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Production of Bioplastic Using Isolated Kernel Starch and Endocarp Cellulose from *Mangifera indica*

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1. INTRODUCTION:

Plastics can be classified as a group of artificial or natural organic materials that can be cast and hard-ened. Plastics are very common and used as packaging material, in bottles, cell phones, printers, even in pharmaceutical to automobiles industries. The reason why plastics are a useful option because their structures can be chemically treated with some strength and shape to obtain higher molecular weights, low reactivity and long-lasting substances. In addition, they are durable and cost-effective. Nowadays, people are more aware about the harmful effects of petrochemical derived plastic materials in the environment by conducting many studies for managing plastic waste on earth by finding eco-friendly alternatives to plastics. This ecofriendly alternative is bioplastics, which when disposed in the environment, will easily degrade through the en-zymatic actions of micro-organisms. The degradation of biodegradable plastics gives rise to carbon dioxide, methane, water, biomass, humid matter and various other natural substances which can be read-ily eliminated (Azios 2007).

The impact of renewable material becomes even bigger and leads to a more sustainable way of manufacturing materials. Today, bioplastic materials represent a valid alternative to the conventional plastics and their applications. Actually; the bioplastics market share is around 1% of the 370 million tons of total global plastic produced. But their annual growth rates however around 30% until 2025 vegetables, fat and oil corn starch, straw, wood chips, sawdust, recycled food waste etc.,

However, a number of bio-based plastics and products have been researched, developed and are growing rapidly. Bioplastics will be a new plastics generation. Renewable raw materials such as corn, potato, or plants are used to manufacture bioplastics. They are a feedstock for bioplastics to get starch, cellulose, lactic acid etc. They are not dangerous, hazardous and very environmentally friendly raw materials to produce. Reduce the problem of plastic waste that is suffocating the planet and contaminating the environment.

Types

Starch-Based - Simple bioplastic derived from corn starch

Cellulose-Based - Produced using cellulose esters and cellulose derivatives

Protein-Based – Produced using protein sources such as wheat gluten, casein and milk

Bioplastic production significantly reduces greenhouse gas emissions and decreases non-renewable energy consumption. (Vincenzo Piemonte, 2012) Firms worldwide would also be able to increase the environmental sustainability of their products by using bioplastics (Sebastian, ***et al.,*** 2016)

Environmental impact

Materials such as starch, cellulose, wood, sugar and biomass are used as a substitute for fossil fuel resources to produce bioplastics; this makes the production of bioplastics a more sustainable activity compared to conventional plastic production.

(Gironi, F., 2011). The environmental impact of bioplastics is often debated, as there are many different metrics for "greenness" (e.g., water use, energy use, deforestation, biodegradation, etc. (Miles, Lindsay, 2018). Hence bioplastic environmental impacts are categorized into non-renewable energy use, climate change, eutrophication and acidification. (Martin, ***et al.,*** 2012).

IMAM PASAND

Hamam Mango, Imam Pasand or Himayat or Himam Pasand is a lesser known and exclusive mango cultivar, grown in Srirangam, Tamil Nadu in India. The names suggest regal origins and it is said to have been the fruit of choice for India's royalty.

It is a large, not too attractive looking mango, mottled green that lightens to blotched yellow-green as it ripens. It is available only during the months of May and June and each mango could grow up to 400 grams to 1000 grams in weight for its unique taste and it has been considered as the 'King of Mangoes'. Many mangoes are initially sweet and incredibly rich smoothness of its aftertaste.

ALPHONSO (MANGO)

The Alphonso mango is a named **mango cultivar** that originated in **India**. The variety is named after Afonso de Albuquerque, an appointed viceroy and a distinguished militarian of the Portuguese in Goa and Bombay, from 1509 to 1515. (Sukhadwala, Sejal, 2012). Alphonso is also one of the most expensive varieties of mango

The Alphonso is a seasonal fruit, available mid-April through the end of June. (Sukhadwala, Sejal, 2012)

The fruits generally weigh between 150 and 300 grams have a rich, creamy, tender texture and delicate, non-fibrous, juicy pulp. As the fruit matures, the skin of an Alphonso mango turns golden-yellow with a tinge of red across the top of the fruit. (Sarmishta, 2010).

