

# **DECONSTRUCTING DECAY-INDUCED PECTIN DEGRADATION: ELUCIDATING THE IMPACT OF DECAY ON PECTIN EXTRACTION FROM CITRUS PEELS**

## **PROJECT WORK**

Submitted in partial fulfilment of the requirements for the award of  
Bachelor of Technology in Biotechnology

By

**DURGA GOMATHI A (40230009)**



**DEPARTMENT OF BIOTECHNOLOGY  
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

## **SATHYABAMA**

**INSTITUTE OF SCIENCE AND TECHNOLOGY  
(DEEMED TO BE UNIVERSITY)**

**Accredited with Grade "A++" by NAAC  
JEPPIAAR NAGAR, RAJIV GANDHISALAI,**

**CHENNAI - 600119**

**APRIL - 2024**



# **SATHYABAMA**

**INSTITUTE OF SCIENCE AND TECHNOLOGY**

**(DEEMED TO BE UNIVERSITY)**

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## **DEPARTMENT OF BIOTECHNOLOGY**

### **BONAFIDE CERTIFICATE**

This is to certify that this Project Report is the bonafide work of **Durga Gomathi A (40230009)** who carried out Project Work entitled "**DECONSTRUCTING DECAY-INDUCED PECTIN DEGRADATION: ELUCIDATING THE IMPACT OF DECAY ON PECTIN EXTRACTION FROM CITRUS PEELS**" under my supervision from October 2023 to April 2024.

#### **INTERNAL GUIDE**

**Dr. P Prakash**

#### **HEAD OF THE DEPARTMENT**

**DR. V RAMESH KUMAR**

---

Submitted for Viva-voce Examination held on \_\_\_\_\_ .

**Internal Examiner**

**External Examiner**

## DECLARATION

I, **Durga Gomathi A (40230009)**, hereby declare that the Project Report entitled **“DECONSTRUCTING DECAY-INDUCED PECTIN DEGRADATION: ELUCIDATING THE IMPACT OF DECAY ON PECTIN EXTRACTION FROM CITRUS PEELS”** done by me under the guidance of **DR P PRAKASH** (Internal) at the Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfilment of the requirements for the award of **Bachelor of Technology degree in Biotechnology**.

**DATE:**

**PLACE:** Chennai

**SIGNATURE OF THE CANDIDATE**

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## ABSTRACT

Pectin, a bioactive polysaccharide, is primarily located in the intercellular regions of dicotyledons. This significant compound possesses a wide array of applications in various industries, such as nutraceuticals, pharmaceuticals, and the food industry. The food processing industries discard substantial quantities of fruit by-products, which serve as a plentiful and valuable reservoir of pectin. Thus, it is plausible to establish a sustainable production of pectin through the utilization of food industry waste. Despite being a scientifically supported concept, the implementation of producing pectin from food industry waste on a large scale has not yet been realized. This is due to several factors like the impact of food waste on the yield and quality of pectin, as well as the lack of infrastructure to manage such substantial amounts of waste. Among these factors, the primary aim of this research is to identify an optimal period for the utilization of food waste and determine the duration in which food waste yields a desirable quantity and quality of pectin. Three different citrus fruits were selected for evaluation and divided into four sample sets spanning four days to investigate the impact of decay on pectin yield and quality. Pectin can be extracted using a variety of conventional, non-conventional, or hybrid methods, among which the microwave extraction method was chosen for its simplicity and time efficiency. The extracted pectin is subsequently assessed using titrimetric analysis, FTIR, FESEM, TGA and NMR to evaluate its characteristics and cytotoxicity of pectin to human colorectal cancer cells are evaluated using MTT Assay. Finally, the optimum time for utilization of citrus to obtain best quality pectin with highest yield was determined.

**Key words:** Extraction, Decay, Esterification, Characterization, Cytotoxicity

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## LIST OF ABBREVIATIONS

- g - gram
- % - percentage
- DE - Degree of Esterification
- FESEM - Field Emission Scanning Electron Microscopy
- FTIR - Fourier Transmission Infra-Red
- TGA - Thermogravimetric Analysis
- DSC - Differential Scanning Calorimetry
- DTG - Differential Thermogravimetry
- NMR - Nuclear Magnetic Resonance
- MTT Assay - 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay
- DMSO - Dimethyl sulfoxide
- PBS - Phosphate buffered saline
- HCT 116-CCL-247 - Human colorectal carcinoma cell line initiated from an adult male

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# CHAPTER 1

## INTRODUCTION

Pectin is an intricate and versatile structural bioactive polysaccharide found in the intercellular regions of dicotyledons, particularly in the middle lamella of the cell wall of higher plants, primarily consists of galacturonic acid residue that is connected through  $\alpha$ -(1-4) linkages (Kamal *et al.*, 2021). Pectin is a type of hydrocolloid and is widely recognized for its diverse array of distinctive structural and biochemical characteristics which makes it an excellent contributor for its application in numerous fields. It plays a crucial role as a stabilizer or thickener in a multitude of organic food products, along with other applications such as being used as a binding agent in pharmaceuticals, for the production of bioactive film, packaging materials to increase the bioavailability of nanomaterials etc., However pectin also has other remarkable qualities such as antitumor activity, prebiotic properties etc..., (Nadar *et al.*, 2022).

Pectin is predominantly obtained from cereal sources however it is proven that food industry by-products such as fruit and vegetable peels, seeds etc..., also have a significant amount of pectin which can be extracted and processed for various applications. These pectin rich food wastes make up a significant portion of the food waste generated by food industries. The amount of fruit waste alone makes up about 16% of all food waste. The Food and Agriculture Organization of the United Nations has provided an estimation indicating that the global production of fruits reached a significant milestone of one billion tonnes in the year 2017, consequently generating a substantial quantity of waste and by-products throughout the process. These fruit wastes, which include peels, seeds, and other fruit leftovers, can harm the environment and human health if they are discarded as such. The peels alone make up approximately 50 to 65% of the total weight of the fruits and remain the predominant by-product (Dalal *et al.*, 2020).

Citrus fruits are noteworthy for their unique fragrance, to some extent due to ascorbic acid, flavonoids, and limonoids contained in the peel. Each year, approximately 50% of residues are generated from the citrus-processing industries,

which are either used as animal feed or disposed of improperly which may cause environmental pollution. However, it can be a major pool of different value-added products, e.g., pectin to be used in different industries. So, three different varieties of citrus *C. sinensis*, *C. limetta*, *C. reticulata* were selected for the evaluation of the impact of decay and composition (Mahato *et al.*, 2018).

Food waste especially fruit wastes have proven to be an excellent source of many nutrients. Which is already being used to produce many value-added products in small scale such as dietary intake by direct incorporation of powder of the dried peels in cakes, breaks, batters, dough etc., which also plays a role in enhancing flavour, colour and aroma. Few examples include the use of mango peel and orange peel powders etc to make cakes, biscuits which are known to have high antioxidant capacity and increased shelf life of these products (Ciriminna *et al.*, 2021). They can also be used for green synthesis of nanoparticles as they act as reducing agents. However, this concept can also be used for industrial production of pectin which can later be used in its application in food, pharmaceutical and other applications just like the commercially produced Pectin which is not sustainably made. But the concept still remains in the laboratory scale and yet to applied industrially (Sharma *et al.*, 2022). This is due to the number of limitations such as the nature of food waste affecting the yield, inability to standardize processes that use organic materials as raw materials, the decay of fruit waste, seasonal variation affecting availability of produce etc..., However hard it maybe it is not impossible to overcome these challenges to formulate a solution to overcome these limitations (Taghian Dinani *et al.*, 2022).

It is obvious that the sooner we utilize the waste to produce products the better, but how soon? Does mixing different varieties affect the yield? To answer these questions, it is absolutely necessary to know this “safe window” for utilization of wastes for production to produce high yield and high-quality products which is hygienically, economically and practically best (Banerjee *et al.*, 2018) .To evaluate this, three citrus varieties have been chosen which were evaluated for the difference of yield for the different decay and the difference in yield produced by individual and

a mixture of citrus fruit peels along with other properties to evaluate the quality of the pectin extracted (Suleria *et al.*, 2020).

There are a variety of methods of extraction that are applied for the extraction of pectin from fruit peels which includes number of conventional, non-conventional or hybrid methods of extraction among which the microwave extraction method had been chosen for the ease of application and time efficacy. The extracted pectin was dried and evaluated using various techniques and it was further evaluated for results (Dranca *et al.*, 2021).

Pectin has been known to have different biological activities and is classified as an anticarcinogen due to its capability to detoxify metal/carcinogen. It is not digested in the small intestine, but is fermented by colon bacteria producing short fatty acid chains that are involved in inhibiting colon inflammation and preventing carcinogenesis. Different studies attributed the importance of these fatty acids in lowering the pH of the colon and the reduction of colon cancer. This is due to its interaction with galectin-3 to stop cell proliferation or metastasis. Galectin-3 is a target protein in anticancer research because when overexpressed, it promotes metastasis and protects cancer cells from apoptosis (Freita *et al.*, 2021). Thus, Pectin can either be modified or can be used to deliver drugs to produce anticancer effect. Thus, we tested the cytotoxicity of pectin against human colorectal cancer cell to evaluate the cytotoxic effect of pure pectin as the final step of our project.

## **1.1 PECTIN AND ITS APPLICATIONS:**

Pectin represents a family of complex polysaccharides, making up the highest percentage composition of plant mass of approximately 35% in dicotyledonous plant cells, while grasses contain 2–10%, and woody tissues contain 5%. It is found particularly in the middle lamella of the cell wall of higher plants, primarily consists of galacturonic acid residue that is connected through  $\alpha$ -(1-4) linkages. Pectin properties include gelation, thickening and stabilization, giving it wide- spread use in food, medical, chemical, textile and other industrial fields (Chandel, V *et al.*, 2022). It is also reported that pectin has several biological and physiological functions, such as reduction of serum cholesterol, delay of gastric emptying,

immune-modulation and inducing apoptosis of colon cancer cells. The pectin and pectin-derived compounds also possess various functional properties; for instance, their antioxidant and anti-inflammatory properties, antibacterial and antiglycation capacity, fat absorption and ion binding capacity, production of edible food coatings, treatment of cancer and wound healing and dressing ability have been recently explored by the pharmaceutical industry (Akin-Ajani & Okunlola, 2022). Apart from these, many novel applications of pectin have been found recently including production of nutraceuticals, active packaging films, Biofunctionalization pectin which provides immense control over its properties which widens its applications.

## **1.2 CITRUS WASTES AS A SOURCE OF PECTIN:**

Fruits and their products, which are known for their high nutrient levels, make up a significant portion of a wholesome human diet. Numerous fruit varieties are consumed as a whole and are used to produce jams, juices, and other edible goods. Large volumes of trash are discarded by the food processing businesses. The amount of fruit waste alone makes up about 16% of all food waste. These fruit wastes, which include peels, seeds, and other fruit leftovers, can harm the environment and human health if they are discarded as such. The peels alone make up approximately 50 to 65% of the total weight of the fruits and remain as the predominant by-product. On the contrary, these fruit wastes are excellent source of pectin industrially. Citrus peel and pulp, which make up 55–60% of the weight of fresh fruit and are by-products of the companies that prepare citrus juice, are projected to weigh more than  $15 \times 10^6$  tonnes globally. These Citrus peels are such excellent sources of pectin that they can be used as such during the process of making jams and jelly for the gelation effect due to its high pectin content. The pectin produced from citrus peels have high yield and quality making it a perfect raw material for sustainable production of pectin (Mahato *et al.*, 2018).

## **1.3 EFFECT OF DECAY IN PRODUCTION OF PECTIN FROM FRUIT WASTES:**

Even though it's a proven concept that pectin can be safely extracted from fruit wastes which has the quality equivalent to commercially produced pectin, the

concept is still not favoured for industrial application since, with fruit wastes the risk of contamination and quality loss is pretty high which is not desired by any producer. The effect of decay is one of the many major challenges that limit the application of this concept in industrial scale thus it becomes inevitable for the need of evaluation of the “safe window” for the application of this concept. It is obvious that we need to process the wastes as soon as possible but the effect of decay is needed to be evaluated to see how the yield and quality changes over time (Siddiqui *et al.*, 2021).

#### **1.4 ANTICANCER EFFECT OF PECTIN:**

The anticancer effect of pectin has been proven in multiple instances. The pectin is particularly active against breast and colon cancer and has the ability to inhibit the progression of cancer by inhibiting the metastasis to liver (Garrido *et al.*, 2023). The interaction of pectin with galectin-3 to stop cell proliferation or metastasis. Galectin-3 is a target protein in anticancer research because when overexpressed, it promotes metastasis and protects cancer cells from apoptosis (Freita *et al.*, 2021). This effect of pectin is contributed by the active fragments of pectin structure. The anticancer effect can also be produced by apoptosis induction by activating caspase 3 and caspase 9 or production of Reactive oxygen species (ROS) within high concentration cancer cell areas. Various other mechanisms are suggested by various other studies however the exact mechanisms of such claims are still unknown. Thus, the pure citrus pectin was evaluated for its cytotoxicity towards human colorectal cancer cells with MTT Assay.

## CHAPTER 2

### LITERATURE SURVEY

#### 2.1 FRUIT WASTE AS A PECTIN SOURCE:

A study by Dalal *et al.*, (2020) explores turning fruit processing waste into valuable products by extracting pectin, a dietary fibre. Fruit waste is rich in pectin (5-35%) and offers a solution to the lack of fibre in modern diets. Traditional methods to extract pectin are slow and harm the environment. Newer methods like microwave and ultrasound extraction are faster and produce more and better-quality pectin. This extracted pectin can be used in baked goods and drinks to add fibre. By reusing fruit waste, we reduce waste and promote a more sustainable future (Dalal *et al.*, 2020). The authors explored using natural antioxidants from fruit waste instead of synthetic ones. Fruits like mangoes and bananas create a lot of peels, seeds, etc. that are difficult to dispose of, especially in developing countries. These residues are rich in phenolics, which have health benefits and antioxidant properties. Authors Babbar *et al.*, (2015) are looking for ways to extract these valuable compounds for use in food, medicine, and cosmetics. This study summarizes the types of phenolics in fruit waste, how to measure their antioxidant activity, and ways to extract them. It highlights the potential of fruit waste as a sustainable source of antioxidants for various industries (Babbar *et al.*, 2015).

A study by Nadar *et al.*, (2022) examines the environmental impact of pectin production using life cycle assessment. They analyse how eco-friendly the process is for every kilogram of pectin produced. This helps pinpoint areas for improvement towards more sustainable pectin extraction. The study also looks at the environmental impact of the acids (citric acid, ethanol) and electricity used in pectin production, focusing on climate change. Their findings aim to guide the development of sustainable and scalable pectin extraction methods that are both environmentally safe and commercially viable (Nadar *et al.*, 2022). Brahma & Ray (2022) examined the jackfruit peel, a discarded part of the jackfruit (*Artocarpus heterophyllus*) tree, for its potential health benefits. Jackfruit peels are often thrown away, but the authors highlight their potential due to the presence of valuable

bioactive compounds. Researchers are looking at the pharmacological (medicine-related) and phytochemical (plant chemical) properties of jackfruit peels. This work summarizes recent studies on these peels, focusing on how to extract and use their functional components. The peels contain flavonoids, polyphenols, and other beneficial compounds that could have applications in medicine and agriculture, such as fighting microbes. By studying these peels, researchers hope to find new uses for this waste product and explore its potential to treat diseases or improve agricultural practices (Brahma & Ray, 2022).

