulose was calculated from the diffraction intensity data. The normal diffraction planes of the cellulose are 101, 101, 021, 002, and 040 and will be present at 14.8°, 16.7°, 20.7° and 22.5° of 20 angle. The crystallinity index was obtained using the equation 2.5 (Segal et al (1951))
\[
\text{Crystallinity index} = \frac{I_{002} - I_{\text{amorph}}}{I_{002}} \times 100 \quad \text{(2.5)}
\]

Where \(I_{002}\) is the maximum intensity of the (002) lattice diffraction and \(I_{\text{amorph}}\) is the intensity diffraction at 18°. The calculation of the X-ray crystallinity order index was performed using equation 2.6. The crystallinity order index was calculated from the fraction of the ratio of (002) to the sum of (101), (021) and (002) refraction areas:

\[
\text{Crystallinity order index} = \frac{A_{002}}{A_{101} + A_{010} + A_{002}} \quad \text{(2.6)}
\]

The above equation was used for calculating amorphous fraction in terms of intensity of the sample.

2.4.3.4 FOURIER TRANSFORMS INFRARED SPECTROSCOPIC (FTIR) STUDIES

FTIR analysis of fibers and composites were carried out using JASCO 400 Infrared spectrometer. Fibers were grinded and mixed with KBr (sample/KBr ratio, 1/99) to prepare pellets. FTIR spectra were recorded in a spectral range of 450-4000 cm\(^{-1}\) with resolution of 2 cm\(^{-1}\), taking four scans for each sample.

2.4.4 EVALUATION OF MECHANICAL PROPERTIES

2.4.4.1 TENSILE STRENGTH

Tensile strength of composites were measured using INSTRON instrument (Model: 3382 INSTRON instrument) as per ASTM standard D-882. The tests were carried out at room temperature and an average of five specimens was taken for each sample.

2.4.4.2 ELONGATION AT BREAK

Elongation at break of composites was obtained from the tensile strength measurement data. The average of five specimens was taken for each sample.

2.4.4.3 TEAR STRENGTH
The tear strength of CMF/EVA composites were measured using Elmendorf tear resistance tester by pendulum method using ATS FAAR Make (ATS model no: 16.40001) as per ASTM standard D-1922. The tests were carried out at room temperature and an average of five specimens was taken for each sample.

2.4.4.4 DYNAMIC MECHANICAL ANALYSIS

The dynamic storage modulus (E') and mechanical damping (tanδ) and stiffness were evaluated using a Dynamic Mechanical Analyzer (Thermo Fisher, DMA Q800, V20.6 Build 24) as per ASTM E-1131. The analysis was carried out in the temperature range of 35 to 100 °C, at a frequency of 1Hz and a heating rate of 10 °C/minutes.

2.4.4.5 AGEING STUDIES – GAMMA RADIATION

The effect of gamma radiation on CMF/EVA composite was studied by irradiating the dumb bell shaped tensile samples with gamma-rays from a $^{60}$Co source. The samples were irradiated for different radiation doses such as 5, 10 and 15 Mrad at a dose rate of 0.321 Mrad/hr exposure time. Tensile properties of CMF/EVA composites before and after gamma-ray irradiation were evaluated as per the method mentioned in 2.4.4.1. FTIR spectrums of the composites before and after radiation were examined as mentioned in section 2.4.3.4.

2.4.4.6 AGEING STUDIES – THERMAL AGEING

The samples for thermal ageing were kept in an oven at 90 °C (±5 °C) for 6 months. The photographs and weight of samples were taken at an interval of one month.

2.4.5 EVALUATION OF THERMAL PROPERTIES

2.4.5.1 THERMOGRAVIMETRIC ANALYSIS (TGA)

Thermo gravimetric analysis of fibers were carried out in the temperature range of 25–400 °C under nitrogen atmosphere with a flow rate of 20 mL/minute using A PERKIN ELMER thermo gravimetric analyzer Pyrisi TGA. A constant heating rate of 20 °C/minutes was maintained. The TGA of CMF/EVA composite
films were also performed using same instrument in these temperature ranges of 50–600 °C.

2.4.5.2 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

DSC of CMF/EVA composites were carried out using Perkin Elmer Diamond Differential scanning calorimetry under nitrogen atmosphere with a flow rate of 20 mL/minutes. Since the previous thermal history of a polymer affects the calculated degree of crystallinity, EVA crystallinity was evaluated after subjecting to a second heating designed to remove the effect of prior thermal history (Brogly et al (1997); McEvoy et al (1998)). The samples were held for 3 minutes at −40 °C and heated from −40°C to 120 °C at a heating rate of 30 °C/minutes. The samples were then cooled from 120 °C to −40 °C at the rate of 30 °C/minutes and held at −40 °C for 3 minutes. Again the samples were heated from −40 °C to 120 °C at the rate of 30 °C/minutes. Percentage crystallinity was calculated using the following equation,

\[
\text{Crystallinity} = \frac{\Delta H_m}{\Delta H_{100}} \times 100
\]  

Where \(\Delta H_m\) is the specific enthalpy of melting of the composite studied and \(\Delta H_{100}\) is the specific enthalpy of melting for 100% crystalline polyethylene (Araujo et al (2012)).

