

**PRODUCTION OF PECTIN FROM TOMATO (*SOLANUM
LYCOPERSICUM*) AND MOSAMBI (*CITRUS LIMETTA*) AND
DEVELOPMENT OF BIOFILMS FOR FOOD PACKAGING**

Submitted in the partial fulfilment of the requirements for the award of
Master of Technology degree in
Biotechnology

By

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of AKSHAYA R (39810002) who carried out the project entitled **“PRODUCTION OF PECTIN FROM TOMATO(*SOLANUM LYCOPERSICUM*) AND MOSAMBI(*CITRUS LIMETTA*) AND DEVELOPMENT OF PECTIN BASED BIOFILMS FOR FOOD PACKAGING”** under our supervision from December 2020 to March 2021.

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TO WHOMSOEVER IT MAY CONCERN

We are glad to permit your student **Ms.R.AKSHAYA, M.TECH,** DEPARTMENT OF BIO TECHNOLOGY, SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY to carry out her Project for **Three months (January to March)** in our research laboratory. We assure to make necessary lab facilities for your student and assist in the successful completion of her research work.

Thanking you.

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DECLARATION

I **AKSHAYA R (39810002)** hereby declare that the Project Report entitled **“PRODUCTION OF PECTIN FROM TOMATO(*SOLANUM LYCOPERSICUM*) AND MOSAMBI(*CITRUS LIMETTA*) AND DEVELOPMENT OF PECTIN BASED BIOFILMS FOR FOOD PACKAGING”** done by me under the guidance of **Dr. PRABAVATHY** and **MS. BRILLIANCE VINCENT** at **ARMATS BIOTEK , MADUVANKARAI GUINDY** is submitted in partial fulfilment of the requirements for the award of Master of Technology degree in Biotechnology.

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ABSTRACT

Natural products play a major role in providing solutions for large biotechnological applications. Concept of waste to wealth is a hot topic with research ongoing globally to reduce carbon footprint. In an effort to follow up this present study focused on Tomato and Mosambi fruit peel waste as a sustainable source for pectin. Pectin extraction was performed using acid method, was optimized by extraction using citric acid, an organic acid. After the extraction process, the residue is dried overnight and this was the dried Tomato and Mosambi peel rind pectin. This peel waste has been investigated for biofilm production, using gelling ability of pectin and strength of cellulose. A casting method was used to form a film from the previously extracted and dried pectin. Further the extracted pectin powder is characterized by FT-IR and UV analysis. The extracted pectin will be casted as biofilm and checked for their morphology (SEM), TGA, Tensile thickness, Soil degradability, Water solubility and Swelling study. The screening was carried out using antimicrobial activity done for biofilms by agar well diffusion method against bacteria. The pectin-based biofilm strongly inhibited the growth of bacterial strains. The purpose of the present work was to develop a sustainable technology for the production of pectin from waste by product and development of biodegradable polymers in food packaging to protect environment. The results will suggest that biodegradable film will replace the plastics made up of petrochemicals. This study tends to indicate that biodegradable film could be potentially explored for a sustainable environment.

Keywords: Tomato and Mosambi peels, Pectin, Cellulose, Biodegradable film

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LIST OF ABBREVIATIONS

°C	- Celsius
µg	- Microgram
µL	- Microlitre
cm	- Centimetre
mm	- Millimetre
FTIR	- Fourier Transform Infrared Spectroscopy
g	- Gram
hr	- Hour
mg	- Milligram
min	- Minutes
mL	- Millilitre
PVC	- Poly Vinyl Chloride
UV	- Ultra Violet
v/v	- Volume/Volume
w/v	- Weight/Volume
w/w	- Weight/Weight
T(S)	- Tensile strength
GLY	- Glycerol
PEC	- Pectin
HD	- High Density
LD	- Low Density

RSM	- Response Surface Methodology
nm	- Nanometer
n	- Newton
MPa	- Megapascal
SEM	- Scanning Electron Microscope

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1. INTRODUCTION

Packaging is the important process for the effectiveness of food preservation. Plastics production has been increasing fastly in the past 100 years, and the materials used for packaging not only give physical protection for the food but also provide some of the physiochemical conditions to the packed product and also extending its shelf life. Packaging materials that has been used for food products are glass, paperboard, paper, plastics and some of the metals. Most the food packaging materials are produced by using petrochemicals and they are not biodegradable. As per an estimate about 450 billion plastic-based packaging materials are derived and distributed each year but in that only 3% of them are recycled. Food packaging alone estimated about 66% of total packaging waste by volume in the world (Marsh and Bugusu 2007). Most of the plastics are prepared by using polyethylene and it usually take about 1000years for degradation in landfills (Manuel, J., B. Amarjit and R.T. Michael,2014). Synthetic plastics are tolerant to natural degradation so their disposition is hiring an international campaign for developing biodegradable polymers (Zheng Y, and K. Ernest. Yanful,2005). In development of biodegradable polymers, industry is finding new applications for this.

Plastic packaging for food and the applications is non-biodegradable, and it also uses valuable and some of the non-renewable petroleum products. Usually biodegradable polymers are obtained from marine food processing industry, agricultural feedstocks and waste material generated from food industry. Now, currently scientists are putting their efforts in finding a new alternative to petroleum and the research has been improved in development of biodegradable food packaging systems thereby reducing the adverse effect on environment. The naturally occurring biodegradable materials has been utilised for the production of biopolymers that gained popularity over recent years because of their low cost. Biocompatibility, non-toxicity, biodegradability, and their availability.

Tomato (*Solanum lycopersicum*) is widely consumed crop in the world as raw material and also in processed forms. Products which remain after pressing of tomato majorly includes 27% peel, 33% seed and 40% part of pomace. Tomato also contains pectin, lycopene, flavonoids, rutin etc... The Mosambi (*Citrus Limetta*) is a citrus

waste globally abundant and environmentally challenging waste, for example, mosambi juice production gives about 50–60% residue of the original mass of the mosambi. This high quantity of waste is high in organic matter content (approx. 95% of total solids) and water (approx. 80–90%) and has a low pH (3-4) and this handling could cause severe damage to the environment. Mosambi waste also contains pectin, soluble sugars, hemicelluloses, cellulose, starch, protein, lignin, ash and fat which have been shown to be beneficial for many yet imperfect disposal and recovery applications. These compounds could be interesting for bioplastics applications.

Mosambi waste and Tomato waste has already been applied as a reinforcement in petrochemical or biobased matrices. In these, the authors reported increased mechanical properties of the products which is compared to the neat polymer. However, pectin, the major component of Mosambi peel and Tomato peel seems to have no significant effect in the above-mentioned composites. Nevertheless, pectin-based composites have been prepared with different reinforcing substances. Cellulose reinforced pectin composites have also been developed, for example, for food packaging applications (A. Soroudi *et al*, 2013).

Pectin is a complex mixture of polysaccharides that contain methylated ester of poly (galacturonic acid), it consists of chains of 300–1000 galacturonic acid units. Pectin is soluble in pure water and it is a naturally occurring polymer and this gets more applications in food industry, pharmaceutical industry and biotechnology industry. It also contains non-sugar substituents, essentially methanol, acetic acid, phenolic acids and occasionally amide groups. Pectin has been used successfully for many years in food and beverage industry as a thickening agent, gelling agent and a colloidal stabilizer.

Pectin is located in the cell walls and middle lamellae of plants. The highest concentration of the pectin is found in middle lamella of cell wall, the slow decrease one passes through the primary cell wall towards the plasma membrane. Though pectin occurs commonly in most of the plant tissues, but the number of sources that are used for the commercial manufacture of pectins is very limited. Pectin normally

has the ability to form gel it depends on their molecular size and degree of esterification.