Kumar *et al.*, (2023) evaluated ways to extract pectin from food waste like citrus peels and melon rinds. Pectin is a valuable thickener and fibre source used in food. Traditionally, acid and alkali methods are used to extract pectin, but newer methods like enzymes and microwaves are becoming more popular. These advanced methods use less energy, produce higher quality pectin, and create less waste, making them more sustainable. Overall, this study highlights how using food waste for pectin extraction can reduce waste and promote environmentally friendly practices in the food industry (Kumar *et al.*, 2023). This study by Campos *et al.*, (2020) explores how to reduce fruit industry waste and create economic value by finding new uses for leftover fruits. Food waste is a major global problem, but these leftovers often contain valuable health-promoting compounds like polyphenols. The authors propose a shift towards a circular economy, where waste is reused or recycled instead of thrown away. It explores how to turn leftover fruits into useful products, particularly antioxidant extracts, which have commercial value. By looking at a real-world example of improved pineapple processing using circular principles, the potential benefits and challenges of such a shift is highlighted. Ultimately, it emphasizes sustainable methods for managing leftover fruit and creating economic opportunities through innovative product development (Campos *et al.*, 2020).

This study analyses current pectin research. It focuses on recent findings such as exploring new sources for pectin extraction, improved methods for pectin purification and fractionation after extraction, and advancements in understanding pectin's physical, chemical, rheological, and functional properties. This highlights the growing demand for pectin and the ongoing research to optimize its extraction and properties (Dranca *et al.*, 2018). The authors investigated acid-based pectin

extraction from sugar beet pulp. They optimized conditions (pH, temperature, time) to achieve pectin yields between 4.1% and 16.2%. The extracted pectins had varying compositions of galacturonic acid, neutral sugars, and esterified groups. Protein residues were also present. Molar mass distribution was broad, ranging from 20,200 to 90,100 g/mol. Most extracts showed surface activity and emulsion stabilizing ability. This suggests that acid extraction conditions significantly affect pectin yield and properties (Yapo *et al.*, 2007). Apple pomace pectin is evaluated for pancreatic lipase inhibition. Pectin from two apple varieties (*Malus pumila* and *Spondaia dulcis*) was extracted using hydrochloric acid or citric acid. The extraction method and apple source affected lipase inhibition. Pectin from *Malus pumila* with citric acid showed the strongest inhibition (94.3%), comparable to commercial pectin. This suggests potential applications of apple pomace pectin in anti-obesity and personal care products (Kumar *et al.*, 2010).

Passion fruit peel, a byproduct of juice manufacturing, is assessed as a potential reservoir of flavonoids and pectin. The utilization of Pressurized Solvent Extraction (PSE) and Ultrasound-Assisted Extraction (UAE) was explored for the effective sequential retrieval of these compounds. It is indicated in the investigation that a blend of alcohol and water demonstrates efficacy in the extraction process, with ethanol emerging as the solvent of choice due to its reduced toxicity profile. In essence, this scholarly inquiry underscores the promise of valorizing passion fruit rind through the extraction of valuable bioactive elements (de Souza *et al.*, 2018). The investigation by Nguyen *et al.*, (2019) delves into the extraction of pectin from the peels of Vietnamese mangoes. The examination reveals that ripe mangoes exhibit the highest pectin content ranging from 18.4 to 31.7 g per 100 g of dry weight. Among the different cultivars, the one with the most notable pectin content was found to be the cultivar, followed by Cat Chu and Hoa Loc. The characteristics of pectin such as water retention capacity, solubility, and emulsion stability were notably influenced by the stage of maturity, except for the degree of esterification and emulsion capacity. On the whole, this scrutiny posits Vietnamese mango peels as a feasible reservoir for pectin extraction (Nguyen *et al.*, 2019). A comparative analysis by Firat *et al.* (2023) scrutinized three distinct methodologies (conventional, ultrasonic, microwave) for the extraction of pectin from the peels of watermelon and pomegranate. The outcomes of the study indicate that a lower pH, elevated

temperature, and prolonged extraction duration led to an increase in yield. Optimal emulsifying pectins were obtained through microwave-based extraction for watermelon peel and ultrasound-based extraction for pomegranate peel. These pectins not only enhanced the quality of ice cream by augmenting its viscosity, prolonging its melting time, and intensifying its firmness but also proposed watermelon and pomegranate peels as sustainable reservoirs of pectin for the food industry. The extracted pectins were recommended as a wholesome and cost-efficient substitute for conventional emulsifiers in ice cream production (Firat *et al.*, 2023).

Gharibzahedi *et al.* (2019) conducted a comparative analysis of four techniques employed for the extraction of fig skin pectin, namely hot-water, ultrasound-assisted, microwave-assisted, and ultrasound-microwave assisted methods. Their findings indicated that ultrasound-microwave assisted extraction (UMAE) demonstrated the highest efficacy, resulting in a pectin yield of 11.71%, surpassing the yields obtained by the other methods which ranged from 6-9%. Antioxidant properties were observed in the pectins extracted using all methodologies. Notably, UMAE-derived pectin exhibited superior quality characterized by a higher content of galacturonic acid and elevated molecular weight, leading to enhanced emulsification and stabilization of emulsions. Furthermore, the analysis revealed that the chemical structure and sugar composition of the pectins remained unchanged irrespective of the extraction method employed (Gharibzahedi *et al.*, 2019). In a study by Ghoshal & Negi (2020), an investigation was carried out on the extraction of pectin from kinnow peels utilizing the hot water method, resulting in a yield of 6.13%. This extracted pectin was identified as a low methoxyl pectin with the ability to form gels. The researchers postulate that due to its characteristic properties, this pectin could potentially find applications in the production of low-calorie jams, jellies, and as a thickening agent in various food products (Ghoshal & Negi, 2020).

## **2.2 PECTIN YIELD FROM VARIOUS FRUIT PEELS:**

In the study conducted by do Nascimento Oliveira *et al.* (2018), an examination was carried out on the influence of pH, temperature, and duration on

the extraction of pectin from mango peels utilizing a central composite design. The pectin yield observed ranged between 18.8 to 32.1 g/100 g of AIR, with a Degree of Esterification (DE) ranging from 62.2 to 86.2%, and viscosity varying from 1.58 to 45.85 mPa·s. A negative correlation between the extraction yield and viscosity was identified. The most favorable conditions for pectin extraction were determined to be 35-48 minutes, 85.4-97°C, and pH 1.66-2.4 or alternatively 80-85 minutes, 94.3-97°C, and pH 1.66-1.79, resulting in a pectin yield of over 30% with DE values equal to or greater than 70% and viscosity equal to or greater than 20.0 mPa·s (do Nascimento Oliveira *et al.*, 2018). In the research conducted by Jong *et al.* (2021), an exploration was undertaken on durian rind, a by-product of durian fruit, as a potential reservoir of pectin. Various durian rind samples from distinct varieties were assessed to ascertain their pectin content and characteristics. The investigators observed variations in the pectin yield based on the type of durian variety, with all samples exhibiting a high level of esterification. These results propose that durian rinds hold promise as an alternative pectin source for diverse sectors, concurrently aiding in the reduction of waste generated from durian cultivation (Jong *et al.*, 2021).

Kamal *et al.* (2021) effectively isolated pectin from sweet orange peels, representing a promising novel origin. The researchers conducted experiments involving temperature, pH, and extraction duration to enhance both yield and quality. Elevated temperatures, slightly acidic pH levels, and extended extraction periods resulted in higher pectin production (ranging from 12.52% to 22.45%). The maximum yield of 23.64% was attained under specific optimized circumstances. Although the quality was generally satisfactory, the anhydrouronic acid content was lower compared to some alternative pectin sources. This indicates the potential viability of sweet orange peels as a pectin source across various industries (Kamal *et al.*, 2021). The investigators optimized the extraction of pectin from pomegranate peels using response surface methodology. Factors such as temperature, duration, and pH had substantial impacts on yield, galacturonic acid levels, and esterification degree. Harsher conditions (low pH, high temperature, prolonged duration) led to increased yield and galacturonic acid levels but decreased esterification. The ideal parameters for optimal pectin yield and quality were determined to be 94.4°C, 200 minutes, and pH 1.24, highlighting pomegranate peels as a promising source of valuable pectin (Nateghi *et al.*, 2022). Wang *et al.* (2014) explored the subcritical

water extraction of pectin from citrus peel and apple pomace. The highest yields obtained were 21.95% for citrus peel and 16.68% for apple pomace. Both types of pectin displayed shear-thinning characteristics and exhibited free radical scavenging capabilities. Citrus peel pectin demonstrated a stronger anti-proliferation effect on colon cancer cells in comparison to apple pomace pectin (Wang *et al.*, 2014).

### **2.3 CITRUS PEEL PECTIN:**

Salma *et al.*, (2014) conducted an investigation into methodologies for the extraction of pectin from lemon peel. The significance of pectin in food processing is well recognized, with lemon peel serving as a favorable source. However, existing techniques yield only 1-1.38%, rendering it unviable for commercial purposes. Efforts are underway to enhance both the quantity and quality of the extracted pectin. Emerging methodologies such as FTIR analysis are being explored to validate the structure of the obtained product. This research underscores the necessity for more efficient approaches to extract pectin from lemon peel, thereby rendering it economically feasible and supporting the sustainable utilization of this byproduct (Salma *et al.*, 2014). The study by Kamal *et al.* (2021) assessed the potential of sweet orange peel (*Citrus sinensis*) as a source of pectin. The optimization of pectin extraction was carried out utilizing response surface methodology (RSM). Various parameters including temperature, pH levels, and extraction duration were scrutinized. Subsequent analysis was conducted on the quality of the extracted pectin. The objective of this method was to determine the most favorable conditions for both the yield and quality of pectin derived from sweet orange peel (Kamal *et al.*, 2021). Mukhtar and team (2022) delved into the utilization of citrus peel pectin as a substitute for commercial pectin in mango jam production. Pectin extracted from different citrus peels exhibited comparable gelling properties to that of commercial pectin. Examination through physico-chemical means indicated minimal alterations in sugar content and acidity over the storage period. Sensory assessments unveiled a decline in color, flavor, and consistency in all jam samples as storage progressed. Nevertheless, citrus peel pectin managed to maintain an acceptable standard in terms of quality and microbiological safety

during storage, hinting at its potential as a feasible and economical alternative to commercial pectin (Mukhtar *et al.*, 2022).

El Fihri *et al.*, (2022) conducted an investigation into the extraction of pectin from citrus peels, including orange, lemon, and bigarade. The highest pectin yield was obtained from lemon peel (28.57%) under specific conditions (90°C, pH 1.5, 120 min). The pectins extracted exhibited a simplistic structure, a high content of galacturonic acid, and significant methyl esterification. Notably, they demonstrated favorable water retention properties and emulsifying capabilities. Among the citrus peels, bigarade peel pectin displayed the highest viscosity, indicating potential applications across various industries (El Fihri *et al.*, 2022). Singhal & Swami Hulle (2022) delved into the multifaceted uses of pectin, a derivative of agricultural waste products. Pectin finds utility in medicinal realms such as drug delivery and wound healing, as well as in food applications for gelling and thickening purposes. The study encapsulates the literature on pectin extraction from citrus fruits and its diverse industrial applications. Emphasis is placed on elucidating the inherent properties of pectin that render it valuable in these contexts, alongside strategies for tailoring pectin to specific requirements. Through an extensive review of existing studies, this work furnishes a comprehensive insight into pectin for both researchers and industrial practitioners (Singhal & Swami Hulle, 2022). The researchers explored the enhanced extraction of pectin from sweet lime peels through the application of sound waves, specifically ultrasound. Investigation of various parameters such as processing duration, power intensity, and peel-to-solvent ratio shed light on their impact on pectin yield and quality. Optimization efforts revealed an optimal combination of these parameters resulting in maximal pectin extraction efficiency and quality. This underscores the potential of ultrasound-assisted extraction as a promising approach for obtaining high-quality pectin from sweet lime peels, thereby minimizing wastage (Arshi Siddiqui & Khan Chand, 2023).

The investigation conducted by Figueira *et al.*, (2023) addresses the issue of citrus waste, which is progressively becoming a significant environmental concern. The majority of existing techniques are capable of recovering only a single valuable component from this waste material. This research delves into a novel approach aimed at obtaining two beneficial products (pectin and hesperidin) from orange

peels in an environmentally friendly manner. Their methodology incorporates an acidic phase for pectin extraction, succeeded by a pH alteration to facilitate hesperidin extraction. By adopting this strategy, the generation of waste is minimized, and valuable commodities are generated for the food sector, all while adhering to the principles of green chemistry. Moreover, this sheds light on related studies that focus on optimizing the utilization of citrus waste and underscores the significance of sustainable extraction techniques for future applications (Figueira *et al.*, 2023). Su *et al.*, (2019) investigated a fresh approach (S-MAE) for the isolation of pectin from orange peels by integrating microwaves and surfactants. Initially, they fine-tuned the microwave parameters to achieve an optimal pectin yield. Subsequently, they evaluated the impact of various surfactants on the extraction process. Among the variants examined, Tween-80 demonstrated superior performance, resulting in a 17% increase in yield. On the whole, this S-MAE technique yielded a greater quantity of pectin with enhanced quality compared to conventional solvent-based extraction methods (Su *et al.*, 2019).

## **2.4 MICROWAVE EXTRACTION METHOD:**

Microwave-assisted extraction (MAE) is emerging as a sustainable option compared to conventional methods for extracting pectin. A recent study conducted by Mao *et al.*, (2023) examines the utilization of microwaves in extracting pectin from various sources. The researchers emphasize that the efficiency of MAE depends on the material's capacity to absorb microwave energy, which is affected by its dielectric properties. Despite the potential benefits of reduced environmental impact, enhanced efficiency, and superior pectin quality, the specific mechanisms underlying MAE in pectin extraction remain ambiguous. It is recommended that future investigations concentrate on refining conditions for different raw materials and developing a more comprehensive comprehension of the underlying processes (Mao *et al.*, 2023). Rahmani *et al.*, (2020) discuss the optimization of pectin extraction from sweet lemon peel (SLP) using microwave assistance through a Box-Behnken design. The ideal parameters (700 W, 3 min, pH 1.5) resulted in a significant pectin recovery rate (25.31%). The pectin obtained exhibited desirable attributes such as a high galacturonic acid content, elevated molecular weight, and effective emulsifying and antioxidant properties. These results indicate the potential

of SLP as a promising source of pectin with valuable industrial applications (Rahmani, Z., *et al.*, 2020). In their research, Thirugnanasambandham *et al.*, (2014) explored microwave-assisted extraction (MAE) for obtaining pectin from dragon fruit peel. By employing a Box-Behnken design in combination with response surface methodology (RSM), the process was optimized. The RSM analysis demonstrated a close correspondence between predicted and actual values. The optimal conditions for pectin extraction included a power of 400 W, a temperature of 45 °C, an extraction duration of 20 min, and a solid-liquid ratio of 24 g/mL, resulting in a pectin yield of 7.5% (Thirugnanasambandham *et al.*, 2014).