2.4.6 SWELLING AND OXYGEN TRANSPORT RATE

2.4.6.1 OXYGEN TRANSPORT RATE

The OTR of composites were measured using gas permeability recorder, DTS FAAR make (Model N 500) as per ASTM 1434. The OTR values were calculated using the following equation.

\[
P = g \left( \frac{h}{t-t_0} \right)
\]

\[
g(x) = -\frac{1}{AT} \left[ V_0 + a \left( P_u + (h_B - h_{20}) \right) \times \frac{1-(h_{20}-h)}{P_W-(h_B-h_{20})} + (2a(h_0 - h)) \right]
\]

\(a\) - area of capillary (mm\(^2\)), \(A\) - area of transmission (mm\(^2\)), \(h_0\) - height of the mercury at the intuition of transmission, \(h\) - height of the mercury in cell capillary leg at any given time (mm), \(h_B\) - maximum height of the mercury in the cell from the datum
plane to the upper calibration line (mm), $h_L$ - height of the mercury in cell reservoir leg from datum plane to the top of the mercury level (mm), $P_u$ - upstream pressure of the gas to be transmitted (kPa), $R$ - universal gas constant, $t_o$ - time at the start of the transmission, $t$ - time, $T$ - absolute temperature (K), $V_{BC}$ - volume from B to C, $V_{CD}$ - void volume of depression, $V_f = (V_{BC} + V_{CD})$.

2.4.6.2 SWELLING AND DIFFUSION PARAMETER

Circular discs (diameter 1.2 cm) of the composites were cut out using a sharp edged steel blade. The sample thickness was measured at several points by using the micrometer screw gauge. The sample was initially dried in a vacuum desiccators and the original weight was taken. They were then immersed in 20 mL of experimental solvent. The samples were removed at regular intervals and then weighed after wiping with a tissue paper. They were then immediately immersed back to the experimental solvent. The time for each sample weighing was kept for maximum of 25-35 seconds in order to minimize the error due to the escape of the solvents from the composites. The experimental work was followed until equilibrium weight was obtained (until constant weight was obtained). The same technique has been adopted by many researchers before (Geethamma and Thomas (2005); Hedenqvist et al (2006); Fraga et al (2006); Rozman et al (1998); Arbelaiz et al (2005)).

2.4.7 EVALUATION OF MICROBIAL GROWTH

50 mL of experimental solvent was poured into 100 mL glass screw cap containers. Circular disc of composite (diameter 1.2 cm) samples were immersed in the experimental solvent and the bottle was capped. The cell was than stored at 3, 10 and 20 °C for 16 days. Total aerobic bacteria were counted in colony forming units (cfu). During the storage period, 0.1 mL of experimental solvent was taken out periodically ($8^{th}$ and $16^{th}$ days) to measure microbial change in the stored milk, curd and orange juice. A series of decimal dilutions was carried out with sterilized distilled water. To enumerate total aerobic bacterial counts, the serially diluted samples were plated on nutrient agar and incubated aerobically at 36 °C for two days.

2.4.8 BIODEGRADABILITY TEST
2.4.8.1 NATURAL SOIL BURIAL TEST

The natural soil burial test of CMF/EVA composites was carried out for 181 days. Samples of pure EVA and CMF/EVA composites with different filler loading were weighed and buried in the soil at 2 feet depth. The buried samples were recovered at frequent interval (30 days) and analyzed for degradation using SEM, tensile strength measurements and weight loss. The tensile strength of composites before and after degradation was carried out as mentioned in section 2.4.4.1. The SEM analysis after degradation was carried out as given in section 2.4.3.1. FTIR spectrums of the composites before and after degradation were examined as mentioned in section 2.4.3.4. The samples were cleaned with soft bristle brush before analysis.

![Figure 2.4.8.1: CMF/EVA composites in natural soil burial test](image)

2.4.8.2 COMPOST SOIL BURIAL TEST WITH EARTHWORMS

Vermicomposting utilizes earth worms as bioreactors to biodegrade organic waste to humus. The compost soil burial test of CMF/EVA composites was carried out for 120 days. The samples of (4 cm × 4 cm) were immersed in the compost which is conditioned with the suitable environment for the growth of vermicompost worm *L. terrestric*. A good amount of cow dung and an average of water content of 70% was maintained throughout the experiment. The samples were buried 3 feet depth in the compost containing cow dung and worms. 50 gm of cow dung was poured below and
above the composites with 10 numbers of earthworms. The samples were taken out and the impurities were removed using soft bristle brush.

**Figure. 2.4.8.2: CMF/EVA composites with earthworms in compost soil burial test**

2.4.8.3 BIODEGRADATION TEST WITH CULTURED FUNGI

The samples were tested for biodegradation by inoculating the CMF/EVA composite films with *A. niger* on a nutrient agar media and incubating for 21 days (Mani and Bhattacharya (1998)). The CMF/EVA film (4 cm × 4 cm) were weighed, rinsed twice for 15 minutes with distilled water, disinfected for 15 minutes with 70% ethanol and then placed on nutrient agar. Before placing the samples, agar surfaces were cultivated with *A. niger*. After 21 days, the films were examined for evidence of colony growth. The morphology study and the weight loss of the inoculated composite films were also performed as discussed earlier.