If the peels are treated as waste materials, it creates an environmental problem, like water pollution. This issue could be turned into an asset, if the potentially marketable by-products such as pectin the pectin can be extracted from the peels.

Pectin has been referred as biodegradable polymer with film forming abilities. They are also successfully used to develop coatings and also edible films with all the possible applications in bioactive packaging. Generally, films made of pectin depict poor moisture barrier, thermal stability and weak mechanical attributes hence it requires some effective strategies to overcome these drawbacks (Sharma, B.R, Naresh L, 2006).

Here, another polymer used for biodegradable film is cellulose. Cellulose is a natural water permeable agro-based polymer which comprised of hydroxyl functional groups that enhance the hydrogen bonding. Cellulose are obtained from cotton, sisal, cornucub, banana rachis, rice husk, soy hulls, cassava bagasse and sugarcane bagasse (Kumar,2014). Various functions for cellulosic waste have increased rapidly. The other major components of plants include hemicellulose with the Ester-type bond linkage they both are very sensitive and insensitive to alkali treatments. Where the lignin and hemicellulose are reported to be amorphous and are known to be crystalline.

Cellulose have been used to prepare composite with higher mechanical strength (Lu and Hsieh,2010). Many methods have been used to prepare cellulose which include ultrasonic technique, acid hydrolysis, and enzymatic hydrolysis (Mashego,2016) but the acid hydrolysis method has been mostly used since this method is simple and fast to generate cellulose. There are several agro-based industries which produce huge amounts of plant biomass (Otieno,2015).

Here bagasse has been causing tremendous environmental pollution in the areas located with sugar industries. Moreover, these wastes cost the industries on storage space and disposal costs. And this results in causing environmental distress, instigating damage to land and its surroundings (Masayi and Netondo,2012). Due to this the extraction of cellulose from bagasse for the production of cellulose was found.

The biologically derived polymer-based packaging methods can resolve the waste disposal to certain level. These polymers comprise naturally occurring cellulose, proteins, starches. The processability of the biopolymers are the other constraints which bound their use as compared to petroleum-based polymers. Addition of other biopolymers like poly-lactic acid (PLA), poly-hydroxybutyrate (PHB), results in the formation of composite film which can potentially serve as an alternative to oil-based polymer.

The bio-based polymers are used for packaging of fresh produce (Bastarrachea L, Dhawan S, Sablani SS, 2011). Cherry tomato which is basically a fruit but it is also known as vegetables which is consumed in the entire world. It is a fruit with short shelf life for 2-3 weeks depends on their storage conditions. It should be stored at 10°C. Storing higher temperature will lead to high respiration rates which cause physiological loss in weight and quality. Usually cherry tomatoes are packed in foam trays wrapped with packaging films like poly-vinyl chloride (P. Srivastava *et al*, 2011). The cost of bio-based polymers is higher than the petroleum-based polymers. Naturally occurring biopolymer-based packaging material have a variety of future for various applications in the food industry.

This study was proposed and carried out on the development of pectin-based biofilms for packaging of cherry tomatoes by following these objectives:

1. To optimize the parameters for extraction of pectin from Tomato and Musambi peel.
2. Developing a bio-polymer using pectin.
3. To assess the mechanical feasibility of the developed polymer.

2. REVIEW OF LITERATURE

In a global industry this food packaging industry created environmental issues like greenhouse gases and soil pollution. As a result, a greater number of non-biodegradable plastics wastes are getting accumulated in many water bodies and soil, this removal leads to consuming of time, energy and money. The petroleum-based packaging materials leads to death for sea animals and birds (Garlotta, 2001). It started becoming a threat to animals, birds and even humans also. Even there is a possibility of recycling the plastics but there is a limitation for their constituents.

2.1. STRUCTURE, TYPES AND USE OF PECTIN

Pectin is a biodegradable polymer which has high water solubility. It is the main polysaccharide structure for higher plant cells. There are numerous applications in beverages and food industries such as thickener, colloidal stabilizing agent, texturizer, gelling agent and emulsifier (Petkowicz CLO *et al*, 2017). Hence, pectin has better prospect and potential as high sustainable biopolymers. There is a novel process for the production of nanocomposite including solid-state blending at normal room temperature just like a ball milling process.

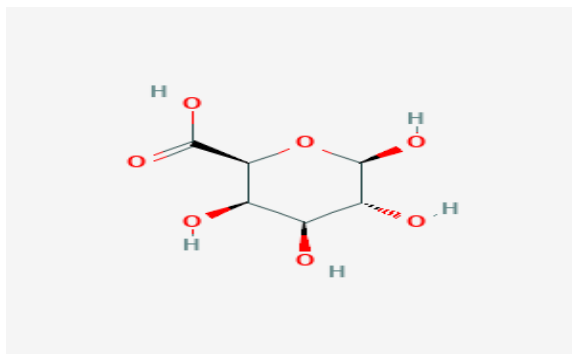


Fig. 2.1 Structure of pectin

More than 50% of the acid units are esterified in pectin normally, and they are categorized as high-methoxyl pectin also known as (HM) pectin. Extended treatment with acid change in the extraction procedure results as low-methoxyl pectin (LM) pectin, which will have less than 50% methyl ester groups.

Naturally pectin is found in plants, but it is more concentrated in citrus fruits like oranges, lemons, musambi and also in apples. The pectin extracted from citrus fruit is called as citrus pectin. On industry base pectins are extracted from citrus peels or

apple pomace, they are the by-products of juice manufacturing industries (Chan SY *et al*, 2017). Those pectins were then employed in production of jams, jellies etc. Thickening agent for sauces, flavoured syrups, ketchups and in milk desserts pectins are used. Likewise, pectin has various applications not only in food industry also in pharmaceutical formulations like pastes, cosmetics etc. For developing low or no sugar jellies LM pectin is frequently used. HM pectin is used to produce fruit preserves. Because it needs presence of sugar and specific degree of acidity. Two types of HM pectin are there, they are rapid set pectin and slow set pectin. Rapid set HM pectin is usually used for jellies that have constituents in the gel structure like hot pepper jelly, whereas slow set pectin is used for making clear jellies like apricot or grape (Brejnholt SM, 2009).

The most useful method for extracting the pectin is direct boiling and microwave heating (Maran JP *et al*, 2014). Two hours required for good yield of pectin but longer heating process cause thermal degradation of pectin. It depends on the types of solvents used and use of extra chelating agents such as EDTA which brings pectin from cell wall (Methacanon P *et al*, 2014).

Pectin is industrially extracted by hot acidified water. Extraction condition of pH for pectin is (1.5 to 3.0), temperature (60 to 100° C) and time (0.5 to 6 hr) this will be varied to gain a material with gelling capability and degree of methylation.

Pectin was extracted from mosambi peel powder at three different temperature, time and Ph by utilizing different acids they are citric and nitric acid. The yield of pectin ranged from 21.4% to 76% when extracted using citric acid at 80°C, 60 min, and the pH is 1.5. But the yield of pectin using nitric acid is 312.5 and 823.33. The percentage of methoxyl and anhydrouronic acid content has higher yield of pectin using citric acid than nitric acid. When compared to the control sample, there are some improvements in the mechanical properties of the films were identified, but these films become fragile after some time. So, in order to increase the film flexibility, glycerol was added as plasticizer. The effects of these films were equated with poly vinyl chloride (PVC) films. The biodegradable film and PVC films showed some changes in weight loss by

the fruits during storage. As a result, the pectin-cellulose films could be regarded as feasible packaging substitute for PVC films ((V. S. Aigbodion *et al*, 2013).