Mada *et al.*, (2022) investigated the utilization of banana and papaya peel waste for pectin extraction through the application of microwave-assisted techniques. Optimal conditions (73°C, 2 min, pH 2) for achieving a pectin yield of 23.74% and anhydrouronic acid content of 69.97% were identified using Design Expert software. The extracted pectin exhibited favorable physicochemical characteristics and functional attributes such as water absorption capacity, oil absorption capacity, and emulsifying activity, indicating its potential suitability for diverse industrial applications (Mada *et al.*, 2022). The research conducted by Picot-Allain *et al.*, (2022) sheds light on the increasing industrial significance of pectin and the viability of utilizing byproducts from tropical fruits as pectin sources. Although citrus peels and apple pomace remain the primary sources in commercial settings, traditional extraction methods involving mineral acids are known to be energy-intensive. The study explores alternative eco-friendly techniques like microwave, ultrasound, and enzyme-assisted extraction as sustainable strategies for obtaining pectin from tropical and subtropical fruits (Picot-Allain *et al.*, 2022). Seixas *et al.*, (2014) delved into the process of microwave-assisted extraction of pectin from yellow passion fruit peels using tartaric, acetic, and nitric acids. Application of response surface methodology revealed that extraction duration and microwave power had a significant impact on the yield when using nitric and tartaric acid, whereas only extraction time influenced the yield for acetic acid. Notably, nitric acid demonstrated the highest pectin yield (18.2%), albeit with reduced purity. Pectin derived from acetic and nitric acid exhibited elevated molecular weight, degree of esterification, and uronic acid content, positioning them as superior options for extraction purposes (Seixas *et al.*, 2014).

Zakaria *et al.*, (2021) found that the amount of acid (pH), the ratio of pineapple peel to solvent, and the power of the microwaves significantly impacted yield, while extraction time did not within the range they tested. The best yield (around 2.5%) was achieved with a low pH, a high solvent ratio, and high microwave power. The pectin they extracted was also high quality. This suggests that MAE is a promising way to efficiently extract pectin from pineapple peels for industrial use. (Zakaria *et al.*, 2021). Lal *et al.*, (2021) combined pulsed electric fields (PEF) and microwave-assisted extraction (MAE). They optimized the process to get the most pectin possible using statistical methods. The best results involved using PEF first, followed by MAE. While the pectin produced this way had a different structure than conventionally extracted pectin, they were able to get similar yields using this new method. This method also resulted in more broken-down cells in the jackfruit waste, which likely helped extract more pectin. Overall, this new method seems like a promising way to get more pectin from jackfruit waste. (Lal *et al.*, 2021). Dranca *et al.*, (2021) compared traditional and microwave-assisted methods for pectin extraction from apple pomace. While the microwave method yielded similar amounts of pectin, it did so in much less time. The quality of the pectin (galacturonic acid content, degree of esterification) was also comparable between methods. The pectin obtained was then used to create films with good oxygen barrier properties, suggesting its potential for applications in food packaging. (Dranca *et al.*, 2021)

## **2.5 ANTI-CANCER EFFECT OF PECTIN:**

Garrido *et al.*, (2023) conducted an analysis on the prospective application of modified pectins (MPs) in the realm of breast cancer therapy. Research conducted both in vitro and in vivo presented favorable outcomes concerning breast cancer cell strains and murine models. While MPs demonstrated potential, further in vivo examinations are imperative to establish definitive preclinical substantiation (Garrido *et al.*, 2023). The research by Guo *et al.*, (2024) delved into exploring the anticancer and immune-boosting attributes of citrus pectins (CP) that were subjected to modification through ultrasound and H<sub>2</sub>O<sub>2</sub>/ascorbic acid. The altered pectins (CP-1 and CP-2) characterized by distinct molecular weights showcased heightened anti-cancer efficacy and enhancement of immune cell activity in contrast

to unaltered CP. Notably, CP-2, distinguished by elevated O-6 methylation and molecular weight, displayed the most potent immunostimulatory impact, indicating a correlation between pectin composition and its bioactive properties. These revelations offer valuable insights into crafting functional food products with enhanced health advantages (Guo *et al.*, 2024). In a study by Kumar *et al.*, (2020), the exploration centered on the antibacterial and anticancer attributes of refined pectin derived from mulberry fruits of BR-2 and S-13 varieties. The findings indicated that BR-2 pectin exhibited more potent antibacterial efficacy against various strains and suppressed the growth of liver cancer cells (Hep G-2) at minimal concentrations. Neither type of pectin demonstrated cytotoxic effects on colon cancer cells (HT-29). Molecular docking simulations allude to the potential of pectin binding to receptors on cancer cells. These outcomes underscore mulberry fruit pectin as a promising reservoir of natural antibacterial and anticancer agents (Kumar *et al.*, 2020).

Emran *et al.*, (2022) examined modified citrus pectin (MCP), a specific type of pectin, is getting attention for its potential to prevent cancers like colon and breast cancer. This study suggest MCP acts as a fiber and may inhibit cancer cell growth. However, more research is needed to confirm these findings and understand how pectin might be used to prevent or treat cancer. (Emran *et al.*, 2022). do Nascimento Santos *et al.*, (2021) evaluated *Caesalpinia pulcherrima* leaves were for their potential health benefits. A pectin-like polysaccharide was extracted and characterized. It exhibited antioxidant activity and immune cell stimulation. The pectin increased CD8<sup>+</sup> T lymphocytes, monocytes, and cytokine production, suggesting its potential as an antioxidant and immunomodulatory agent. This study supports the traditional use of *C. pulcherrima* leaves and opens doors for its exploration in herbal medicine. (do Nascimento Santos *et al.*, 2021). Mellinas *et al.*, (2020) highlight pectin as a promising bioplastic alternative to synthetic polymers due to its biodegradability and natural origin. It covers recent research on pectin structure, types, sources, and its potential applications, particularly in food packaging. The growing interest in pectin for the food and beverage industry, including combinations with other biopolymers to enhance properties, is also addressed. (Mellinas *et al.*, 2020)

## **CHAPTER 3**

### **AIM AND SCOPE**

#### **3.1 AIM:**

The aim is to analyse the pectin yield, quality, and variation among three citrus fruit varieties, investigate the impact of decay on pectin properties, and compare yields and other properties from individual and mixed fruit combinations.

#### **3.2 SCOPE:**

- To analyse the effect of decay of citrus fruits on the yield of pectin and find a “safe window” to extract pectin from waste.
- To analyse the effect of combined composition of citrus fruits on the yield of pectin.
- To Characterize the extracted pectin.
- To check toxicity of the pectin extracted.

## CHAPTER 4

### MATERIALS AND METHODS

#### 4.1 Extraction of pectin from citrus peels:

##### 4.1.1 Preparation of citrus peel powder for extraction:

The three varieties of citrus peels *C.sinensis*, *C.limetta*, *C.reticulata* are collected and washed to remove any dirt. The washed citrus peels are then weighed and divided proportionately till 16 parts are obtained which is shown in the table that follows.

**TABLE 4.1.1: Citrus Peels weight**

| Sample              | Day Wise Weight (g) |       |       |       |
|---------------------|---------------------|-------|-------|-------|
|                     | Day 1               | Day 2 | Day 3 | Day 4 |
| <i>C.sinensis</i>   | 107.1               | 107.1 | 107.1 | 107.1 |
| <i>C.limetta</i>    | 85.65               | 85.65 | 85.65 | 85.65 |
| <i>C.reticulata</i> | 98.22               | 98.22 | 98.22 | 98.22 |
| Mixed fruits        | 103                 | 103   | 103   | 103   |

The divided samples are allowed to rest till the designated day comes. The day 1 sample i.e. Fresh fruit peels are processed as the same day of collection. The processing starts with drying them in hot air oven for 24h at 60°C. Once the peels are dried, they are powdered to get peel powder. Similarly, the process was repeated for Day 2, Day 3 and Day 4 sample which resulted in the production of 16 citrus peel powder.

##### 4.1.2 Microwave assisted extraction of pectin:

Pectin extraction was performed using a domestic microwave apparatus operating at a frequency of 2450 MHz and a maximum power output of 900 W, incorporating adjustable microwave power and irradiation duration. The pectin powder obtained through the aforementioned approach was utilized for extraction. A solution comprising 5 g of orange peel powder and 100 mL of distilled water (pH

1.5) underwent microwave treatment for a period of 90 seconds. Subsequent to this, the solution was cooled to room temperature and filtered to remove peel powder residues. The filtrate collected was centrifuged in a cooling centrifuge at 4°C, 10000 rpm for 15 minutes. The insoluble residue collected at the bottom was discarded, whereas the supernatant was treated with an equal volume of 95% (v/v) ethanol to prompt precipitation. The precipitated pectin mass underwent triple washing with 95% (v/v) ethanol to remove mono and disaccharides. Following this procedure, the coagulated pectin was dried in a Hot air oven for 4 hours at 50°C. The acquired pectin was subsequently quantified, and the pectin yield was determined. The extracted pectin was then stored in an air tight container for subsequent analysis.

#### **4.2 Moisture content:**

Moisture content of the peels was calculated using the weight before and after drying the fruit peels using Hot air oven.

$$\text{Moisture content \%} = \frac{\text{Weight of peels before drying} - \text{Weight of peels after drying}}{\text{Weight of peels before drying}} \times 100$$

#### **4.3 Pectin Yield:**

Pectin yield % was calculated using the weight of pectin extracted and the weight of peel powder used for extraction.

$$\text{Pectin yield \%} = \frac{\text{Weight of pectin (g)}}{\text{Weight of Peel powder (g)}} \times 100$$

#### **4.4 Purification of Pectin:**

The Pectin extracted using microwave extraction method was subjected to purification procedure with bromine water, proceeded by oxidation. Initially, the pectin was dissolved in water at a concentration of 1g/10ml and subsequently subjected to heat to ensure thorough dilution. Following this, 20ml of 0.1N Bromine water was introduced into the solution and allowed to undergo oxidation. Any surplus bromine water was removed through 2–3 cycles of agitation, succeeded by

the introduction of ether until the aqueous layer reached a colourless state. The pectin specimens were then precipitated utilizing 95% alcohol, filtered via a muslin fabric after a duration of 2 hours. The filtered pectin was ultimately exposed to various alcohol concentrations and desiccated in an oven at 50°C for a period of one hour. This methodology was observed to be expeditious and proficient in achieving elevated levels of purity during the purification of pectin from its natural sources.

#### **4.5 Qualitative analysis of pectin using titration:**

##### ***4.5.1 Degree of esterification:***

The degree of esterification, DE, is defined as the proportion of esterified galacturonic acid groups to the total galacturonic acid groups that are present, and this quantification was carried out utilizing a titration method involving ethanol. A quantity of approximately 0.2 g of pectin was soaked in 2 ml of ethanol, after which the substance was dissolved in 20 ml of distilled water and subsequently subjected to agitation in an automated water bath maintained at a temperature of 45°C until complete dissolution of the pectin was achieved. The resulting solution underwent titration with 0.1 N NaOH subsequent to the introduction of three drops of Phenolphthalein, until the manifestation of a faint pink hue. The enumeration of unbound carboxy groups was ascertained based on the volume of 0.1 N NaOH solution utilized during the initial titration process. Following this, 10 ml of 0.1 N NaOH were introduced for the purpose of neutralizing the polygalacturonic acid. The sample was then sealed with a stopper and subjected to vigorous agitation, subsequently left at ambient temperature for a duration of 2 hours to facilitate the de-esterification of the pectin. An additional 10 ml of 0.1 N HCl were incorporated in order to neutralize the NaOH, and the sample underwent agitation until the disappearance of its pink tinge. The subsequent step involved the addition of three drops of phenolphthalein into the sample, followed by further titration with 0.1 N NaOH. The volume of the titrant was meticulously documented as the final titration volume once a discernible pink coloration emerged. The quantification of esterified carboxy groups was deduced from the volume of 0.1 N NaOH solution consumed

during the final titration process. The calculation of DE was performed based on the formula given below.

$$DE\% = \frac{\text{Final Titration volume (ml)}}{\text{Initial Titration volume(ml)} + \text{Final Titration volume (ml)}} \times 100\%$$

## **4.6 CHARACTERIZATION STUDIES:**

### ***4.6.1 Fourier Transform Infrared Spectroscopy:***

Fourier Transform Infrared Spectroscopy, or FTIR, is a method for examining the chemical make-up of a material. FTIR evaluates pectin by shining infrared light on the sample. Different functional groups within the pectin molecule, like carboxylic acids, ester groups (depending on modification), and hydroxyl groups, absorb specific infrared frequencies. By analysing the resulting spectrum, scientists can identify these functional groups and their relative abundance. This information is valuable because it reveals the degree of esterification (important for gelling) and helps ensure the absence of contaminants. While it doesn't provide complete structural details, FTIR offers a rapid analysis of pectin's key chemical features. Pectin can have varying degrees of esterification, meaning some of the galacturonic acid units have methyl ester groups attached. The intensity of the peak corresponding to ester groups (around 1740 cm<sup>-1</sup>) in the FTIR spectrum is proportional to the degree of esterification. The significance of this data cannot be overstated, given that the level of esterification has a direct impact on the characteristics of pectin such as its gelation properties and utility in various food-related scenarios. Furthermore, the application of FTIR is instrumental in identifying any impurities or foreign substances present in the pectin sample. Observing peaks associated with functional groups not commonly associated with pectin may suggest the existence of alternative polysaccharides, proteins, or minerals.

### ***4.6.2 Field-emission Scanning electron microscopy:***

Field-emission Scanning electron microscopy or FESEM is a highly versatile technique used to obtain high-resolution images and detailed surface information of

samples. FESEM evaluates pectin's physical characteristics by focusing a beam of high-energy electrons on the sample. These electrons interact with the pectin's surface, generating signals that create a high-resolution image. This image allows scientists to visualize the morphology (shape and size) of individual pectin molecules or clumps, revealing details like branching, aggregation, and surface texture. Additionally, by analyzing the image, researchers can estimate the size distribution of pectin particles, which is crucial for understanding how pectin behaves in applications like biofilms or drug delivery. However, FESEM primarily focuses on surface features and size, not the chemical makeup, and requires specialized sample preparation that might alter pectin's natural state. Here it offers high-resolution images, allowing visualization of the morphology (shape and size) of individual pectin molecules or aggregates. By analyzing FESEM images, scientists can estimate the size distribution of pectin particles. This information is crucial for understanding how pectin will behave in applications where particle size plays a role, such as in biofilms or controlled drug delivery systems.

#### ***4.6.3 Thermogravimetry analysis:***

Thermogravimetric analysis (TGA) offers valuable insights into the physicochemical properties of biopolymers. Evaluation of pectin through Thermogravimetric Analysis (TGA) involves measuring weight changes with increasing temperature. The controlled heating chamber accommodates the pectin sample for analysis. Elevation in temperature leads to the decomposition or volatilization of distinct pectin components at specific temperatures. Continuous weight monitoring of the sample is carried out by the TGA instrument. Examination of the weight loss profile (weight vs. temperature) allows scientists to identify decomposition temperatures of various pectin constituents, estimating thermal stability, moisture content, and volatile components presence. Thermal analysis (DSC and TGA with DTG) was compared with other analytical techniques (chemical analysis, color measurement, SEM, and FT-IR spectroscopy) to assess its potential as a screening tool for characterizing pectin changes during storage. Citrus pectin samples stored and analyzed for behavior alterations in thermal analysis.