KALAPAD (MANGO)

Kalapad mangoes are medium sized mangoes and are grown in Andhra Pradesh. The flesh is pulpy and luscious and the completely ripened mangoes are reddish yellow in colour. Mango is a rich source of antioxidant and it helps to boost the immune system.

ADVANTAGES OF BIOPLASTICS:

- They reduce carbon footprint
- They provide energy savings in production
- They do not involve the consumption of non-renewable raw materials
- Their production reduces non-biodegradable waste that contaminates the environment
- They do not contain additives that are harmful to health, such as phthalates or bisphenol A
- They do not change the flavour or scent of the food contained
- The common Bioplastic can be injection moulded and shaped to take the same form as traditional thermoplastic
- In some cases, the bioplastic are stronger and lighter
- Common bioplastics such as starch-based PLA and PHB are nontoxic and of no health concern.

Uses of bioplastics

It is expanding its use in various sectors: medical, food (catering products, disposable containers ...), toys, and even in the world of fashion (Versace has a clothing line Ingeo, made from corn) and, of course, biodegradable bags.

2. MATERIALS AND METHODS:

SAMPLE COLLECTION

About 15 pieces of each of three varieties of mangos such as Kalapad, Alphonso, Imampasand were collected from the local market of Pallavaram, Koyambedu and Vadapalani, located in Chennai.

SAMPLE TREATMENT

- The mangoes were washed and peeled to recover the seeds.
- The seeds were washed with the tap water to remove the impurities and dried overnight.
- Then the mango seeds kernel was separated manually using a kitchen knife to recover the seeds from the hull.
- The sample was aseptically transferred in to sterilized bags.

Isolation of Starch

- The recovered embryos of the sample were cut into small pieces and placed in mixing blender and soaked in water
- After mixing process, starch slurry is filtered using a muslin cloth and filtrate is obtained after 30 minutes.
- Starch sediment was separated from the slurry and then washed again with distilled water.
- After the second settling, starch sediment was dried using an oven with temperature ±40ºC for removal of free water.

Confirmative Test for Starch

- The Iodine solution is added to a pinch of extracted starch sample
- The Appearance of Intense blue – black colour indicates the presence of starch

Determination of starch content

$$\frac{\textit{Weight of Starch}}{\textit{Weight of Kernel}} \times 100\%$$

Isolation of Cellulose

- The mango husk was cut into small pieces.
- Dried Husk was delignified by sodium hydroxide in the dosage of 1 g NaOH/100g EFB with consistency 10%
- The digester heated until pressure inside the digester was 6 bar for 5 h.
- Pulp was then washed with water to remove NaOH residue and black liquor.
- The pulp was beaten in laboratory beater to get pulp with freeness level of 300.
- Sodium hypochlorite (5.25% in water w/w) were used for cellulose purification.
- 100 g of pulp was treated in a flask containing 3000ml of deionized water with 33.5g sodium hypochlorite at 70-75ºC.
- The addition of sodium hypochlorite was continued at 2 h intervals until the cellulose become white.
- The cellulose was left in acidified condition for 12 h before washing. Cellulose was washed three times using deionized water.

Confirmative Test for Cellulose

- A drop of sulfuric acid is added to a pinch of extracted cellulose sample.
- Change of colour in the fiber thickenings to black confirms the presence of cellulose.