2.2. PECTIN-CELLULOSE BASED BIOFILMS

Thermal and mechanical properties of pectin-cellulose based films and their consequence on the storability of strawberries. In this study the pectin-cellulose based biofilms were used as packaging to stretch the storage life of strawberries. Pectin based films after incorporation with cellulose (1, 2, 4 and 8% w/v) were developed by casting method on the teflon sheets and their mechanical and thermal properties pectin were studied (Lu and Hsieh, 2010).

However, thermogravimetric analysis showed the decomposition and glass transition temperature increased. The barrier properties like moisture absorption and water solubility test gave best results.

2.3. ATTRIBUTES OF BIODEGRADABLE FILMS

Biopolymers are appealing as pectin for making bioactive food packaging. They are complex polysaccharide present in the primary plant cell wall in the form of soluble fibre. They are usually recognised for their splendid film making capability, so they are developed as coatings or edible films with possible applications in bioactive packaging. Pectin films mostly have inadequate moisture barrier, some feeble mechanical properties and low thermal stability, so it requires a substitute to replace these drawbacks. Some research has been utilized to modify the physical properties of the films (Kulkarni, S.G *et al*, 2010).

In order, they developed a biodegradable cellulose/starch film in various starch-based materials using a polymer melt processing technique. The mechanical properties and the structural attributes of the film resulted in an increase of mechanical properties, such as tensile strength and elongation (Reddy, N., Yang, Y., 2005).

Another chitosan based biopolymer made by N-deacetylation of chitin, discovered from shellfish waste, which has been used to make edible films or some coatings to

increase the shelf life of foodstuffs. The effect of different concentrations of the fibre material (cellulose fibre) and plasticizer (glycerol) were assessed in order to optimize the properties of tensile, temperature and water solubility of the films (Rosa, M.F *et al*, 2010).

Applications of biodegradable packaging for fresh-cut fruits and vegetables and the effects on the product quality. This study gave better results for combined usage of coatings and biodegradable films and further these results can be improved in order to enrich the films. Though, the actual usage of the materials is limited because of their high cost of the raw material and the production is limited (Ahmed, M *et al*, 2005).

2.4. SHELF LIFE EXTENSION OF FRESH PRODUCE THROUGH PACKAGING IN PETROLEUM-BASED AND BIO-BASED POLYMERS

A material is significantly well known for contributing the shelf life of fresh produce packed with it. The effect of packaging films (PP, LDPE, HDPE, PVC and Cling wrap) in order to gases and water vapour with and without the application of vacuum packaging on some of the physical characteristics (weight loss, colour, texture) and the sensory aspects of the shredded cabbage at different storage conditions were studied. Among all packaging films PP could extend the shelf life of the processed cabbage almost for up to 3 weeks with minimum colour change, reduction in weight loss of the sample and deterioration in sensory properties and marginally some low changes in other tested parameters. PVC cling wrap showed least effective packaging film. Vacuum packaging showed some significant difference in almost all parameters tested for all different types of packaging films used (Galdi MR *et al*, 2008).

PLW showed decrease in thickness of film but it increased with level of perforations for carrots and shelled peas. It was a minimum non-perforated shrink film. When compared to HD films and LD films for treatment PLW was lower in perforations and under room temperature conditions (Liu, Y *et al*, 2017).

The LDPE films are highly suitable for preventing moisture content on the spinach within the packages has no water accumulation in the LDPE films, whereas in PP

packages water accumulation didn't take place only under treatment A as compared to B and treatment C.

There is a study used a combination of micro perforated films and a modified atmosphere packaging to increase the shelf life of tuber truffles. They were stored at 4, 10 and 23°C and their respiration rates were noted. The quality of the truffle was reduced and showed weight loss, microbial content and the fruits retained firmness. The shelf life was prolonged to 21 days with this intervention (Odriozola-Serrano, I *et al*, 2008).

Experiment carried out on storage of cherry tomatoes grown under different environmental conditions and packaging materials. In LDPE, cassava starch-based biofilms, carnauba wax the fruits were stored and in PVC films for period of 30 days. Physiochemical tests were conducted regularly and in that the cherry tomatoes had a shelf life of 25 days when stored in LDPE, PVC and carnauba wax. But the samples stored in the cassava starch-based films were degraded within 15 days (Cuq B *et al*, 1995).

2.5. PROCESS OPTIMIZATION USING RESPONSE SURFACE METHODOLOGY

Response surface methodology (RSM) is a statistical tool which explores the relationship between various variables and one or more response variables. Box and Wilson in 1951 introduced this method. The aim of the RSM is to sequence of planned experiments to achieve an optimum response. Peculiar situations where numerous input variables affect the performance measure or quality characteristic is known as response this is the main application of RSM. The input variables are designated as control (Sudhakar, D.V. and Maini. S.B, 1995).

Most of the RSM process are from chemical or engineering process, industrial research and also in biological experiments with focus on optimizing process.

RSM for quantitative assessment of solids and moisture transfer during osmotic dehydration of sugar beet in an aqueous solution of sodium chloride (0-8%w/w) and sucrose of (30-70%w/w) at different temperature conditions (30-50°C) in different times (30-240 mins).

Research on rheological evaluations and design of pectin based functional gels. Low methoxyl pectin (LMP), High methoxyl pectin (HMP), sorbitol, gardenia, apple flavour, quercetin and calcium chloride were used as general ingredients for formulation. Further it is assessed for their effect on experimental responses. Finally, a gelled product with the application of best levels of ingredients (Chan SY and Choo WS, 2013).

On the basis of the above-mentioned systematic investigation, current research was planned and executed to develop novel, biodegradable and antimicrobial packaging material from waste tomato and musambi peels for packaging of fresh produce.

3. OBJECTIVES OF THE RESEARCH WORK

- Production of Pectin from Tomato and Musambi peel waste.
- Extraction of pectin through acid method.
- Characterization of pectin powder through FT-IR and UV.
- Extraction of cellulose from sugarcane bagasse.
- Further, extracted pectin is investigated for biofilm production.
- Morphology identification for Thin film by SEM analysis.
- Determination of antimicrobial activity of pectin-cellulose based biofilm.
- Mechanical properties for films were analyzed.
- Biodegradability test, Swelling test and Solubility test for thin film.
- Determination of Tensile strength and Thermogravimetric analysis (TGA) for thin film.
- Validation of developed pectin-based biofilm.
- To produce biodegradable eco-friendly product.

4. MATERIALS AND METHODS

All the glassware used in the study was immersed in cleaning solution prepared following the method described by (Mahadevan and Sridhar, 1996) for a few hours. Then the glassware was washed thoroughly with tap water, followed by detergent solution and finally rinsed with distilled water. The cleaned glass wares were dried in hot air oven and stored.

4.1. STERILIZATION

All the glass wares, media and solutions used in the present study were autoclaved at 121°C with 15 min psi for 20 min.

4.2. CHEMICALS

Analytical grade chemicals supplied by Hi Media, SD Fine Chemicals, Merck, Qualigens and Sigma Chemicals were used. General laboratory techniques recommended by (A. Ghaffari *et al*, 2007), (Purvis *et al.*, 1966), (Tuite, 1969) and (A. Soroudi *et al*, 2013) were followed for the Extraction of pectin, preparation of media, inoculation, maintenance of cultures and production of polymers.