#### **4.6.4 Nuclear Magnetic Resonance:**

Nuclear Magnetic Resonance, commonly known as NMR spectroscopy, assesses pectin through the examination of the magnetic characteristics exhibited by its atomic components. The various sugar units present in pectin, such as galacturonic acid and rhamnose, display distinct "NMR signatures." Through the analysis of the NMR spectrum, researchers can distinguish these sugar units and their interconnections, thereby unveiling the sequential arrangement and overall configuration of the pectin. Moreover, NMR has the capacity to ascertain the level of esterification (methyl groups binding) and pinpoint the side chains that are evident on the pectin molecule. This intricate depiction facilitates a comprehensive comprehension of the pectin's formation and potential functional attributes. It stands as a spectroscopic methodology utilized for the determination of molecular structures and compositions. The analysis of Pectin NMR is conducted utilizing  $^1\text{H}$  NMR with a solvent such as DMSO. This technique yields even more detailed structural insights into pectin, offering information regarding individual sugar molecules, the degree of esterification, and the analysis of side chains.

#### **4.6.5 MTT ASSAY:**

The utilization of the MTT assay presents a method for evaluating the potential influence of pectin on cell viability. Within this assay, viable cells are subjected to pectin for a specified duration. Subsequently, the addition of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) occurs. Viable cells possess the capability to metabolize MTT into a pigmented byproduct. Through the assessment of the intensity of this pigmented byproduct, researchers are able to indirectly approximate the quantity of viable cells. Any reduction in intensity resulting from pectin treatment compared to untreated cells implies a likely decline in cell viability, suggesting potential anti-proliferative or cytotoxic effects of pectin. The MTT assay delivers a quantitative evaluation of cellular well-being subsequent to pectin exposure. An investigation into the impact of isolated pectin on the viability of human colon cancer cells was conducted utilizing the MTT assay. The HCT 116-CCL-247 cells were cultivated in 96-well plates, and medications were administered to the cellular monolayer. Subsequent to a 24-hour observation period to assess

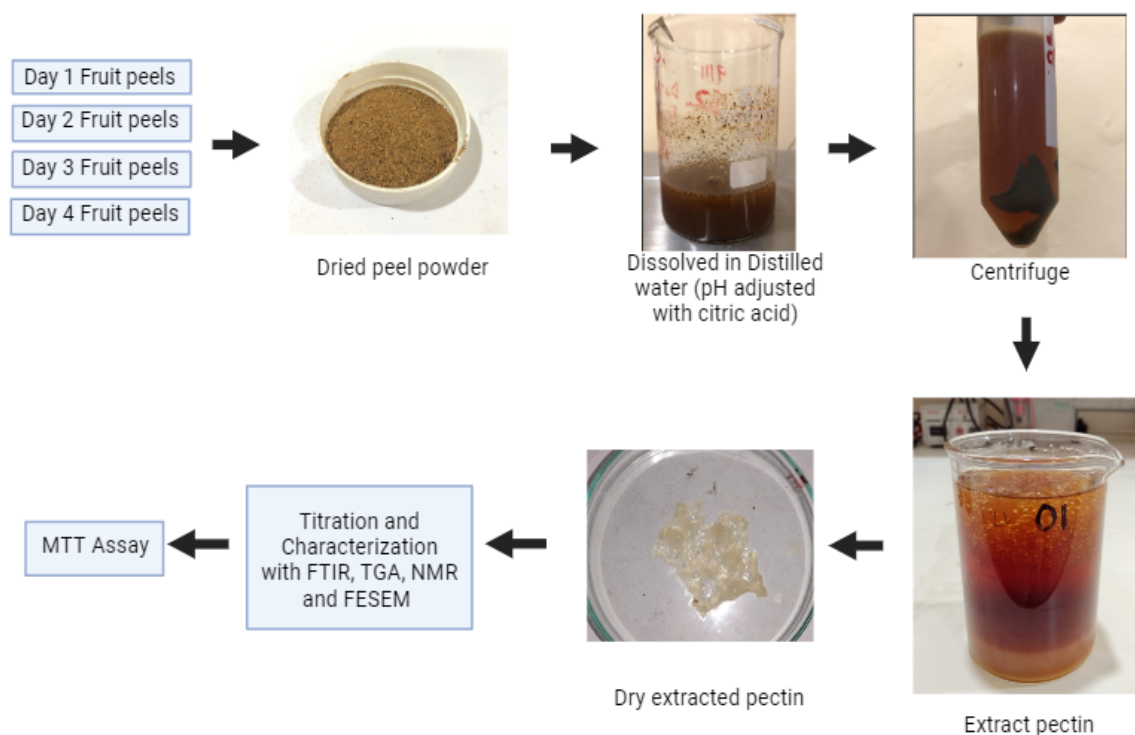
cell morphology, the cells underwent two washes with 1XPBS and were incubated for three hours with 0.2 mg/ml of MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide). The resultant purple-hued byproduct was dissolved using isopropyl alcohol, and the optical density was gauged at 620nm employing an ELISA Reader.

#### **4.6.6 Statistical analysis:**

MATLAB is a powerful software program specifically designed for scientific and engineering data analysis. It excels at handling complex datasets, from intricate time series to medical research results. MATLAB offers features to organize, clean, and manipulate your data, along with a vast library of built-in functions for statistical analysis, signal processing, and even machine learning. This allows you to uncover trends, identify relationships, and ultimately extract meaningful insights from your scientific data. MATLAB was applied to compare the degree of esterification of commercial pectin and pectin extracted from citrus peels.

## CHAPTER 5

### RESULTS AND DISCUSSIONS



**Fig:5.1: Overall Process flow chart**

#### 5.1 Moisture Content:

**TABLE 5.1: Moisture content**

| Sample              | % of Moisture content |       |       |       |
|---------------------|-----------------------|-------|-------|-------|
|                     | Day 1                 | Day 2 | Day 3 | Day 4 |
| <i>C.sinensis</i>   | 76.75                 | 79.92 | 78.89 | 79.83 |
| <i>C.limetta</i>    | 75.48                 | 77.58 | 72.21 | 75.59 |
| <i>C.reticulata</i> | 76.17                 | 74.85 | 73.93 | 80.35 |
| Mixed fruits        | 78.05                 | 79.22 | 80.67 | 79.12 |

The moisture content was more or less equal in all 16 samples following a general range between 72-82%.

## 5.2 Pectin Yield%:

**TABLE 5.2: Pectin yield%**

| Day   | Yield %           |                  |                     |              |
|-------|-------------------|------------------|---------------------|--------------|
|       | <i>C.sinensis</i> | <i>C.limetta</i> | <i>C.reticulata</i> | Mixed Fruits |
| Day 1 | 14                | 6.6              | 4.1                 | 5.1          |
| Day 2 | 8                 | 4                | 2.9                 | 3.59         |
| Day 3 | 3.2               | 2.09             | 1.21                | 1.7          |
| Day 4 | 1.23              | 1                | 0.556               | 1.3          |

Out of all 16 samples, the *Citrus sinensis* peels had the highest yield. But all day 1 and day 2 pectins had relatively good yield as well while the yield percentage drops drastically after day 2.

## 5.3 Qualification Analysis of Pectin:

**TABLE 5.3: Degree of Esterification**

| Day | <i>C.sinensis</i> | <i>C.limetta</i> | <i>C.reticulata</i> | Mixed Fruits |
|-----|-------------------|------------------|---------------------|--------------|
| 1   | 77%               | 50%              | 44%                 | 57.14%       |
| 2   | 62%               | 31%              | 14.2%               | 22%          |
| 3   | 14.2%             | 7.3%             | 10.7%               | 15%          |
| 4   | 7.8%              | 2.5%             | 6.2%                | 8%           |

Out of all 16 samples, the *Citrus sinensis* peels had the highest Degree of Esterification. Comparatively, all day 1 and day 2 had highest Degrees of Esterification but it drops drastically after day 2.

With the Yield and Degrees of Esterification data it was safe to conclude that the desirable yield and quality can only be observed till day 2 in all cases. In our case the *Citrus sinensis* has both highest Yield and Degree of esterification so, we decided to proceed with analysis of *Citrus sinensis* for further analysis.

### 5.3.1 Comparing extracted pectin with commercial pectin:

MATLAB was used to compare commercially pectin and day 1 pectin extracted from *Citrus sinensis*. t test was performed in MATLAB to find out the most significant one among the two.

#### Proposed Hypothesis:

The null hypothesis ( $H_0$ ) now states that the mean DE of commercial pectin is higher than the mean DE of citrus peel pectin.

The alternative hypothesis ( $H_1$ ) states that the mean DE of citrus peel pectin is higher than the mean DE of commercial pectin.

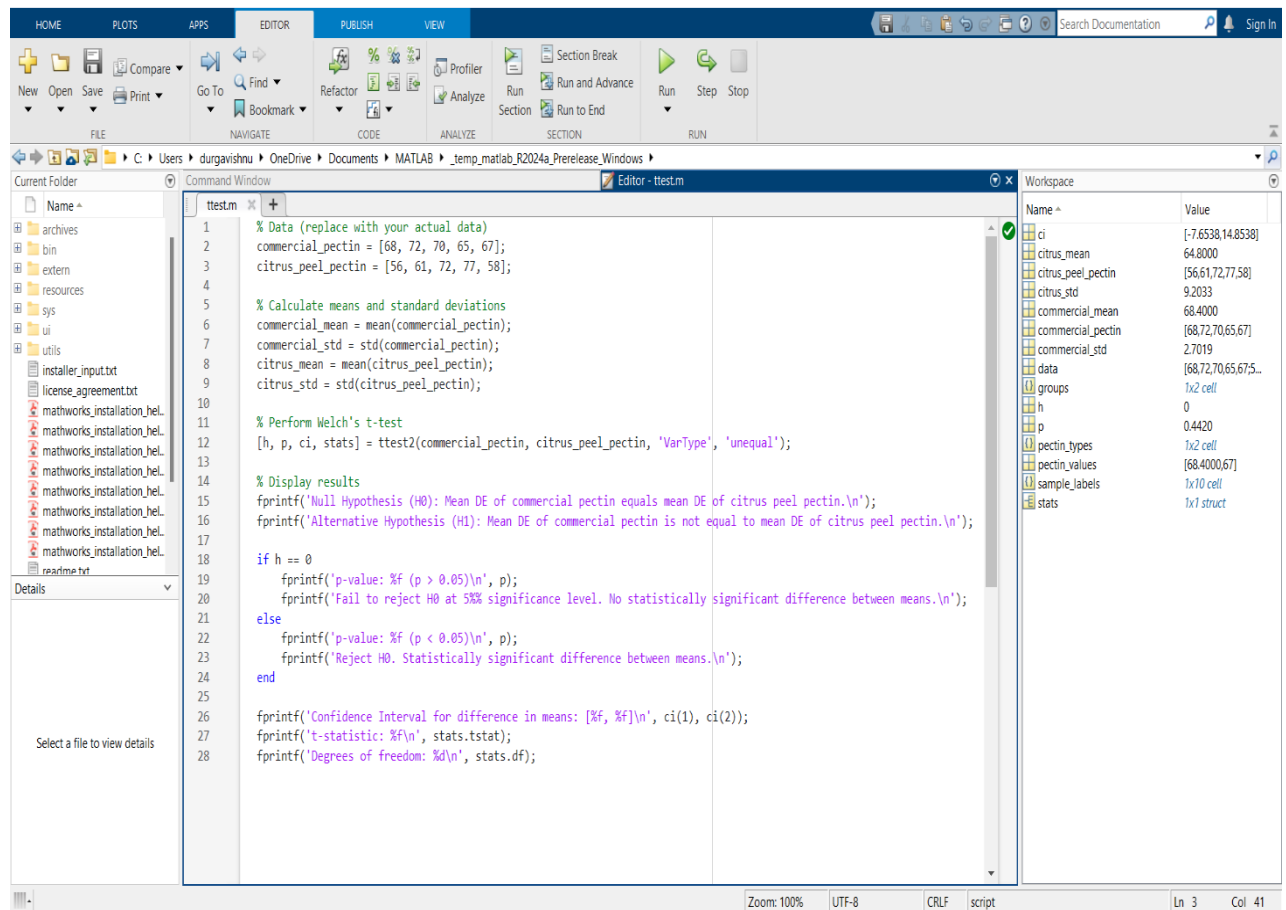


Fig:5.2: MATLAB t test

#### Interpretation:

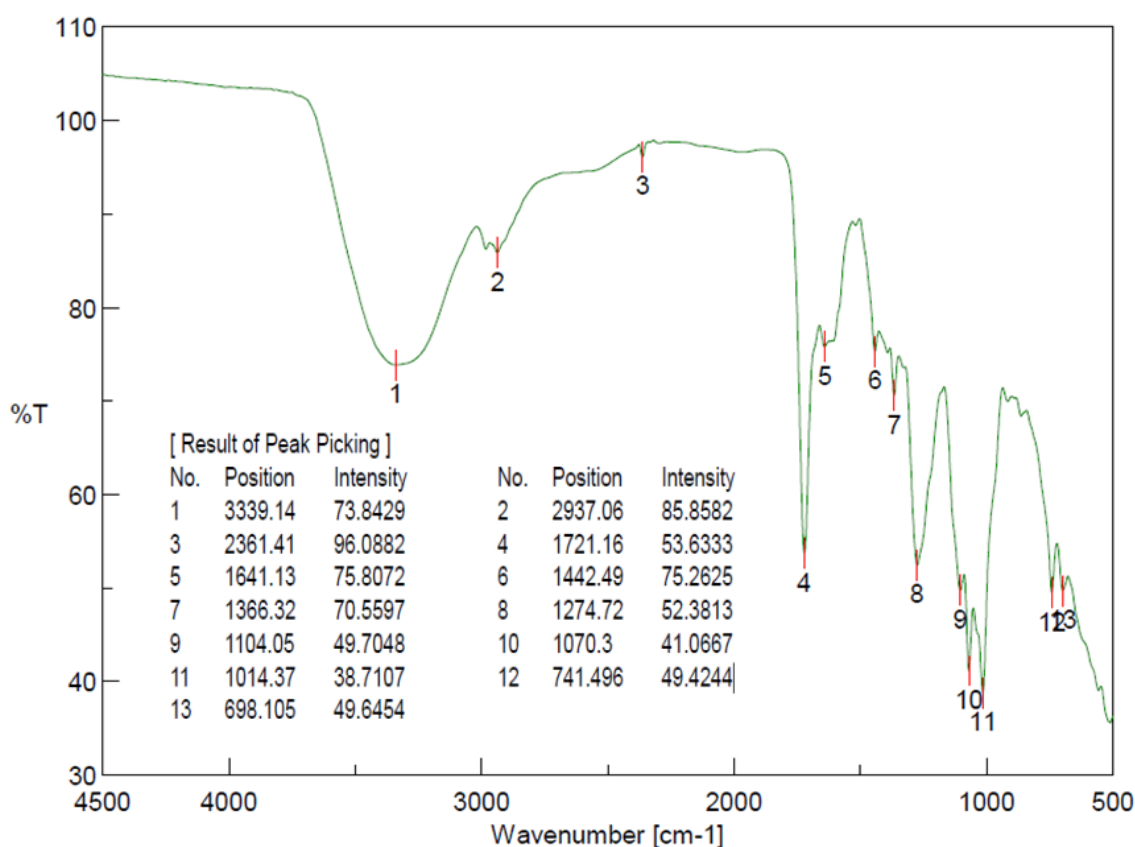
Rejecting  $H_0$  ( $p$ -value  $< 0.05$ ) suggests that citrus peel pectin has a higher DE compared to commercial pectin.