Determination of Cellulose Content

$$\frac{\textit{Weight of Isolated cellulose}}{\textit{Weight of Husk}} \times 100\%$$

- **Isolated starch and cellulose from three varieties of mango seed was used for Bioplastic preparation.**

PREPARATION OF BIOPLASTIC

Starch – Cellulose Bioplastic preparation

- Bioplastic composite of starch – cellulose composite was prepared by solution casting and evaporation process using mango starch the polymers matrix and glycerol as plasticizer.
- In each sample 3 g of mango starch was suspended in 100 ml distilled water and heated at 60ºC for 15 minutes for gelatinization.
- Cellulose solution was added slowly to the gelatinized starch and stir until all cellulose mixed well in the gelatinized starch.
- Cellulose was added in varying concentration such as 0, 0.12g, 0.25g, 0.375g, 0.5g
- Then Glycerol was added at varying concentration such as 0, 0.12g, 0.25g, 0.375g, 0.5g
- The mixture was cooled and cast on acrylic plates and air dried. The film produced was peeled of and kept in zipper bag and stored in desiccator.

FT IR Analysis *(Imam Pasand, Alphonso & Kalapad)*

- To determine the functional groups in the prepared bioplastics from three varieties of mango seeds, FT-IR was performed using **Shimadzu** in the range of 400 – 4000 cm^{-1}

SEM Analysis *(Imam Pasand, Alphonso & Kalapad)*

- To view the morphology of the prepared bioplastics from three varieties of mango seeds, SEM was performed using **Hitachi-S 4800**

3. RESULTS AND DISCUSSION:

In the present investigation PRODUCTION OF BIOPLASTIC USING ISOLATED KERNEL STARCH AND ENDOCARP CELLULOSE FROM *mangifera indica* the following results were gained and the discussion is presented here.

Isolation of Starch from Kernel.

Table No 1 Isolation of starch from kernel

Fig No 1 Isolated starch from three varieties of mango (Imampasand, Alphonso, Kalapad)

Table No 1 & Fig No 1 represents the isolated starch in terms of grams and yield (as percentage) Starch a polysaccharide was isolated from three varieties of mango kernel (Imampasand, Alphonso, Kalapad).

By comparing the percentage yield of Starch in three varieties of mango kernel it is found that Alphonso mango seed kernel has produced the highest yield followed by Imampasand and Kalapad. The yield of starch in Kalapad is found to be very less.

The isolated starch was in greyish powdered form, also this result represents as that normally mango kernel is considered as a waste by common man and thrown away, But from the kernel starch can be isolated and can be used as one of the source for bioplastic production.

CONFIRMATIVE TEST FOR STARCH

Iodine test

Fig No 2 Iodine test

Fig No 2 represents the iodine test performed for isolated starch from kernel of three varieties of mango Appearance of blue black colour represent the presence of starch.

ISOLATION OF CELLULOSE FROM ENDOCARP

Cellulose is a tough fibrous and water insoluble polysaccharide. The cellulose helps in maintaining rigidity and stiffness in plant and parts of plant. Hence, from the review papers it is identified that the endocarp of mango seed is rich in cellulose and hence it was used in the present investigation.

Table No 2 Isolation of cellulose; Fig No 3 Isolated cellulose from three varieties of mango (Imampasand, Alphonso, Kalapad)

Table No 2 highlights the weight of cellulose extracted from mango seed endocarp of three varieties and Fig No 3 represents the isolated cellulose. The yield range was higher in Immapasand with (56%) , followed by Kalapad (40%) Alphoso (38%) respectively.

By undergoing review studies in isolation of cellulose from different plant sources, it is identified that the content of cellulose present in mango endocarp of the present study has gained a good yield, which can be subjected to further studies.

CONFIRMATIVE TEST FOR CELLULOSE

Fig No 4 Test for cellulose

Fig No 4 depicts the confirmative test for the isolated cellulose. It represents the appearance of black colour after the addition of sulphuric acid.

BIOPLASTIC PRODUCTION

Bioplastic are considered to be easily degradable. The other considered advantage is that, it reduces the usage of fossil fuel resources, a smaller carbon foot print. It is less toxic, and does not contain bisphenol A (BPA), a hormone disruptor presents in other traditional plastics.