4.3. OPTIMIZATION PROCESS PARAMETERS FOR EXTRACTION OF PECTIN

4.3.1. *Sample collection and preparation*

The sampling scheme was designed with the intention of production of pectin from Tomato sample and Musambi sample peel waste, it's a by-product of luice manufacturing units, were procured from the local market of Chennai, Tamilnadu for experimentation. The peels were washed in order to remove the dirt, dust and traces of possible pesticide spray. Afterwards these samples were cut into smaller pieces (for uniform drying) and they were dried using the hotair oven at 60° C, until their weight became constant. Finally, the dried peels were grinded and made into fine powder and the powder were tightly sealed in polythene bags under humid conditions and kept at room temperature till further use.

4.3.2. Extraction of pectin from tomato and musambi peel with citric acid(C₆H₈O₇)

Pectin was extracted as per the method given by (A. Ghaffari *et al*, 2007). Ten grams of dried powder sample of tomato and musambi peel was treated with 85% ethanol at 70°C for 20 min in a hot water bath and then it was filtered by using muslin cloth to get rid of solid impurities present in the sample. These steps were carried out another three times followed by the extraction of the residue by adding citric acid and the Ph was adjusted to (1.5-2.5) using Ph meter (Elico Model No L-614, India). After extraction process, the residue was treated with five times the volume of 95% ethanol for precipitation of pectin and centrifuged at 2500 rpm for 15 min to separate the pectin from supernatant. Discard the supernatant and the pellet was washed with 70% ethanol followed by 95% ethanol for its purification. Lastly, the washed pellet was dried overnight at room temperature to remove the moisture content and this was the dried tomato and musambi pectin.

4.3.3. Physiochemical properties of extracted pectin

4.3.3.1. Pectin yield

Pectin yield was calculated as the amount of pectin extracted from the quantity of tomato and musambi peel. It was determined by the equation and expressed in percentage.

Pectin yield=Weight of the pectin extracted(g) / Weight of the tomato and musambi peel(g) ×100

4.3.3.2. Degree of esterification (DE)

Degree of esterification was conducted by measuring the acid base titration (Levigne S *et al*, 2002). About 1ml of propanol (65%) was added to 50 ml of distilled water and 0.25g of pectin was dissolved it by placing the solution in an electronic shaker. Afterwards, titration of the solution was conducted with 0.1 N sodium hydroxide was named as 'a'. That 30 ml of NaOH was added and the solution was left undisturbed for 30 min. Again, 10ml of sulphuric acid was added to it with continuous stirring. Then followed by titration of mixture with 0.1N NaOH and volume (ml) of NaOH expended was designated as 'b'. Then, the degree of esterification was computed for different pH, time and temperature combinations by using equation.

$$DE (\%) = 100b(a-b)$$

4.3.4. Characterization of extracted pectin

4.3.4.1. Fourier Transform Infrared Spectroscopy:

The dried pectin (2 mg) was given for FTIR spectra of citric acid extracted pectin (Sigma-Aldrich, St. Louis, USA) in the range of 400-4000 cm^{-1} , resolution 4 cm^{-1} (Ravishankar *et al.*, 1995) Perkin Elmer.

4.3.4.2. UV-Visible Spectroscopy:

UV-Visible spectrum was generated by scanning the pectin powder (10 mg/mL) dissolved distilled water at wavelength ranging from 200 nm to 800 nm. The spectrum generated was recorded to confirm the identity of the presence of compound (Yuan *et al.*, 2007).

4.4. DEVELOPMENT OF PECTIN AND CELLULOSE BASED BIOFILMS

The pectin required for preparation of bio-polymer was extracted by previously explained methods. However, pectin-based films usually have poor moisture barrier, less mechanical properties and low thermal stability. So, for that, cellulose from sugarcane baggase were selected in order to develop the pectin-based biofilms and to overcome these drawbacks. The reason for selecting is also inexpensive, it also combines the advantages of being strong polymer.

4.4.1. Extraction of cellulose from sugarcane baggase

Sugarcane baggase sample was collected from local shop, Chennai, Tamilnadu. It was primarily washed with deionized water to remove the unwanted particles. Baggase was then sundried before grinding into small pieces using a blender and sieved under double mesh sieves. Then it was oven dried at 105°C for about 5 hr and then stored at room temperature and sealed in air tight polythene bags. The isolation of cellulose was done by previously modified methods of (Johar *et al.*, 2012).

70g of baggase powder was mixed with 700 ml 6% (w/v) of HNO_3 for 2 hr in a hot water bath placed at 80°C then washed with deionized water until neutral. The solution was then treated with 500 ml of 1% NaOH at constant stirring for 2 hr at

80° C in water bath. Further it was washed with deionized water and it is bleached with 250 ml of sodium hypochlorite of 0.735% (w/v). Acetic acid was further treated to the lignocellulose extract and stirred for 2 hr at 80° C. The residue was then washed with deionized water and bring to pH and let it to dry for three days at room temperature. And the dried cellulose was stored in air tight polythene bags.

4.4.2. Procedure for development of pectin-based biofilms

Pectin based biofilms were prepared by casting process by adding fixed concentration of pectin (1% w/v) and glycerol as a plasticizer (2.5% v/v) in a determined volume of distilled water which was used as a solvent (Zink, J *et al*, 2016). The levels of pectin, glycerol and cellulose were determined as per the data and trials. The control for the film was prepared by dissolving 2 g of pectin, 5 g of cellulose was added in beaker with 200 ml of distilled water and stirred then add 5 ml glycerol as a plasticizer. Solution was heated at 90° C for 15 min in water bath and then continuous stirring was done by magnetic stirrer for 30 min. It was followed by casting the solution in a petridish. These films were then dried in an incubator at room temperature of 35° C for 30 hr. Afterwards this film was peeled off from the plates manually. Then the films were conditioned at 50±5% relative humidity for 48 hr prior to further analysis. Further the film was ready for testing. The morphology of the films was analysed by scanning electron microscope (SEM).

4.4.3. Characterization of the developed film

4.4.3.1. Thickness and area of film

The thickness of the developed pectin-based bio film was measured by using a micrometer (Model: Mitutoya Corp; Japan) which has a sensitive of ± 0.001 mm. In different locations on each film the thickness of the film was measured and an average value was calculated. The length and breadth of the film were measured after its drying and conditioning using a scale. Then the area of the film was calculated as the product of length and breadth.

4.4.3.2. Antimicrobial analysis of films

The antimicrobial action of biofilms was investigated using four bacterial strains as model bacteria. For this purpose, approximately 10^8 colony forming units (CFU) of bacteria were cultured on a nutrient agar plate, which was placed overlaid

on to petridishes. The films were cut into circular shape from the test films and placed on the bacterial plate. The plates were incubated for 24 hr at 37°C in the aerobic conditions. Laterally, the petri dishes were examined for zones of inhibition around the film disc, and the size of the zone diameter was measured at two cross sectional points and also the average was taken as the inhibition zone of the films.

4.4.3.3. Morphological characteristics of the developed biofilm

The scanning electron microscopy (SEM) was used to observe the morphological characteristics of developed biofilms. The sample to be tested was placed on aluminium stubs with double-sided adhesive tape and covered with a gold layer in an ion sputter (E-1010, Hitachi, India). The samples were observed in scanning electron microscope (SEM) at an acceleration voltage of about 15Kv. The secondary detector was also used for this purpose.

4.4.3.4. Biodegradability of films

The films were to be tested initially weighed and put inside a mud pot already filled with soil having a moisture content of 25%. Afterwards this mud pot was placed in which the humidity was adjusted to 25°C. The loss in the weight of the films was measured after every 3 days (Meulen I *et al*, 2008).