But with  $p = 0.4420$  means that there is only a slight difference between the two pectins, meaning that they could be of similar quality.

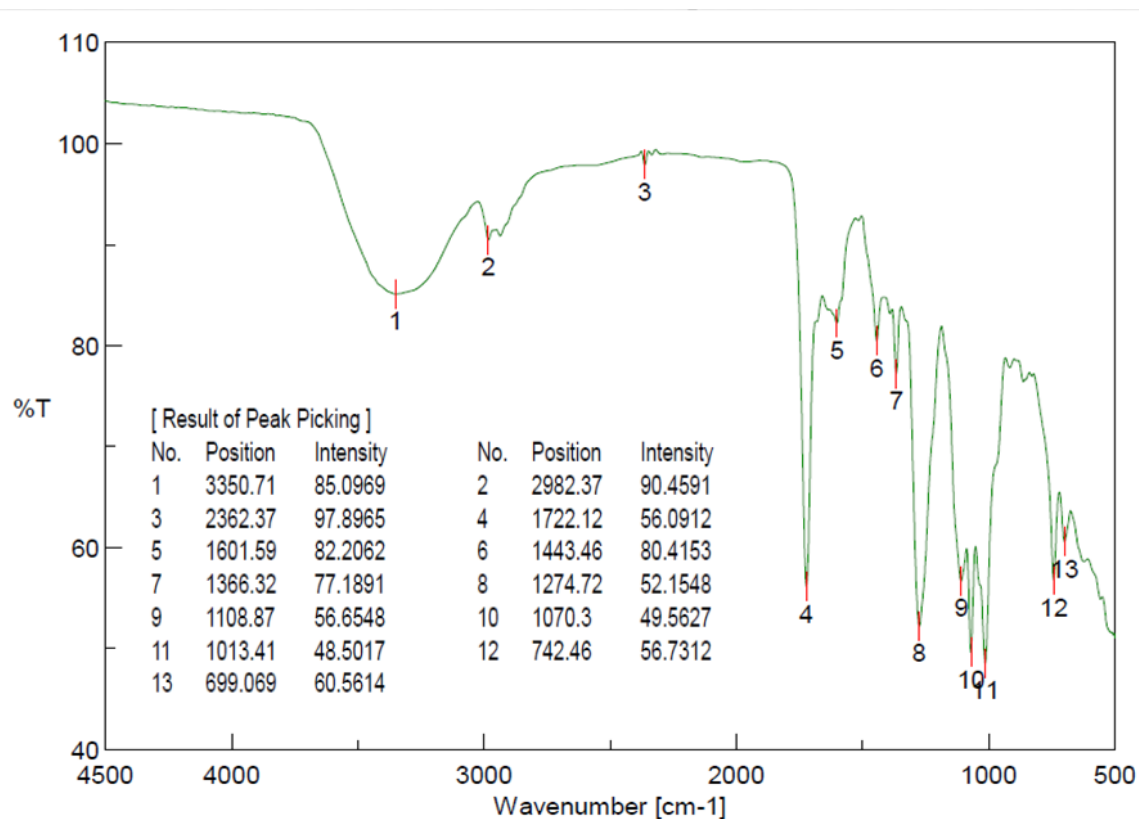
## 5.4 Characterization studies:

### 5.4.1 Fourier Transform infrared spectroscopy:

FTIR analysis of pectin extracted from both day 1 and day 2 of *Citrus sinensis* peels was done at Centre for advanced studies, Sathyabama Institute of Science and technology (Model Name FT/IR-6600typeA, Serial Number B049361790) was used to evaluate the extracted pectin.



**Fig:5.3: Day 1 Pectin FTIR**



**Fig:5.4: Day 2 Pectin FTIR**

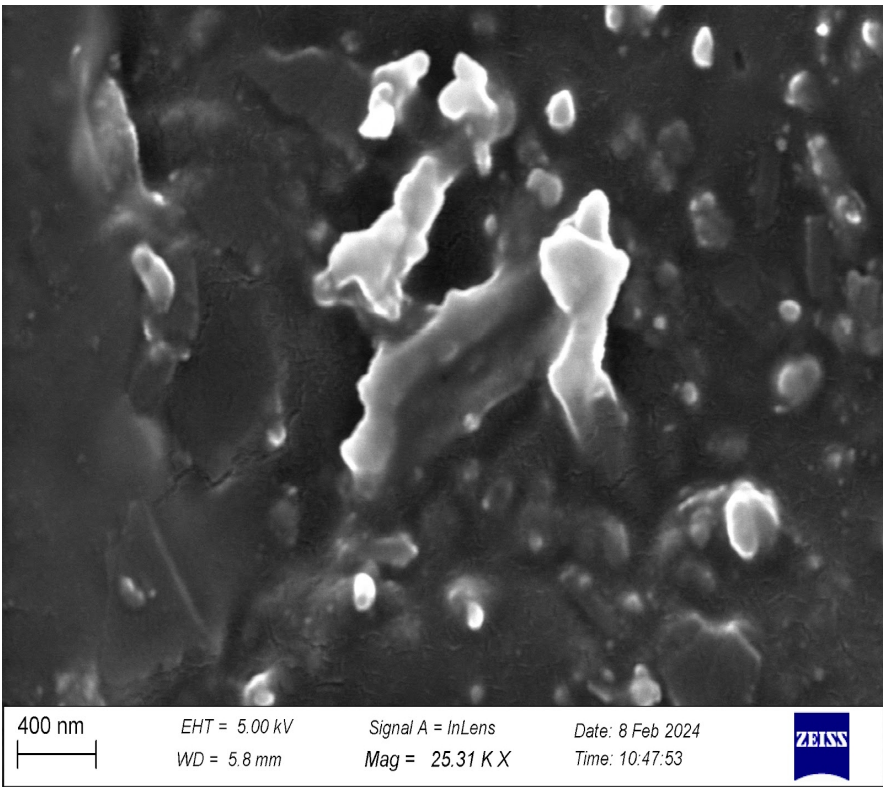
The FTIR spectrum of pectin shows peaks corresponding to various functional groups present in the molecule. Carboxylic acids (around 1740  $\text{cm}^{-1}$ ) peak indicates the presence of galacturonic acid, the main building block of pectin. The intensity of Ester groups (around 1740  $\text{cm}^{-1}$ ) peak relative to the carboxylic acid peak reflects the degree of esterification. A higher intensity suggests more methyl ester groups attached to galacturonic acid units. Hydroxyl groups (around 3000-3500  $\text{cm}^{-1}$ ): This broad peak represents O-H stretching from the carbohydrate units in pectin. From the differences observed between day 1 and day 2 pectin, it can be concluded that day 1 pectin was of higher quality in terms of the aspects of evaluation.

#### **5.4.2 Field-emission Scanning electron microscopy:**

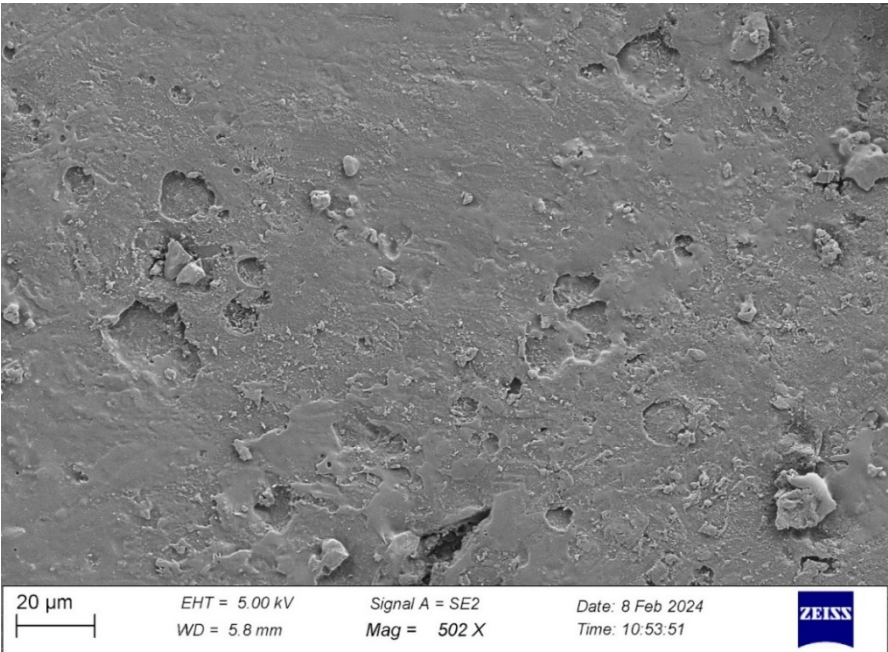
The Pectin extracted from day 1 and day 2 *Citrus sinensis* peels was sent for FESEM analysis to get high-resolution images of surface materials. This analysis

was done at Centre for advanced studies, Sathyabama Institute of Science and technology.

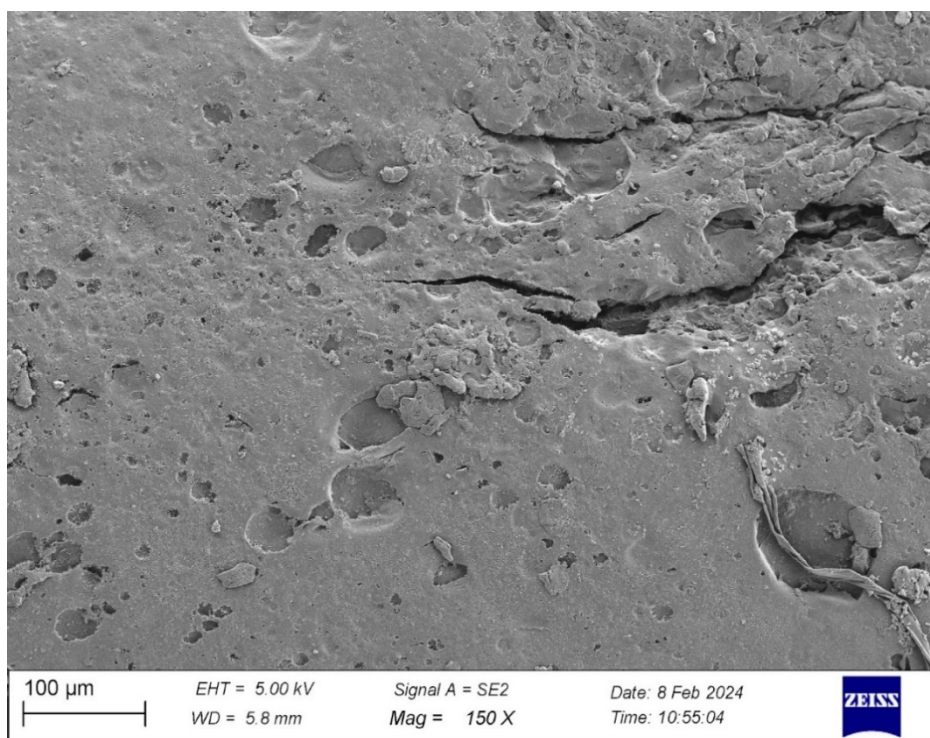
Day 1 Pectin FESEM images:



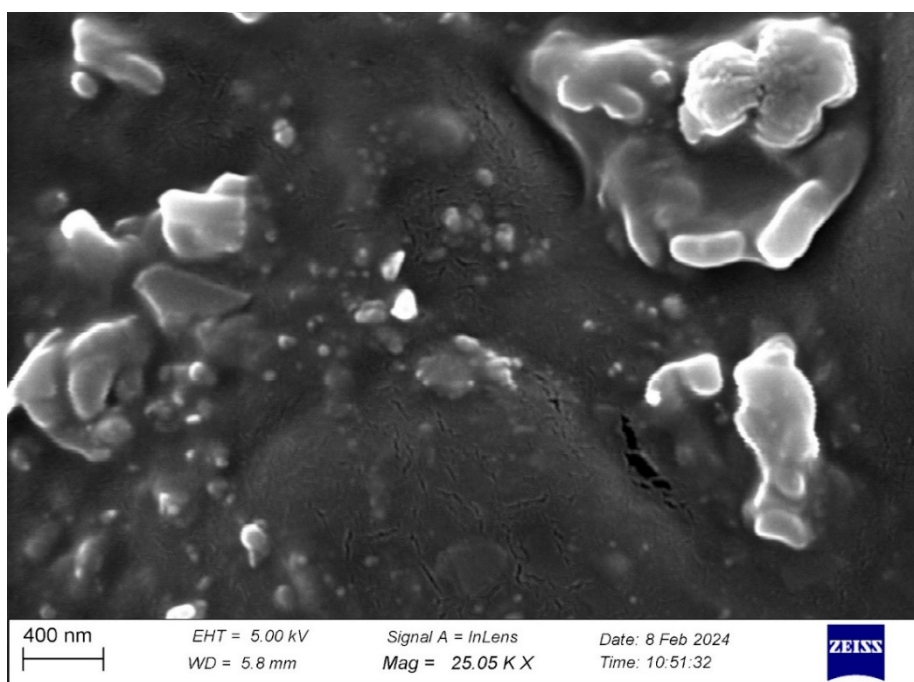
**Fig:5.5: Day 1 FESEM Images at 25.31 K X**



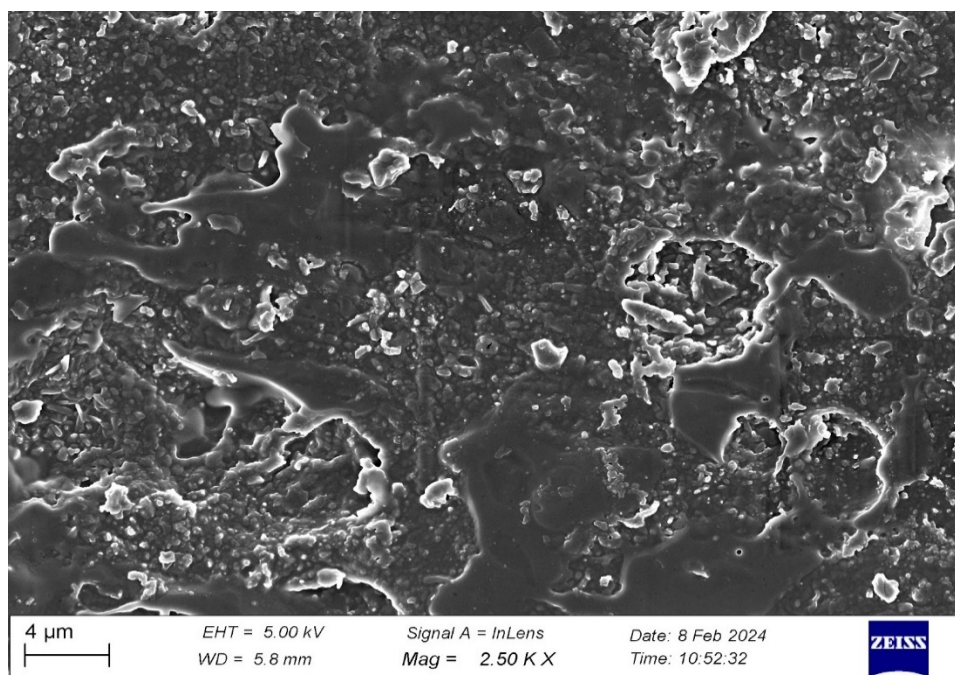
**Fig:5.6: Day 1 FESEM Images at 502 X**