Hence, the cheapest source i.e., mango used is chosen as a source of bioplastics. The starch was isolated from kernel of three varieties of mango seed and cellulose was isolated from the endocarp of three varieties of mango seed. Since the mango seeds served as a good yield producer of starch and cellulose, it was used in the production of bioplastics. Literature proves that starch itself can be used in the production of bioplastics, but to increase the tensile strength, along with starch, cellulose was also used as the copolymer along with glycerol.

Bioplastics production was tried using four samples [Standard starch & cellulose, starch & cellulose isolated from Imampasand, starch & cellulose isolated from Alphonso, starch & cellulose isolated from Kalapad] with fixed concentration of **starch** (3g) and varying concentration of **cellulose** (0.12g, 0.25g, 0.375g, 0.5g) and **glycerol** (0.12g, 0.25g, 0.375g, 0.5g) which is depicted in Fig No 14,15,16 & 17.

Fig No 5 Bioplastic standardized using starch and cellulose

Fig No 6 Bioplastic produced using starch and cellulose isolated from Imampasand

Fig No 7 Bioplastic produced using starch and cellulose isolated from Alphonso

Fig No 8 Bioplastic produced using starch and cellulose isolated from Kalapad

Fig. No 9 represents the bioplastic sheets produced using starch, cellulose & glycerol used for further studies.

By analyzing the produced bioplastic, it is seen that for all four samples at concentration (**starch**: 3g, **cellulose**: 0.375g and **glycerol:** 0.375g) the bioplastic has gained the properties of good rigidity, increased tensile strength, high flexibility when compared with reduced concentration of starch and cellulose such a 0.12g & 0.25g and increased concentration of starch and glycerol 0.5g.

Fig No 9 Good quality bioplastic sheet

FT IR

The functional groups predicted from the graph

The isolated starch was reinforced with cellulose fibers with glycerol as plastizer, Due to this binding there occurs changes in the Chemical structure of normal starch cellulose, which is studied and confirmed using **FT IR**

FT IR Spectra of the produced bioplastic [standard (1), Imampasand (2), Alphonso (3), Kalapad (4)] is depicted in Fig No 10,11,12,13.

Fig No 10 FT IR analysis of standard starch and cellulose Fig No 11 FT IR analysis of Imampasand starch and cellulose

Fig No 12 FT IR analysis of Alphonso starch and cellulose Fig No 13 FT IR analysis of Kalapad starch and cellulose

From the FT IR graph, it is noticed to view two regions of absorbance. One set of absorbance at lower wave lengths (500-1700 cm^{-1}) and the other at higher wave length (2500-3500 cm^{-1}). The wide band was observed is the range of 3600 to 3000 cm^{-1}which contributes to the hydroxyl group O-H for all four bioplastic samples, the peak at 2937.59, 2887.44 (Bioplastic from Imampasand mango seed represents to aliphatic saturated – CH stretching

The peak in the range of 2198.85 (Imampasand), 2243.21 (Alphonso), 2210 (Standard) represent to C≡C Stretching. The peak indicated at 1645.28 for all four-bioplastic sample indicates C C Stretching and peak at 1417.68 (Imampasand), 1415.75 (Alphonso), 1417.68 (Kalapad), 1421.54 (Standard) represents to O-H binding of absorbed water by the cellulose. The absorbance bands around 1151.50 (Imampasand), 1151.50 (Alphonso), 1151.50 (Kalapad), 1153.43 (Standard) and absorbance around 1105.2 for all four bioplastic samples and peaks around 920 (four for four samples) and peak around 852 (for four samples) represents C-O stretching (a type of alcohol, carboxylic acid, ester).

The presence of these functional groups represents the interaction between starch and cellulose during the formation of bioplastics.

SEM Analysis

The morphology of the isolated bioplastic was subjected to SEM studies

Fig No 14 SEM analysis of bioplastic using standard starch and cellulose

Fig No 15 SEM analysis of bioplastic using Imampasand starch and cellulose

Fig No 16 SEM analysis of bioplastic using Alphonso starch and cellulose

Fig No 17 SEM analysis of bioplastic using Kalapad starch and cellulose

Fig. No.14,15,16,17 represents the SEM images of the produced bioplastics using starch and cellulose standard, Imampasand, Alphonso, Kalapad at 5μm magnification.