4.4.3.5. Swelling study of films

Swelling study is usually conducted to check whether developed material retains its original properties when it was formed during the preparations. A piece of weighed samples were taken in the test tube to check the protuberance and the other morphological changes. Further, it was carried out on the medium containing various solvents such as chloroform, water, methanol and ethanol medium. The delibrated samples were kept for about 2 hrs in the medium and the results were recorded accordingly.

4.4.3.6. Solubility test of films

The films were prepared and solubility studies were conducted to check its solubility stage of the films. Samples were soaked in the eppendorf containing different solvents.

4.4.3.7. Mechanical properties

These properties were expressed as tensile strength (Ts). The tensile strength of the developed films was assessed by using double column texture analyzer (Stable Micro System, Model: TA.TXT. Plus). The tensile strength was employed to ascertain the strength of the developed film. The strain value was set to 50 % and the grip separation was set at 50 mm with a cross head speed of 5 ms⁻¹ (Nadras O *et al*, 2011). Ts was calculated by using the equation and expressed in newtons (N).

$$Ts = F_{\max} / A$$

Where, F_{max} is the maximum load needed for tearing the sample and A is the cross-sectional area (m²) of the film used (thickness×width) which is equal to 50×50mm (Tongnuanchan, P *et al*, 2016).

4.4.3.8. Thermal attributes

Thermogravimetric analysis (TGA), (Q500 TA instruments, Waters LLC, USA), was used to perform the chemical film properties, such as decomposition. Approximately about 6-8mg of each sample was heated in room temperature at 250 °C at rate of 10 °C/min. Further, the analysis was performed under nitrogen atmosphere, and finally an average is reported.

4.4.4. Carrot experiments

4.4.4.1. Carrot wrapping

The selected carrots were cut into uniform size, color and without any physical damages and fungal infections. They were divided into two groups. One for control and the other for sample as following: 1% PEC +2.5% GLY +5 g CELLULOSE film (W+PEC+GLY+CELLULOSE) and the other for control. These samples were stored at 4°C the quality of both the samples were evaluated during storage at 0,2,4,6 and 8 days (Vandendriessche, T *et al*, 2013).

4.4.4.2. Weight loss

The weight loss of the samples was calculated in triplicate at 0,2,4,6 and 8 days of storage using the equation

$$\text{Weight loss (\%)} = (W_o - W_1) / W_o \times 100$$

Where, W_o and W₁ represents the initial and final weight of the carrot.

5. RESULT AND DISCUSSION

5.1. COLLECTION OF SAMPLES

The main objective of the present study was to extract the pectin from waste tomato and musambi peels and assessing its potential applications. The samples were collected from Local market Chennai, Tamilnadu (Fig 5.1)



Fig. 5.1 *Solanum lycopersicum* and *Citrus limetta*

5.2. EXTRACTION OF PECTIN WITH CITRIC ACID

The experiments were designed and performed. The response parameters were pectin yield and degree of esterification. Depending upon the different combinations of variables, the parameters were observed. The pectin yield varied from 14.8% to 17.2% with a mean of 15.408% and standard deviation of ± 0.717 ; and the degree of esterification from 75 to 87.02%.

5.2.1. *Pectin yield*

The yield of pectin extracted with citric acid increased with increase in pH, till the pH value of 2.5 was attained and pectin yield was observed. The effect of extraction temperature was non-significant upto 70° C and effect on pectin yield increased. The extraction time had significant effect on the pectin yield followed by the pH, extraction time and temperature as shown in Table. 5.2 (Chan SY *et al*, 2017).

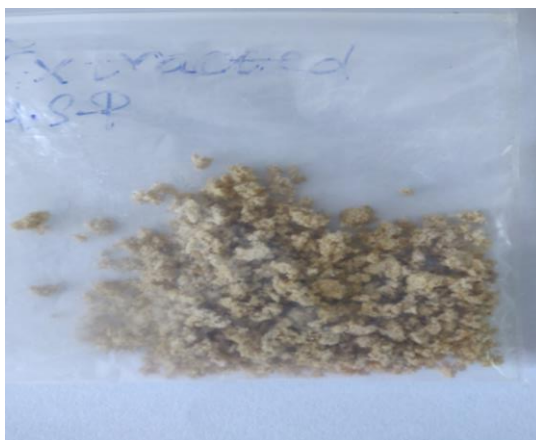


Fig. 5.2. Extracted pectin

5.2.2. Degree of esterification (DE)

The degree of esterification of pectin extracted with citric acid increased with increase in pH till the limiting pH value of 2.25 was attained and beyond in which the DE is observed. The temperature during extraction is a non-significant effect on DE of the extracted pectin. And it also increased slightly in temperature. The maximum DE (87.04%) of the extracted pectin was found when the time, temperature and pH values had 60 min, 80°C respectively. As the DE was more than 50% so the extracted pectin is confirmed as high methoxyl pectin (A. S. Oliveira *et al*, 2016). Indicated in Table. 5.2.

Table 5.1. Effect of extraction of pectin with citric acid

Time(min)	Temperature (°C)	pH	Pectin yield (%)	Degree of esterification (%)
30.00	80.00	2.00	15.10	83.00
30.00	70.00	2.50	14.60	85.24
60.00	80.00	2.25	16.10	87.04
45.00	60.00	1.50	14.80	76.69

5.3. CHARACTERIZATION OF EXTRACTED PECTIN

5.3.1. Fourier Transform Infrared Spectroscopy

Validation of the pectin extracted from tomato and musambi was done using FTIR. The fingerprint regions of pectin are from the range of 950-1200 cm^{-1} which indicate the presence of polysacchrides vibration and also the bands ranging from 12000-1800 cm^{-1} indicate the presence of carboxylic groups in pectin. And the absorption bands observed from 1011-1220 cm^{-1} indicates the presence of glycoside ring bond stretching. This region is same for all the pectin, which is insignificant to method of extraction (Jiang *et al*, 2012).

Table 5.2 Fourier Transform Infrared Spectroscopy

PEAK	FUNCTIONAL GROUP	
1715	C=C	Ester
1025	C=O	Carbonyl
3334	C-H	Alkyne
2917	C-H	Methylene
1624	C=C	Alkene

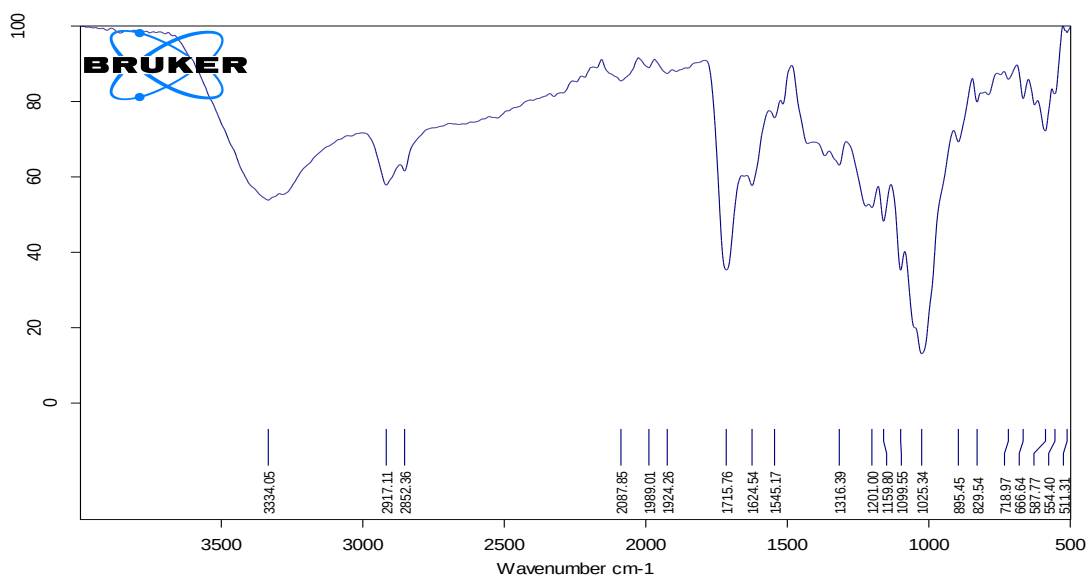


Fig. 5.3. FTIR Spectrum of extracted pectin

5.3.2. UV-Visible Spectroscopy:

The optical properties of the bioactive compounds were monitored by UV–visible spectrophotometer (Shimadzu UV–Vis 3600). The compound 10 mg/mL is dissolved in distilled water at wavelength ranging from 200 nm to 800 nm.