**Fig:5.7: Day 1 FESEM Images at 150 X**

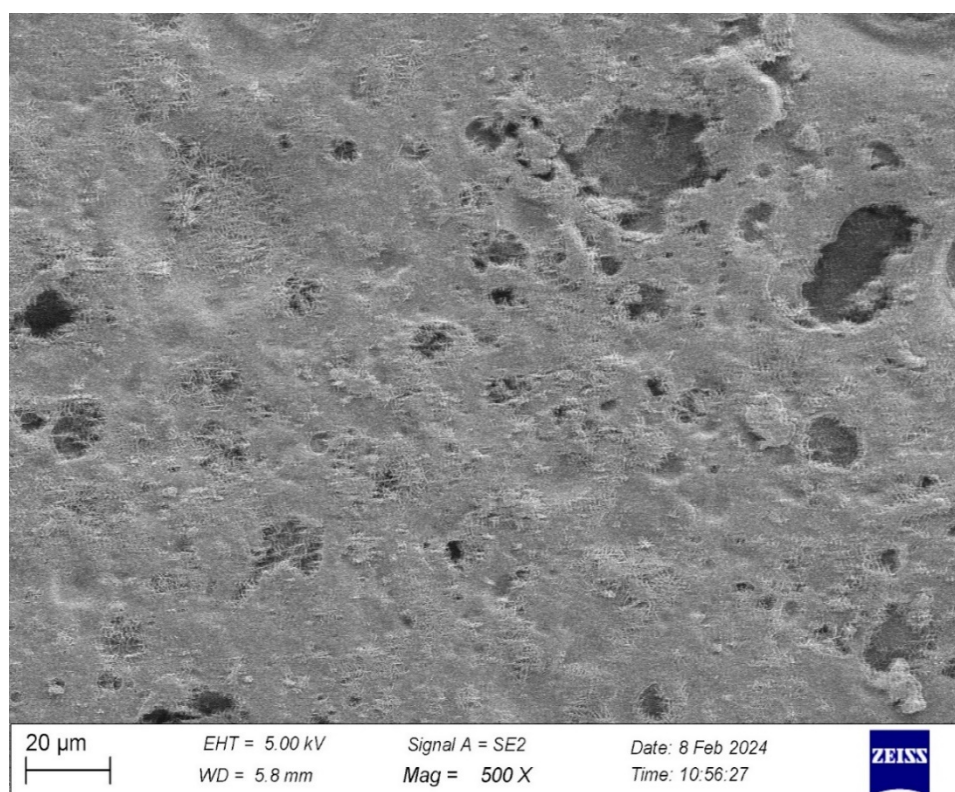


**Fig:5.8: Day 1 FESEM Images at 25.05 K X**

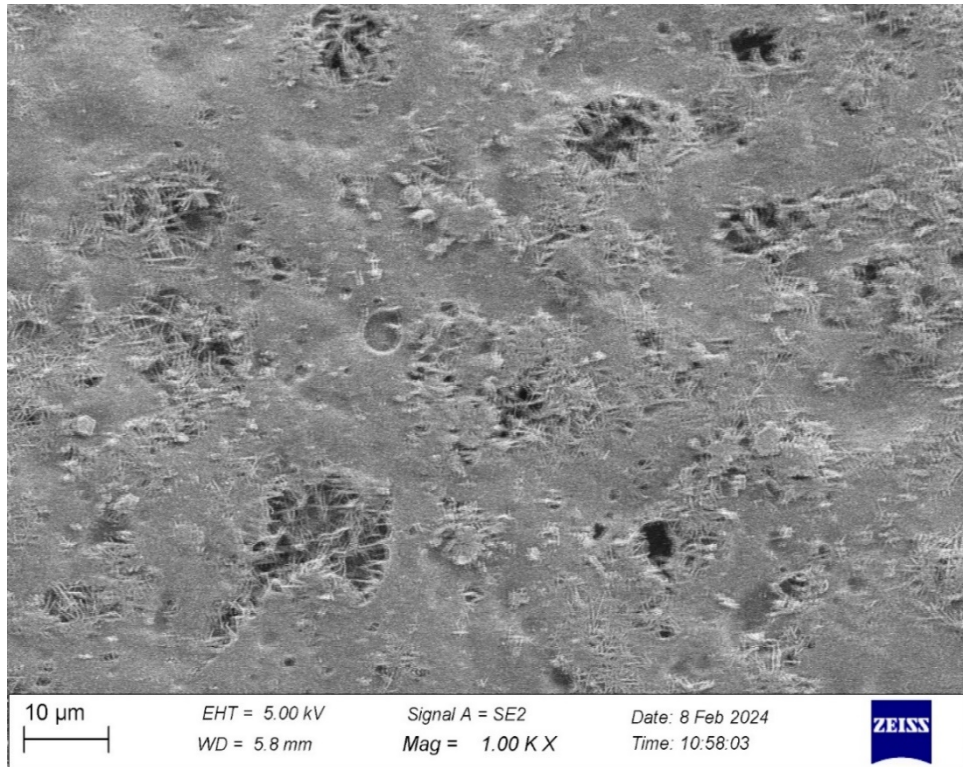


***Fig:5.9: Day 1 FESEM Images at 2.50 K X***

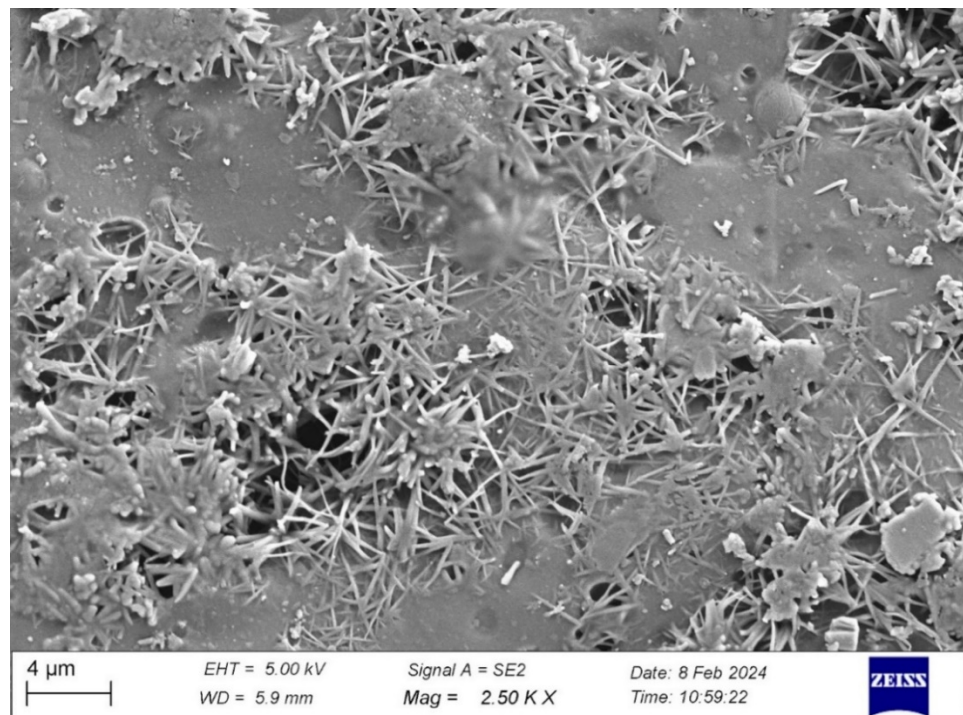
Day 2 Pectin FESEM images:



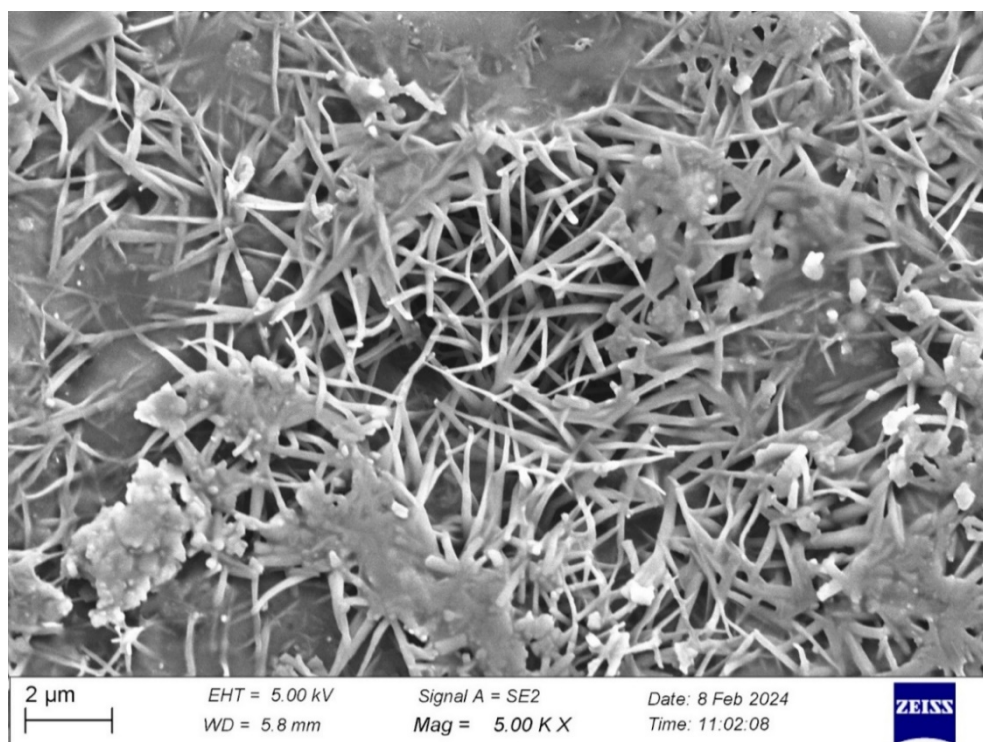
***Fig:5.10: Day 2 FESEM Images at 500 X***



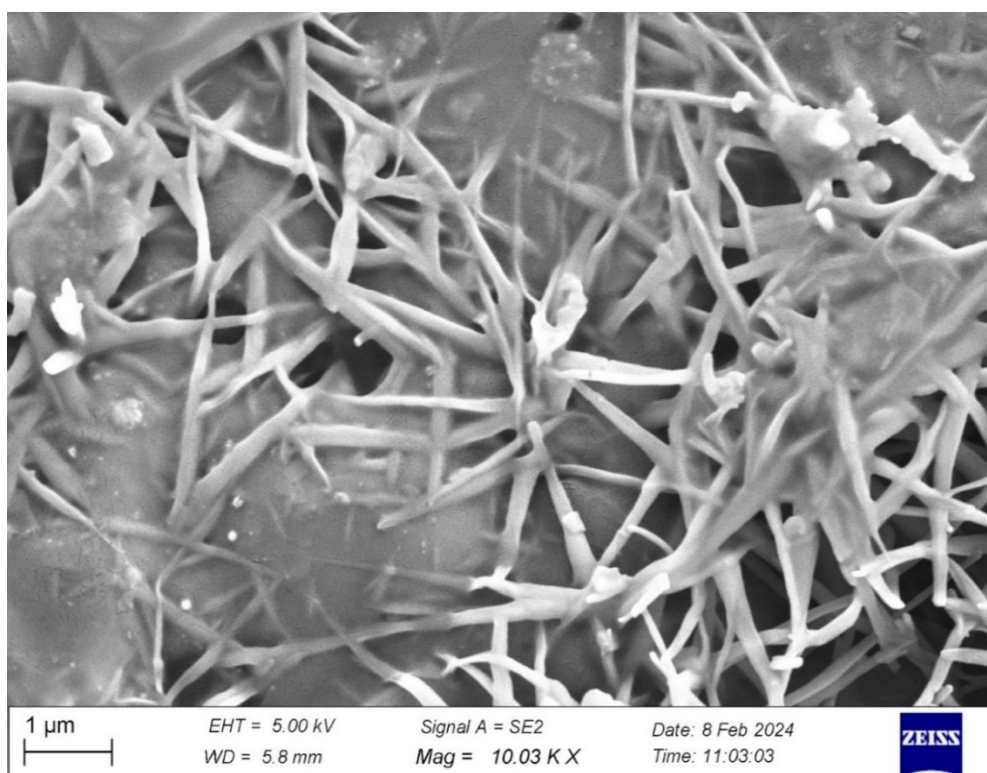
**Fig:5.11: Day 2 FESEM Images at 1.00 K X**



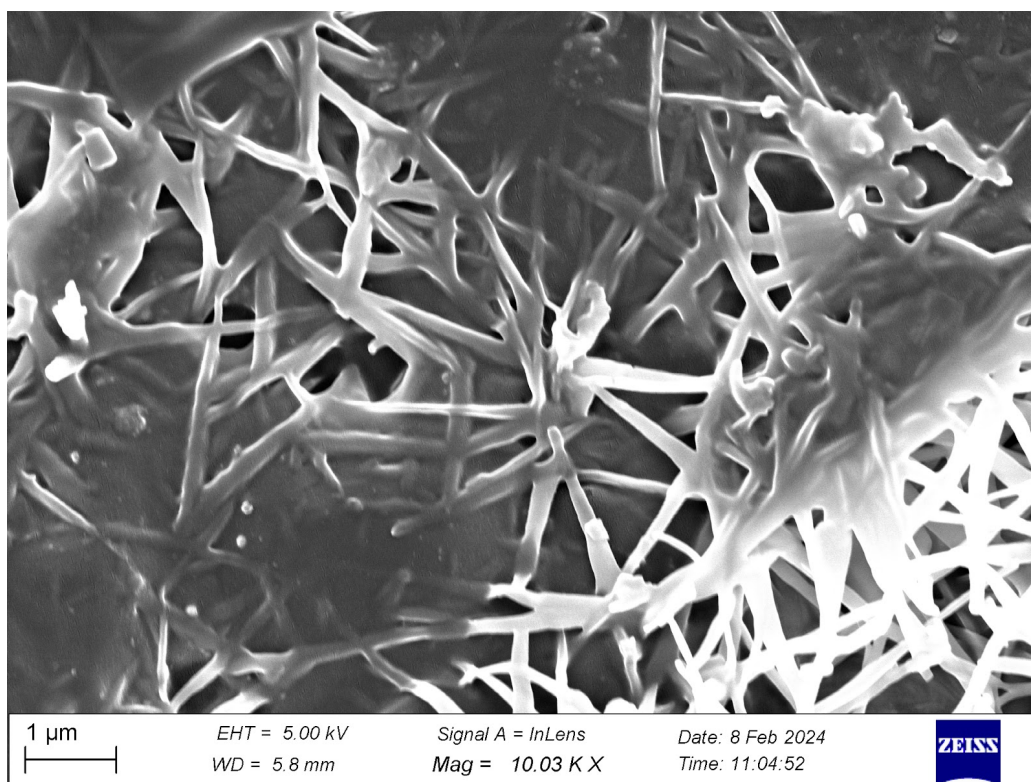
**Fig:5.12: Day 2 FESEM Images at 2.50 K X**