By analyzing the images, it is understood that the images are smooth, and rigid in appearance with no cracks. Thus, it confirms that bioplastics produced using isolated starch and cellulose from different mango seeds are of good quality and texture.

Table No 1 Isolation of starch from kernel

S.No.	Name of the Source	Weight of Kernel	Starch in grams	Yield %
1	Imam Pasand	50g	4.152g	8.304%
2	Alphonso	50g	8.182g	16.36%
3	Kalapad	50g	0.511g	1.022%

Fig No 1 Isolated starch from three varieties of mango (Imampasand, Alphonso, Kalapad)

Iodine test

Fig No 2 Iodine test

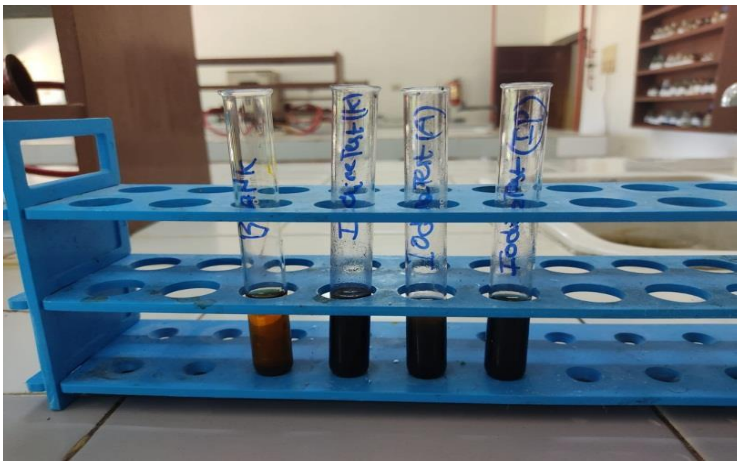

Table No 2 Isolation of cellulose

S.No.	Name of the Source	Weight of Endocarp	Cellulose in grams	Yield %
1	Imam Pasand	50g	28g	56%
2	Alphonso	50g	19g	38%
3	Kalapad	50g	22g	40%

Fig No 3 Isolated cellulose from three varieties of mango (Imampasand, Alphonso, Kalapad)

CONFIRMATIVE TEST FOR CELLULOSE

Fig No 4 Test for cellulose

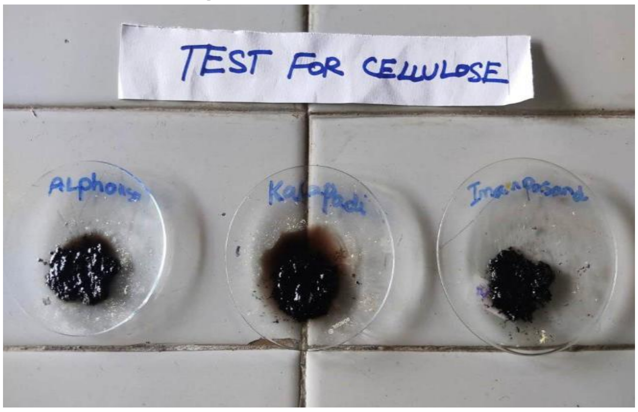

Fig No 5 Bioplastic standardized using starch and cellulose.