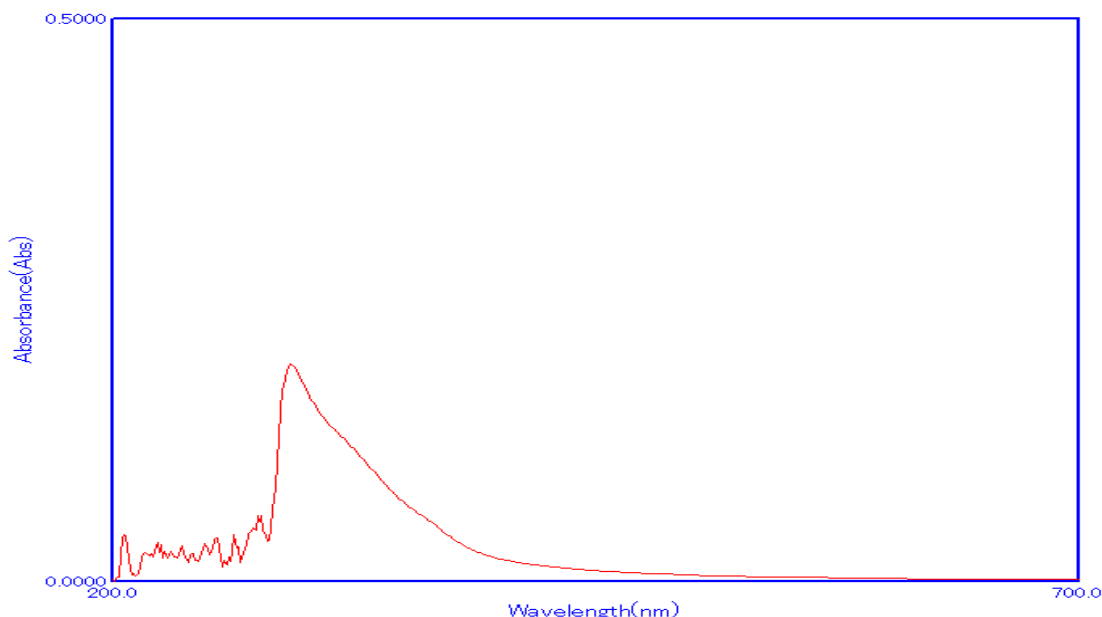


Fig.5.4. UV-Vis Spectrum of extracted pectin powder

5.4. PREPARATION OF PECTIN AND CELLULOSE FILM

The pectin base biofilms were developed and its effect of pH and temperature on various properties of the films was studied and atlast derived from it. The moisture content of the films dried overnight at 105°C was 18.20 ± 0.145 (PECT: GLY: CELLULOSE:1%:2.5%:5%w/v). Visual observation of the films revealed characteristic features as shown in (Fig.5.5). The blended compound showed equal proportions which displayed a smooth surface appearance and was flexible. The analysis also revealed that cellulose was an effective polymer blended in the film (V. B´atori *et al*, 2016).



Fig.5.5. Extracted cellulose



**Fig. 5.6. Visual appearance of the film prepared (Bioactive compound:
PECT GLY: CELLULOSE:1%:2.5%:5%w/v).**

5.5. CHARACTERIZATION OF DEVELOPED FILM

5.5.1. Thickness of the film

The thickness was measured at different spots using screw gauge revealed a smooth surface morphology with 8 mm thickness. This might be increase in cross linking activities at higher pH (Abdorreza, M.N *et al*, 2011).

5.5.2. Tensile strength (TS)

The mean value of the tensile strength of the film was about 2.06 ± 0.1 MPa. Tensile strength increased due to when there is increase in pH and temperature. The presence of pectin not only prevented the self-aggregation during testing but also improved its mechanical strength (Silva M. A, *et al*, 2009).

5.5.3. Biodegradability of the film

There are reduced in the weights of film and these are very much desired properties for the typical bioplastic. The biodegradability of the bioplastic material is indicated as shown in Table 5.3. The biodegradability test for the films is helpful in preparation of the environmentally friendly product as they are derived from natural polymers, and they can be reused in bio compost preparation. This plays an important role in green applications (Voon, H.C *et al*, 2012).

Tab 5.3 Indicated the biodegradability of film

Biofilm	Quantity taken in (g)	Observation after 3 days	6 days	9 days	12 days
Pectin based cellulose film	1 g	0.7871	0.6742	0.4213	0.4215



Fig. 5.7. Degradable film after 12 days

5.5.4. Swelling study of films

There is no much change in the internal of biofilm material when the material is soaked in chloroform and methanol solvents. Further, there is a slight increase in the weight of biofilm when it is soaked in water it has a reliable material than other materials. But the appearance of the film hasn't changed but there is slight increase in the weight of the samples after 2 hrs. Results for swelling test of bioplastic is shown in Table 5.4. There is swelling higher in water as a medium and this is the desirable result because some are prepared with solvents, and it will help in stabilize product synthesis and development.

Tab 5.4 Results for swelling test

Solvent medium	Quantity (ml)	Initial weight of sample(g)	Final weight of sample(g)	Difference in weight (g)	Swelling (%)
Chloroform	5	1	1.01	0.01	1%
Methanol	5	1	1.08	0.08	8%
Ethanol	5	1	1.1	0.1	10%
Water	10	1	1.10	0.1	10%

5.5.5. Solubility test for film

Some of the samples were not completely soluble in the different mediums used and these are the results for preparation of biofilms. This shows the film prepared by pectin-based cellulose is stable. Results for the solubility test is shown in Table 5.5. Solubility is the main properties to check whether the material is sustainable or not. If the film possesses the property of less or zero property that can be considered as good material with stability.

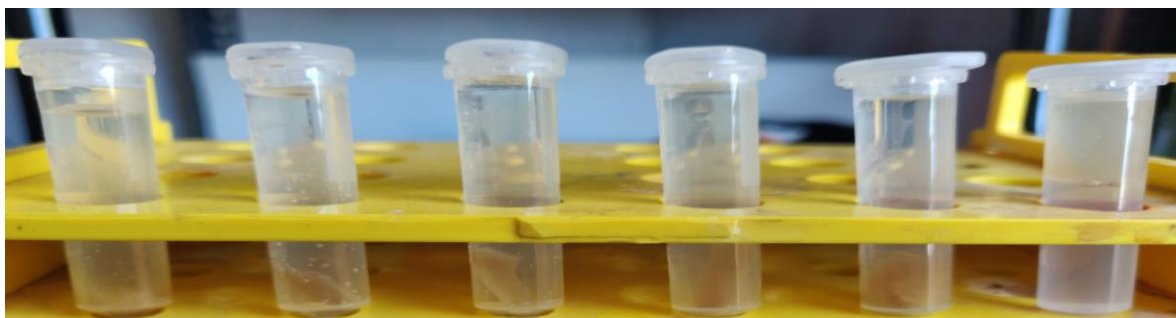


Fig.5.8. Results for solubility test of films

5.5.6. Antibacterial activity of film

The films were assessed for their anti-microbial efficacy against the bacterial strains which was taken as model bacteria by zone inhibition method. The petridish comprising the films demonstrated the inhibition action which was visible by the appearance of circular inhibition zones.