**Fig:5.13: Day 2 FESEM Images at 5.00 K X**



**Fig:5.14: Day 2 FESEM Images at 10.03 K X**

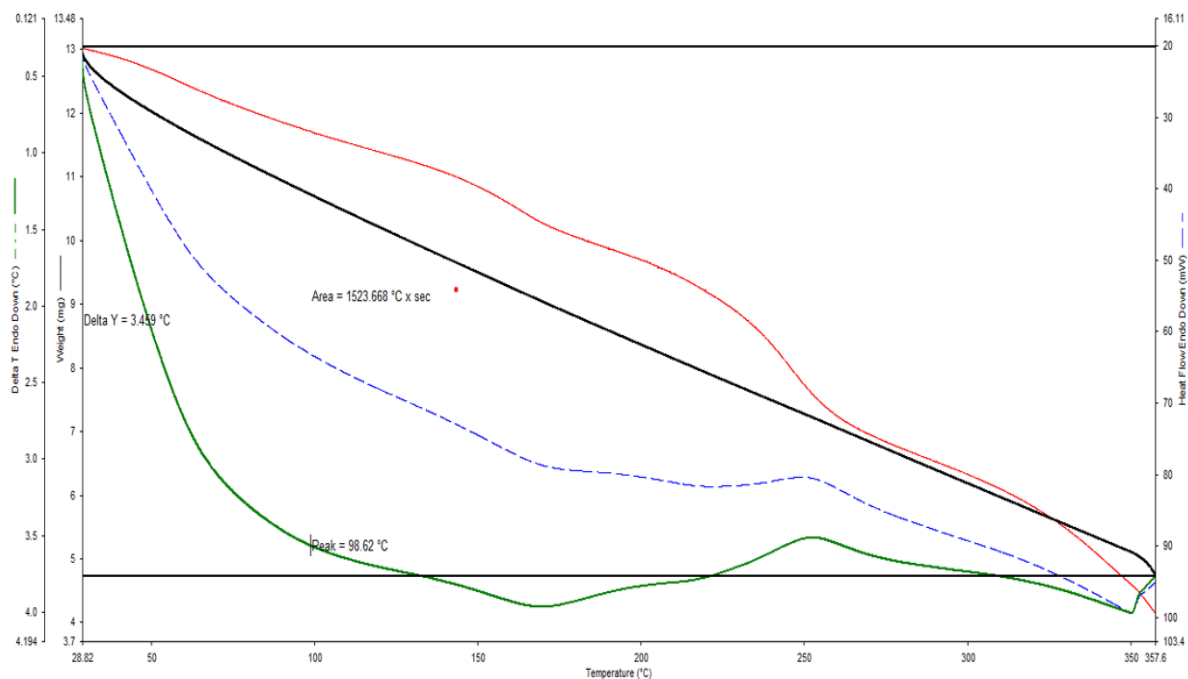


**Fig:5.15: Day 2 FESEM Images at 10.03 K X**

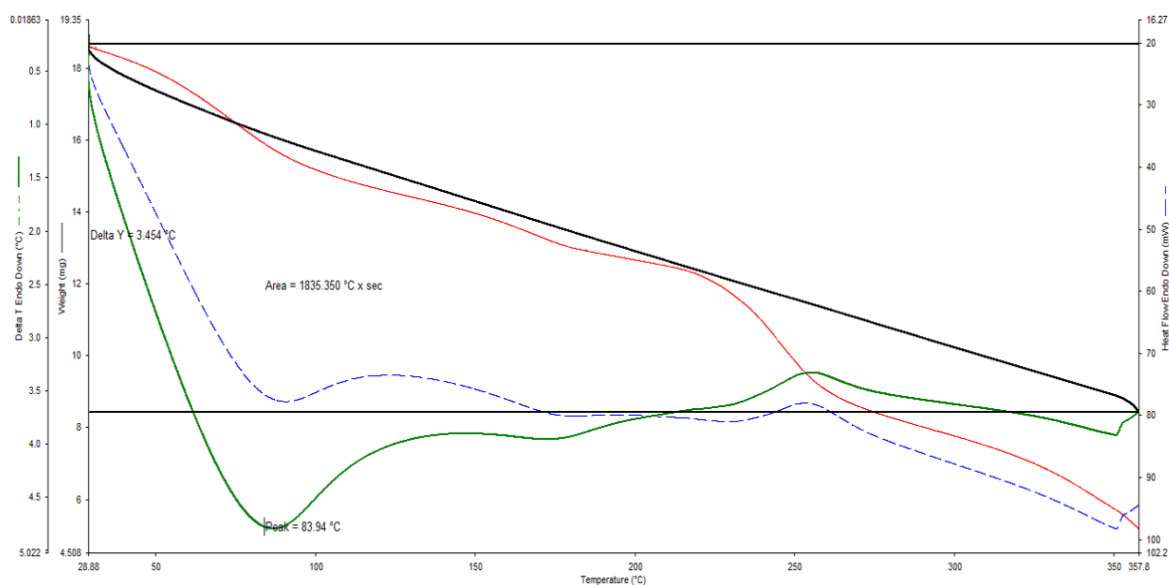
The day 2 pectin had uneven surface characteristics which often arise as a result of changes in sugar units' composition which affects the drying characteristics and gelation ability giving rise a Pectin that dries faster as the gelation ability is lower. Thus, day1 can be considered as a better fit.

#### **5.4.3 Thermogravimetry analysis:**

The Pectin extracted from day 1 and day 2 *Citrus sinensis* peels was sent for TGA and DSC to analyse the thermal stability and decomposition behaviour of pectin. This analysis was done at Centre for Ocean Research, Sathyabama Institute of Science and technology.



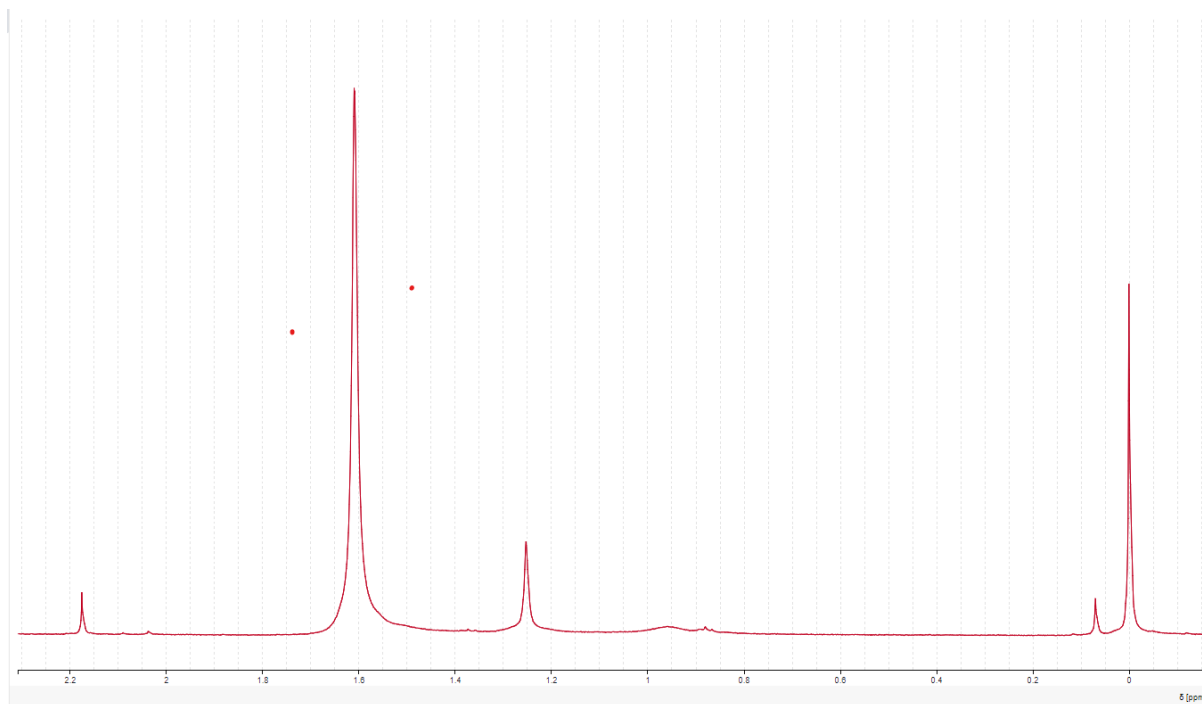
**Fig:5.16: Day 1 Pectin TGA**



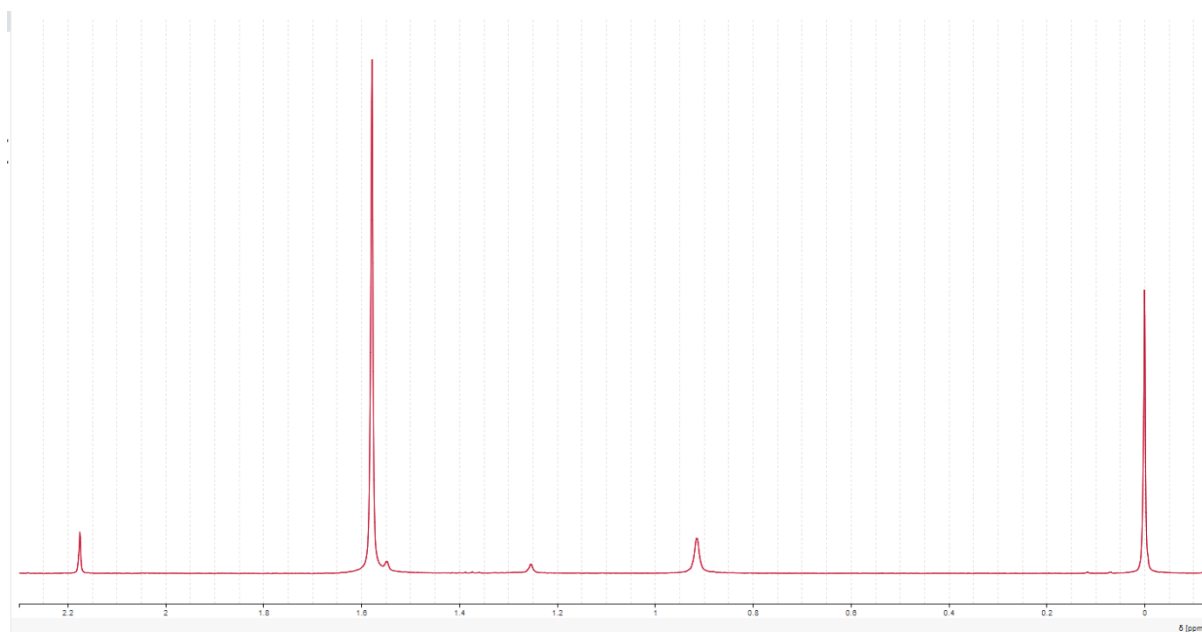
**Fig:5.17: Day 2 Pectin TGA**

From the TGA results shown above, the thermal stability of the day 1 and day 2 are observed as 98.62° C and 83.9° C respectively. This infers that the thermal stability of day 1 pectin is higher than day 2 pectin.

#### 5.4.4 Nuclear Magnetic Resonance:



**Fig:5.18: Day 1 Pectin NMR**

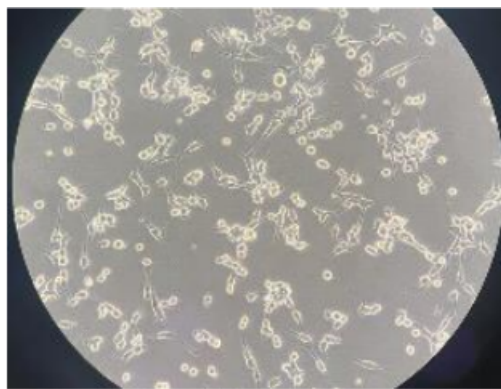


**Fig:5.19: Day 2 Pectin NMR**

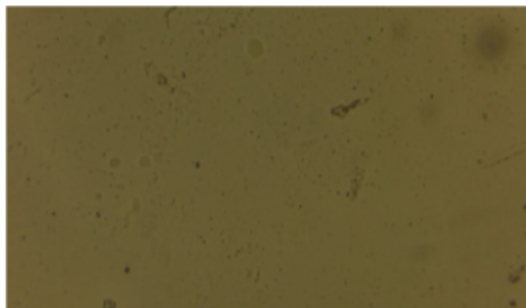
The primary sugar units in citrus pectin are galacturonic acid and rhamnose. Signals from protons in these sugar units typically resonate between 1.0 ppm and 5.5 ppm in a  $^1\text{H}$  NMR spectrum. The NMR results reveal that the Day 1 and Day 2

Pectin have structural differences at the molecular level. This proves that the quality of pectin changes at the very molecular level itself just within a span of 24 hours. The galacturonic acid peak which is observed between 1.0 and 1.5 is more pronounced Day 1 pectin compared to Day 2 pectin. This quality must be taken into consideration as the galacturonic acid is one of the major factor that influences the nature of pectin and thus its applications.

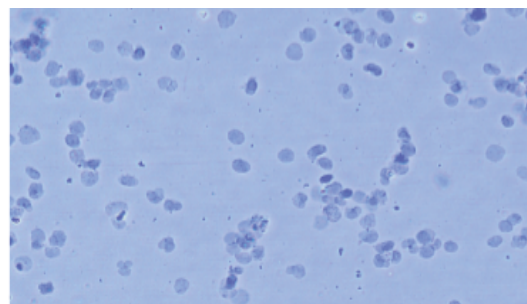
#### **5.4.5 MTT Assay:**



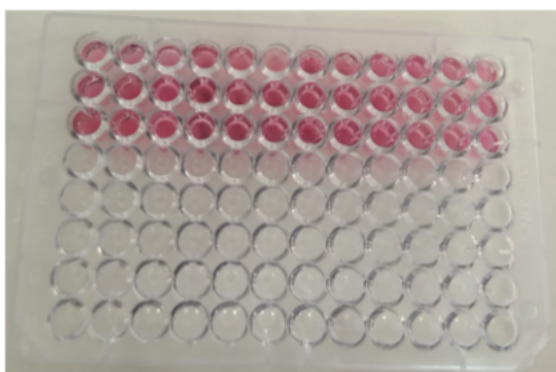
***Fig:5.20: HCT 116 - CCL-247***



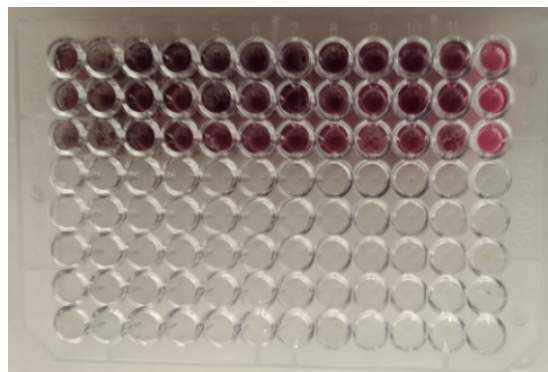
***Fig:5.21: Cells before staining in 4X***



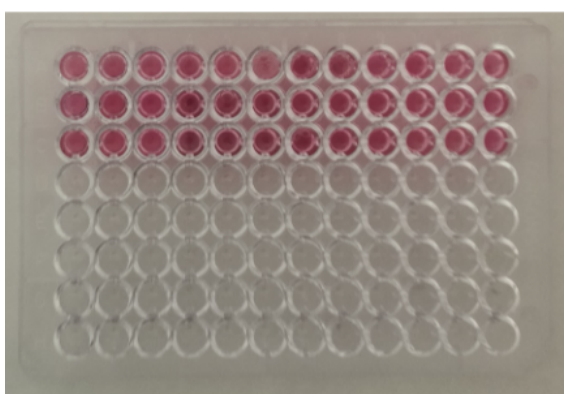
***Fig:5.22: Cells after staining in 100X***