Fig No 6 Bioplastic produced using starch and cellulose isolated from Imampasand

Fig No 7 Bioplastic produced using starch and cellulose isolated from Alphonso

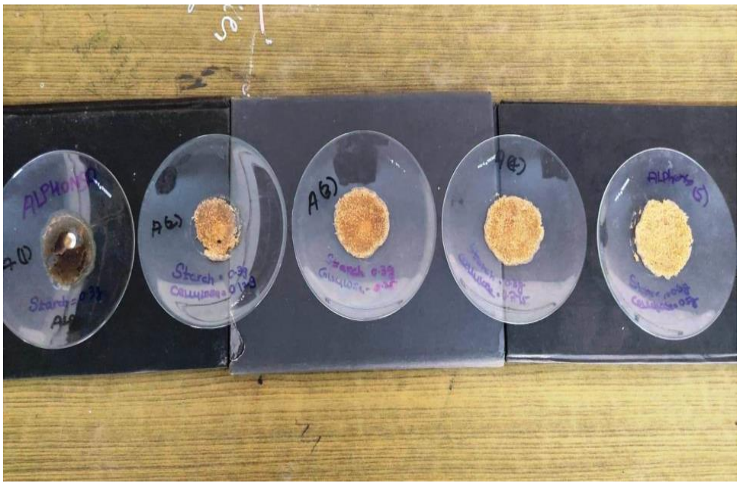

Fig No 8 Bioplastic produced using starch and cellulose isolated from Kalapad

Fig No 9 Good quality bioplastic sheet

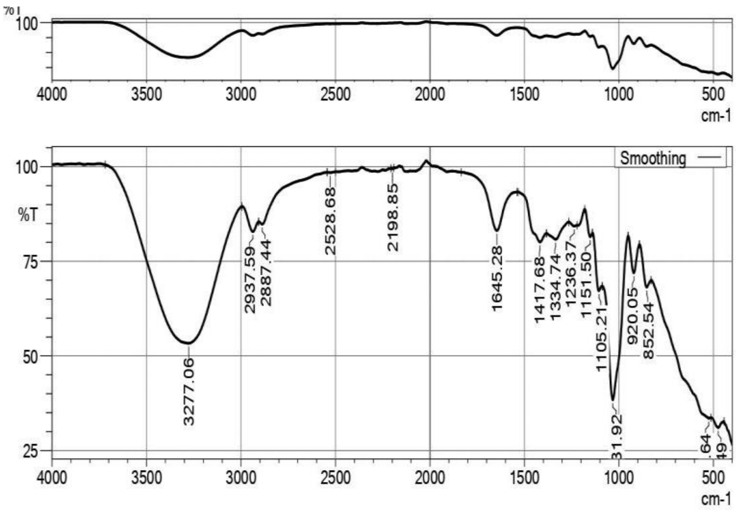

Fig No 10 FT IR analysis of standard starch and cellulose

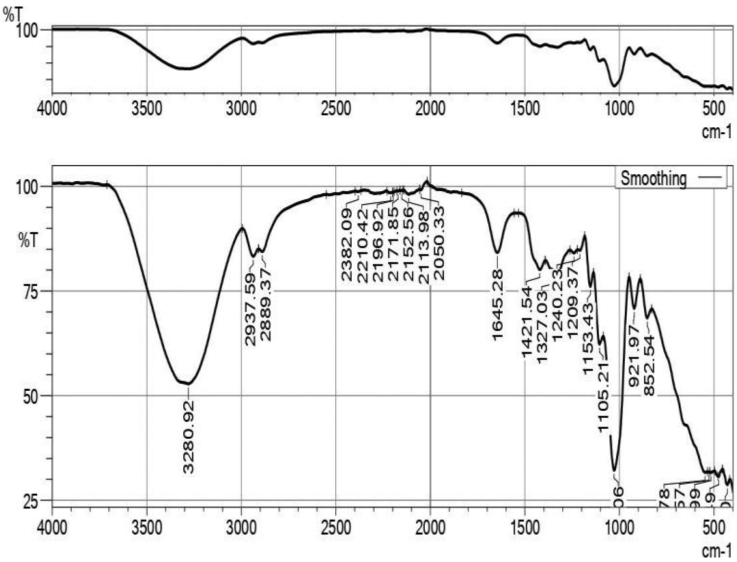

Fig No 11 FT IR analysis of Imampasand starch and cellulose

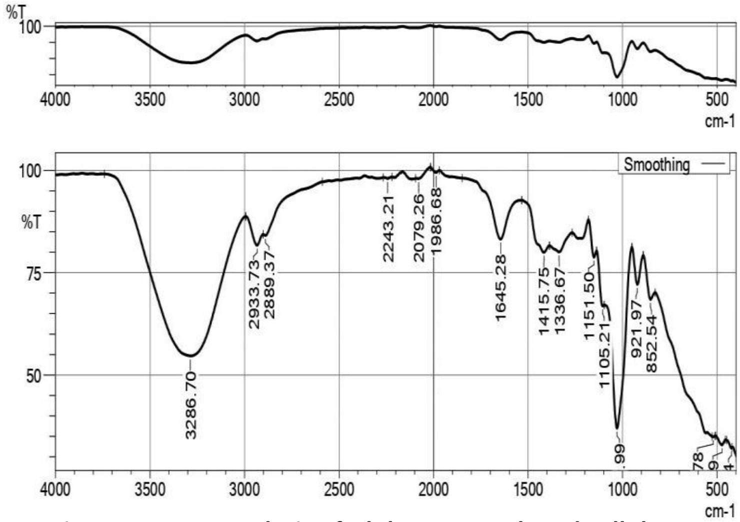

Fig No 12 FT IR analysis of Alphonso starch and cellulose

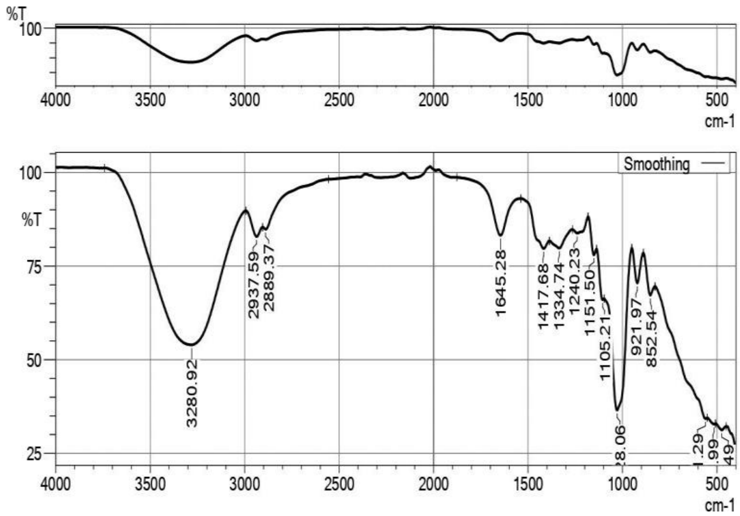

Fig No 13 FT IR analysis of Kalapad starch and cellulose

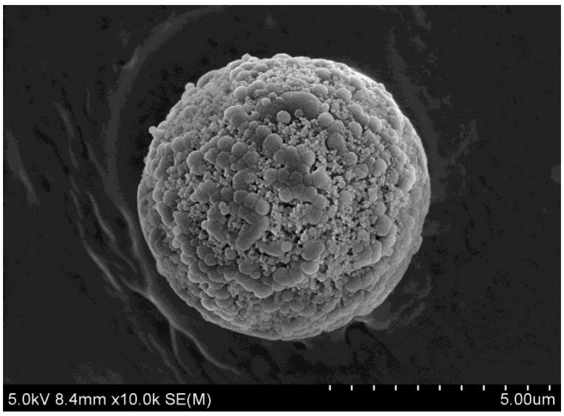

Fig No 14 SEM analysis of bioplastic using standard starch and cellulose

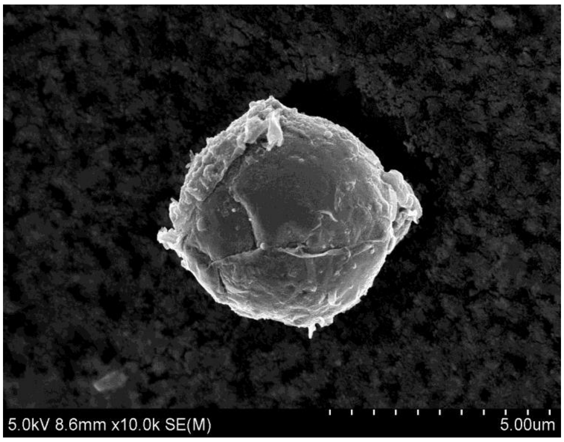

Fig No 15 SEM analysis of bioplastic using Imampasand starch and cellulose

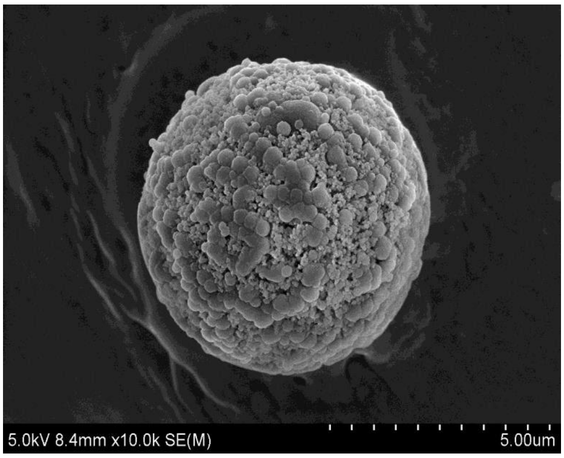