Tab 5.5 Zone of inhibition(mm) of antimicrobial activity of film

Organism	(1)	(2)	(3)	(4)
<i>Enterococcus faecium</i>	7	5	2	0
<i>Escherichia coli</i>	9	7	2	0
<i>Staphylococcus aureus</i>	6	4	3	0
<i>Pedomicrobium ferrugineum</i>	7	5	3	0



Enterococcus faecium



Escherichia coli



Staphylococcus aureus



Pedomicrobium ferrugineum

Fig.5.9. Antimicrobial activity of biofilm from pectin

5.5.7. Morphological characteristics of the developed film

The SEM images in Fig.5.10 reveals the second layer was formed on the surface of the films. In drying films, where there is more than one type of particles, which means that one particle ends on top of another. This led the formation of smooth surface of the biofilms.

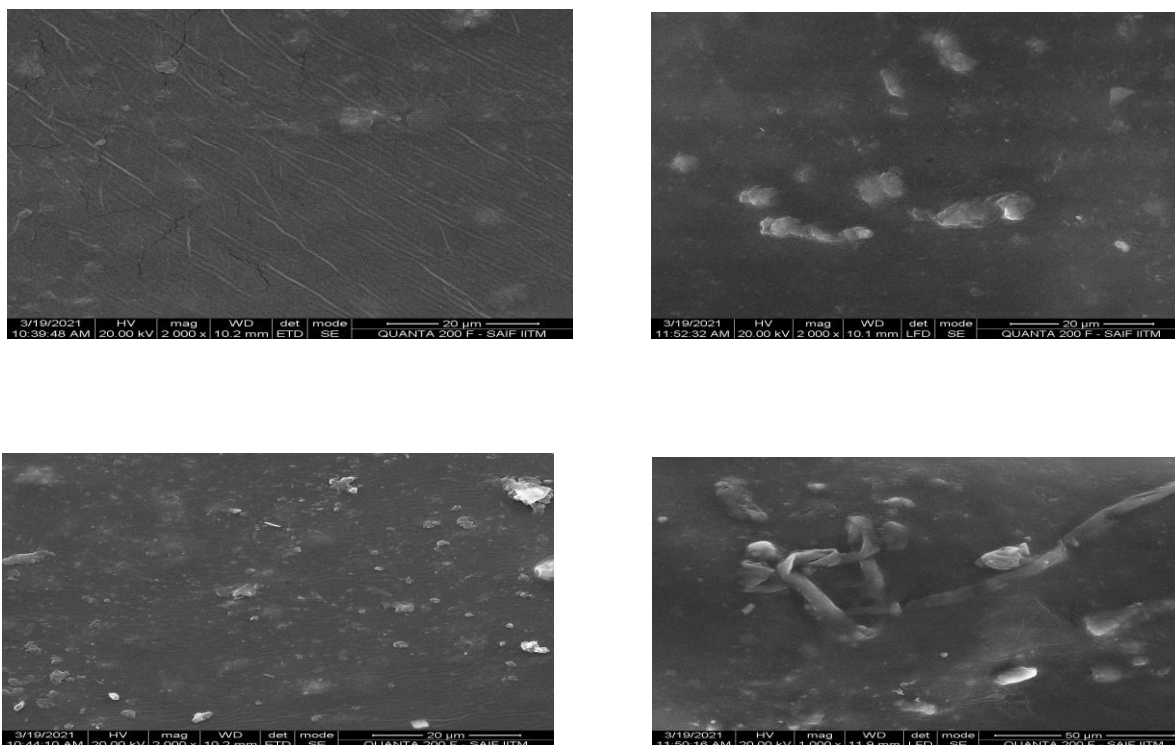


Fig.5.10. SEM of developed pectin-based biofilm

5.5.8. Thermal properties

In Fig.5.11 shows the TGA curves. The weight loss curves of the sample appeared from room temperature to about 110 °C, representing evaporation of water molecules, present mainly as free water. The degradation of the sample started at about 115 °C and having a residue of about 70% approximately at 290 °C. And the weight loss of the film was observed at the range of 100-290 °C.

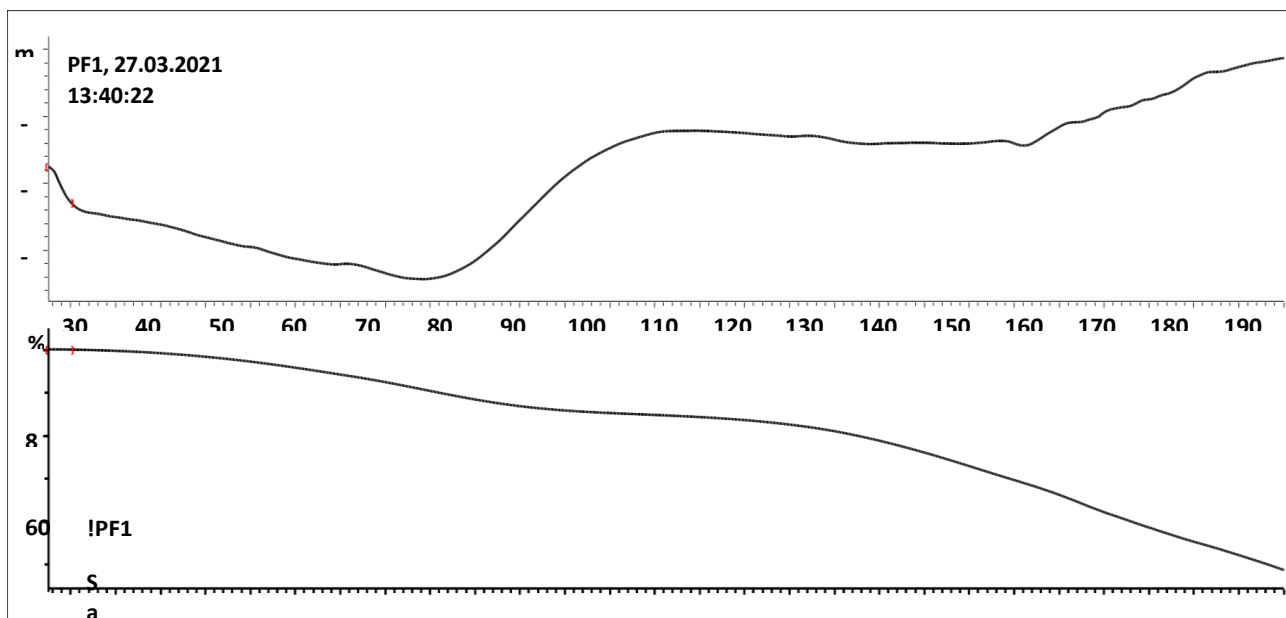


Fig.5.11. Thermogravimetric analysis of pectin-based biofilm

5.6. QUALITY OF WRAPPED CARROT DURING STORAGE

Due to their characteristics (1% PEC +2.5% GLY +5 g CELLULOSE film) and the other for control, this film were used to wrap carrot. The results were compared to the control sample. The performed analysis was finalized to study the quality of carrots from 0 to 8 days (Tyagi, V et al, 2017).