Fig No 16 SEM analysis of bioplastic using Alphonso starch and cellulose

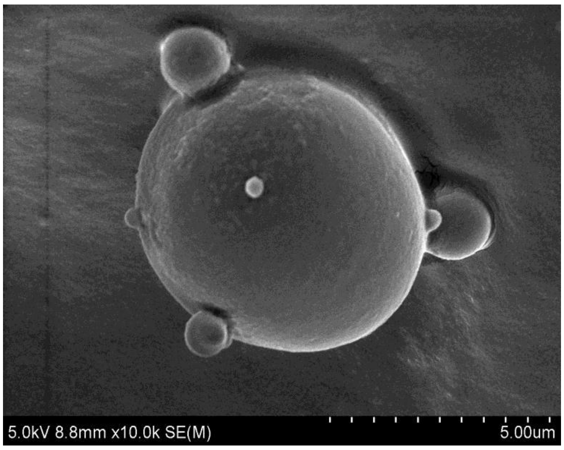

Fig No 17 SEM analysis of bioplastic using Kalapad starch and cellulose

4. SUMMARY:

- In the present investigation "**PRODUCTION OF BIOPLASTIC USING ISOLATED KERNEL STARCH AND ENDOCARP CELLULOSE FROM**

Mangifera indica" the following results were gained and is summarized

- The three varieties of mango such as (Imampasand, Alphonso, Kalapad) were collected from pallavaram market, Koyambedu market and Vadapalani market,located at Chennai.
- The seeds were extracted dried and the endocarp and kernel was chosen for the present study.

- From the endocarp, cellulose was isolated and from the kernel, starch was isolated and the weight are recorded as (**Kernel**: Imampasand = 50g, Alphonso = 50g, Kalapad = 50g) and (**Endocarp**: Imampasand = 50g, Alphonso = 50g, Kalapad = 50g)
- The percentage yield of the isolated starch from three varieties of mangoes kernel were gained as Imampasand = 8.304%, Alphonso = 16.36%, Kalapad = 1.022%. The highest yield of starch was received for Alphonso, likewise the percentage yield of isolated cellulose from three varieties of mango endocarp was Imampasand = 56%, Alphonso = 38%, Kalapad = 40%. The highest yield of cellulose was received for Imampasand.
- The isolated starch and cellulose from three varieties of mango was subjected to bioplastic production.
- The produced bioplastic was obtained with good quality, texture, rigidity and plasticity.
- To further confirmation, it was subjected **FT IR** studies. The functional groups gained revealed the formation bioplastic.
- The morphology of isolated bioplastic was studied using **SEM,** which revealed a smooth appearance with no cracks.

5. CONCLUSION:

Thus, the present investigation has gained a good quality of product which is isolated from a waste source and tend to the nature of degradability.

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