**Film packaging with carrot (25°C)
(PEC+GLY+CELLULOSE)**



Control

Fig.5.12. Validation of developed pectin-based biofilm



Fig.5.13. Validation of film after 8 days

5.6.1. Weight loss of the film

The analyses were performed from 0 to 8 days. Up to two days, no significant differences were found among all the analyzed samples. Weight loss is reduced when it is wrapped with pectin-based film. The content of citric acid was also higher in both PEC-containing films, even after eight days. The weight loss is tabulated below.

Tab 5.6 Weight loss of the wrapped carrot

Food sample	1 st day	8 th day
Carrot	0.7 g	0.4 gm

5.7. DISCUSSION

On industry base pectins are extracted from citrus peels or apple pomace, they are the by-products of juice manufacturing industries (Chan SY *et al*, 2017). For developing low or no sugar jellies LM pectin is frequently used. HM pectin is used to produce fruit preserves. Because it needs presence of sugar and specific degree of acidity. Rapid set HM pectin is usually used for jellies that have constituents in the gel structure like hot pepper jelly, whereas slow set pectin is used for making clear jellies like apricot or grape (Breinholt SM, 2009). On the basis of industrial scale, hydrochloric acid, nitric acid and sulphuric acid are some of the mineral acids commonly used to extract pectin (Petkovic CLO *et al*, 2017).

Previous studies on pectin extraction from watermelon rind using hydrochloric acid and nitric acid as an extractant. Therefore, the objective of this study was to obtain pectin from tomato and musambi peel powder using citric acid and compare to those using hydrochloric acid as an extractant. The yield of pectin from watermelon rind using hydrochloric acid were 10.02% and the yield of pectin from tomato and musambi peel were 15.48%, respectively. Citrus acid, has been shown to increase the yield of pectin extraction from the sources (Methacanon P *et al*, 2014).

The DE (71.5%) of the watermelon rind extracted pectin was determined when the time, temperature and pH values had 4°C for 1.5h, when compared to this the maximum DE (87.04%) of the extracted pectin was found when the time, temperature and pH values had 60 min, 80°C respectively (A. S. Oliveira *et al*, 2016). Validation of the pectin extracted from tomato and musambi was done using FTIR. The bands ranging from 12000-1800 cm⁻¹ indicate the presence of carboxylic groups in pectin. And the absorption bands observed from 1011-1220 cm⁻¹ indicates the presence of glycoside ring bond stretching (Jiang *et al*, 2012). This study was compared with watermelon rind pectin, bands ranging from 11000-1800 cm⁻¹ indicate the presence of carboxylic groups in pectin (Emaga TH *et al*, 2018).

The pectin base biofilms were developed and its effect of pH and temperature on various properties of the films was studied and at last derived from it. The moisture content of the films dried overnight at 105° C was 18.20±0.145 (PECT: GLY: CELLULOSE:1%:2.5%:5%w/v). The thickness was measured at different spots using screw gauge revealed a smooth surface morphology with 8 mm thickness (Abdorreza, M.N *et al*, 2011). Similar results were reported by (Bae *et al*, 2009).

The mean value of the tensile strength of the film was about 2.06±0.1 MPa (Silva M. A, *et al*, 2009). This result was consistent with the findings reported by (Kadam *et al*, 2013). In biodegradability test there are reduced in the weights of film and these are very much desired properties for the typical bioplastic (Voon, H.C *et al*, 2012). When compared to this result, previously a study reported 80% degradation within six months (M. Semsarilar *et al*, 2012).

Solubility is the main properties to check whether the material is sustainable or not. Some of the samples were not completely soluble in the different mediums used and these are the results for preparation of biofilms. This shows the film prepared by pectin-based cellulose is stable. The films were assessed for their anti-microbial efficacy against the bacterial strains which was taken as model bacteria by zone inhibition method. The SEM images in Fig.6.0 reveals the second layer was formed on the surface of the films. The analyses were performed from 0 to 8 days. Weight loss is reduced when it is wrapped with pectin-based film. The content of citric acid was also higher in both PEC-containing films, even after eight days.

Hence the synthesized bioplastic product has got all the substantial properties for commercial viability and use of fruit and vegetable waste, a renewable resource will always be the best raw material for producing sustainable bioproduct. This versatility of the bioproduct plays key role in green applications.

6. SUMMARY AND CONCLUSION

6.1. SUMMARY

The main aim to pack any food material is to contain, sell and market. It serves as a shelflife of packaged food and also protects it from physical, chemical and biological factors which might affect the packed commodity. Most of the polymers used in packaging of food products are derived from petrochemicals and are non-biodegradable which affects the environment and human health. So, there is a need to search for possible sustainable alternatives. The best solution for this problem will be able to utilize the waste materials/by-products and convert them into resources. In order to that biodegradable sustainable food packaging materials is developed. Such a material is tomato and musambi peel, which is a by-product.

It is well known that citrus peel and in tomato peel there is a good source of bioactive compounds like pectin which is naturally occurring biodegradable polymer. The production cost can be low by simple inexpensive method to develop low cost product. Such an attempt was made by this research work, by utilizing agricultural wastes (tomato and musambi peel) for extraction of pectin and cellulose. Pectin is extracted from waste tomato and musambi peel with citric acid. This method was compared on the basis of some parameters like pectin yield (%), degree of esterification (%). Further, the extracted pectin is characterized for FTIR and UV-Vis to confirm the presence of compound.

The extracted pectin was then used for casting pectin-based films, which were prepared by casting process by adding pectin (1%w/v), glycerol (2.5%v/v) and cellulose (5%w/v) in a determined volume of distilled water. Cellulose was extracted from the waste sugarcane baggase these were used for the preparation of pectin-based film. The morphology of the film was studied by SEM analysis.

The response parameters were thickness (mm), tensile strength (TS), thermal analysis. Besides these parameters, biodegradability, swelling study, solubility test, validation of developed film and antimicrobial properties.

The following results and conclusion were drawn out of this research: Depending upon the various combinations of independent variables, there is a wide variation in the response parameters was observed during extraction of pectin with citric acid. The pectin yield varied from 14.8% to 17.2% with a mean of 15.408% and standard deviation of ± 0.717 ; and the degree of esterification from 75 to 87.02%.

- The extracted pectin was further characterized by FTIR and UV-Vis and confirmed the presence of pectin
- Depending upon the process conditions of pH and temperature levels in films, the response parameters like, thickness, tensile strength of the film were 8mm thickness and 2.06 ± 0.1 MPa.
- The film depicted a biodegradable behaviour and lost about 42.1% of their original weight in 8 days.
- In SEM the film morphology indicated that the films presented a matrix filmogenic continuous and compact layer.
- Characteristics of the films can be concluded that this contributes to the decreased solubility in water and providing increased resistance to the films during swelling study.
- The developed films were assessed for their antimicrobial activity against four strains of bacteria. The inhibition zones were measured.
- The biofilms behaved quite similar and at low temperature storage, the shelf life of the carrot almost doubled as the ambient storage.

6.2 CONCLUSION

Overall, it could be concluded that pectin can be extracted from waste tomato and musambi peels and it can be successfully used to provide biofilms. Modification of the films with cellulose improved physical and other barrier properties. The obtained results showed the positive influence of the films prepared in the presence of pectin and cellulose on weight loss. Based on these studies it is possible to suggest PEC-based films prepared in the presence of cellulose and glycerol as adequate content for extending the shelf- life of different fruits and vegetables.

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