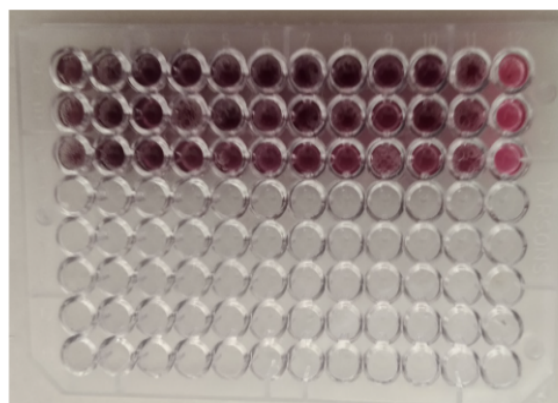
**Fig:5.23: Cells before MTT**



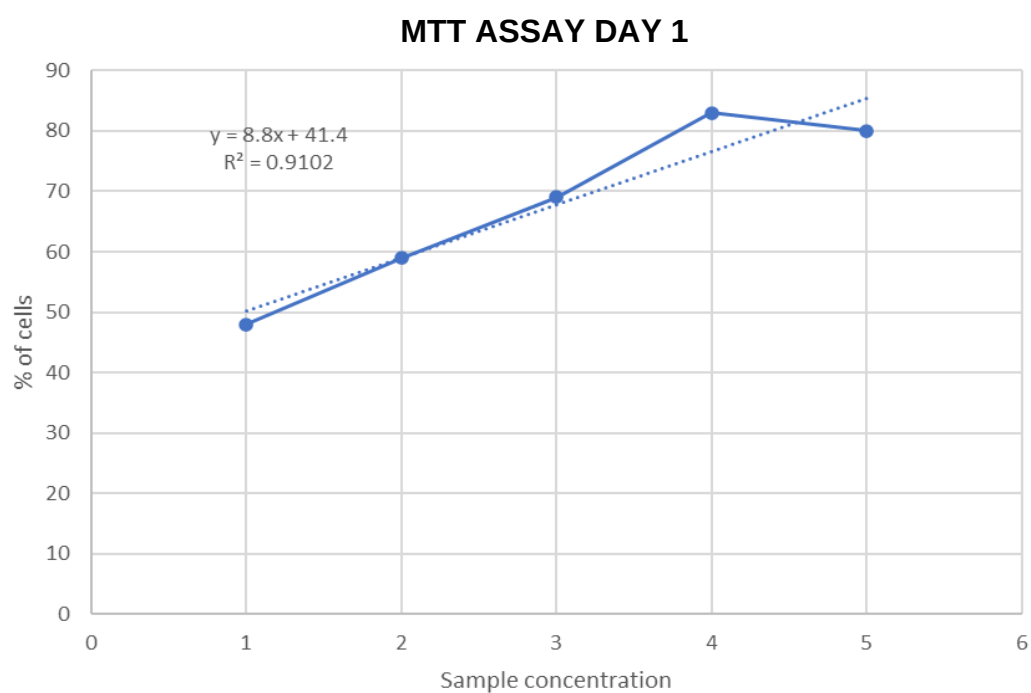
**Fig:5.24: Cells after MTT Day 1**



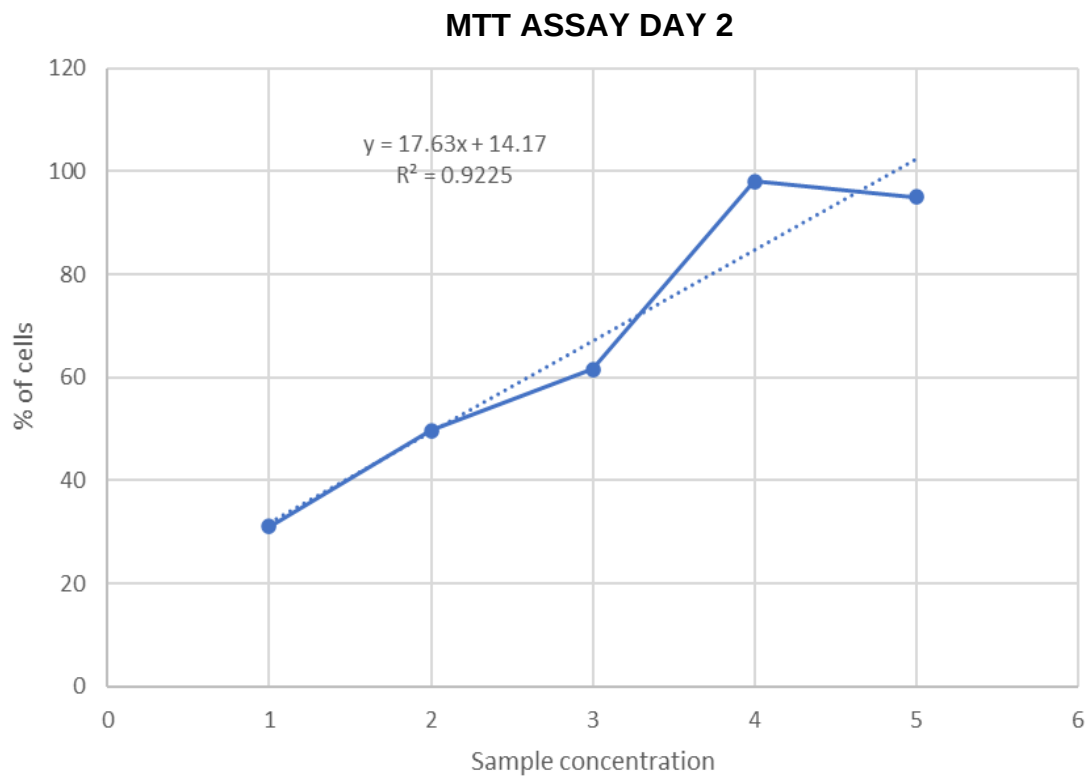
**Fig:5.25: Cells before MTT**



**Fig:5.26: Cells after MTT Day 2**



**Fig:5.27: MTT Assay Day 1 Pectin**



***Fig:5.28: MTT Assay Day 2 Pectin***

The IC<sub>50</sub> value of Pectin on Day 1 was determined to be 0.97727, whereas the IC<sub>50</sub> value for Day 2 Pectin was established at 2.3233. This observation demonstrates that the efficacy of Day 1 pectin is nearly twice as strong as that of Day 2 pectin, highlighting a considerable excellence of Day 1 pectin over Day 2 pectin.

## 5.5 DISCUSSION:

Pectin, a naturally occurring substance present in the cell walls of all higher plants, has traditionally been valued for its ability to create gels and provide thickening and stabilizing effects. Its versatility finds application across various sectors including food, pharmaceuticals, and cosmetic (Abebe Alamineh, 2018). In this study, pectin extraction was performed on various citrus fruit samples using the Microwave extraction method. Among these, *Citrus sinensis* yielded 14%. Likewise, in a study conducted by Abebe Alamineh (2018), pectin extracted from citrus yielded 9%. Similarly, Pandharipande & Makode (2012) 12% pectin was extracted from citrus fruits.

Further purification of the extracted pectin was carried out using Bromine water method. Among the 16 pectin samples extracted from 4 distinct samples over a span of 4 days, it was evident that only up to Day 2 did the pectin exhibit a substantial yield, beyond which there was a sharp decline in the yield. This signifies that utilizing three- or four-day old fruit peels for pectin production may not be advantageous. This observation was confirmed by the Degree of esterification determined through titration method. The values of the degree of esterification also indicated that mixed citrus peels might not result in a superior quality pectin as anticipated. This trend was also reflected in the pectin yield. When comparing all other three citrus peel pectin samples from Day 1 and Day 2, it was observed that *Citrus sinensis* demonstrated the most remarkable yield and quality, as discerned from the degree of esterification values. Similarly, Pandharipande & Makode (2012) conducted a study on the purification of pectin by treating it with solutions of various pH levels. Among the treatment conditions, a pH of 2 resulted in the purification of 3.4% pectin. (Pandharipande & Makode, 2012)

Degree of Esterification is the ratio of esterified galacturonic acid groups to total galacturonic acid groups present. DE is an important property that determines the gelling nature of pectin (Altaf *et al.*, 2015). In the present study, *Citrus sinensis* and other citrus fruits underwent analysis to determine the degree of esterification, resulting in a value of 77%. A similar study conducted by Hannington Twinomuhwezi (2020) reported a degree of esterification of 85% from citrus peel. However, this range did not exceed the findings of our study.

Following the selection of *Citrus sinensis* for assessing the impact of decay on yield and quality, various factors such as surface characteristics, chemical structure, anti-cancer effects, etc., were evaluated using FTIR, FESEM, TGA, NMR, and MTT Assay, and the outcomes were compared. The analysis revealed that the pectin from Day 1 exhibited the most desirable attributes, although the pectin from Day 2 also demonstrated noteworthy results. Subsequently, a statistical comparison was made between the pectin extracted from citrus peels and commercially available pectin, confirming that the pectin from Day 1 shared similar quality characteristics with the commercially extracted pectin.

FTIR characterization was used to know the functional group contained in the pectin. In our study, Carboxylic acids (around  $1740\text{ cm}^{-1}$ ) peak indicates the presence of galacturonic acid, the main building block of pectin. The intensity of Ester groups (around  $1740\text{ cm}^{-1}$ ) peak relative to the carboxylic acid peak reflects the degree of esterification. A higher intensity suggests more methyl ester groups attached to galacturonic acid units. Hydroxyl groups (around  $3000\text{--}3500\text{ cm}^{-1}$ ), Similarly done by Hastuti & Kurniawati (2020). The results of FTIR characterization showed OH group at wave number  $3448.72\text{ cm}^{-1}$  and carbonyl group at wave number  $1604.77\text{ cm}^{-1}$ . Pectin spectrum has typical absorption of a strong band between  $1630\text{--}1600\text{ cm}^{-1}$  which is a stretching band of carboxylic ions.

According to the literature, the cytotoxic effects of pectin on cancer cell lines may be related to different factors involving their structure, as conformation of the molecule, degree of esterification and side chain of these polysaccharides. In the present study, purified pectin was undergoing MTT assay against cancer cell line. The IC<sub>50</sub> value of Day 1 Pectin was found to be  $0.97727\text{ mg}$  and The IC<sub>50</sub> value of Day 2 Pectin was found to be  $2.3233\text{ mg}$ . This proves that the day 1 pectin is almost double times as potent as the day 2 pectin.

The report reveals that in order to extract pectin from citrus peels while maintaining a yield and quality comparable to commercially produced pectin, the waste must be dehydrated and processed within 24 hours of peeling the fruits. Adhering to this “safe window” facilitates the production of pectin equivalent in quality to commercially available pectin, even from fruit waste, thus establishing a competitive and sustainable production approach.

## CHAPTER 6

### SUMMARY AND CONCLUSION

Pectin was extracted from all 16 samples which includes 4 samples for each day for 4 categories of Citrus peels like *Citrus sinensis*, Citrus limetta, Citrus reticulata and Mixed fruits to evaluate the effect of decay on the yield and quality of pectin. Out of which *Citrus sinensis* had the highest yield and quality which was evaluated by calculating the degree of esterification with titration. From which specifically day 1 and day 2 *Citrus sinensis* pectin was most significant thus further characterization studies including FTIR, FESEM, TGA, NMR and MTT Assay was done. The results showed the differences between day 1 and day 2 in various aspects and day 1 turned out to be the best of the two. The day 1 *Citrus sinensis* pectin was compared with commercial pectin using t test and the results showed that the pectin produced from day 1 *Citrus sinensis* peels was just as significant as commercial pectin which means that with this knowledge, sustainable production of pectin from fruit peels can be upscaled without compromising the yield and quality which is a common challenge faced by processes that use wastes for production.

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# ANNEXURE

← Submissions Being Processed for Author ⓘ

Page: 1 of 1 (1 total submissions)Results per page 10 ▾

| Action  |  Manuscript Number  | Title  | Authorship  | Initial Date Submitted  | Status Date  | Current Status  |
|------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|
| <a href="#">Action Links</a>                                                             | IJBIMAC-D-24-06004                                                                                                                                                                    | A Review of Pectin Extraction from Fruit By-products and Its Industrialization Hurdles  | Other Author                                                                                 | Mar 23, 2024                                                                                               | Mar 25, 2024                                                                                    | With Editor                                                                                        |

Page: 1 of 1 (1 total submissions)Results per page 10 ▾



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ABSTRACT

Pectin, a bioactive polysaccharide, is primarily dicotyledons. This significant compound possesses various industries, such as nutraceuticals, pharmaceuticals, food processing industries discard substantial waste. Pectin can serve as a plentiful and valuable reservoir of polysaccharides for the sustainable production of pectin through the utilization of food waste. Being a scientifically supported concept, the impact of food industry waste on a large scale has not yet been fully explored. Factors like the impact of food waste on the yield and quality of pectin, lack of infrastructure to manage such substances

Match Overview

|   |                                                                                                                                                                              |     |
|---|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 1 | <b>Crossref</b> 171 words<br>Anil B. Kute, Debabandya Mohapatra, Nachiket Kotwaliwal e, Saroj Kumar Giri, B. P. Sawant. "Characterization of Pectin from Citrus Peel Waste". | 2%  |
| 2 | <b>Internet</b> 171 words<br>crawled on 07-Sep-2022<br>www.mdpi.com                                                                                                          | 2%  |
| 3 | <b>Internet</b> 135 words<br>crawled on 06-Dec-2016<br>www.fhjdj.com                                                                                                         | 1%  |
| 4 | <b>Internet</b> 123 words<br>crawled on 06-Dec-2021<br>link.springer.com                                                                                                     | 1%  |
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| 6 | <b>Crossref</b> 56 words<br>Nicky Rahmana Putra, Dwila Nur Rizkiyah, Azrul Nurfaiz Mohd Faizal, Ahmad Hazim Abdul Aziz. "Mini review of unloc                                | 1%  |
| 7 | <b>Crossref</b> 49 words<br>Somya Singhal, Nishant Rachayya Swami Hulle. "Citrus pe ctins: structural properties, extraction methods, modifica ...                           | <1% |
| 8 | <b>Crossref</b> 46 words<br>Florina Dranca, Mircea Oroian. "Extraction, purification and characterization of pectin from alternative sources with a                          | <1% |



## GRADUATE APTITUDE TEST IN ENGINEERING 2024

अभियांत्रिकी स्नातक अभिक्षमता परीक्षा २०२४

ORGANISING INSTITUTE: INDIAN INSTITUTE OF SCIENCE, BENGALURU

### SCORE CARD

Name of the Candidate

**DURGA GOMATHI A**

Name of the Parent/Guardian

**ARUMUGANAINAR P**

Registration No.

**BT24S57402448**

Test Paper

**Biotechnology (BT)**

Date of Examination

**February 10, 2024**

GATE Score

**342**

Marks out of 100

**38.33**

All India Rank (AIR)  
in the test paper

**2970**

**Qualifying Marks**

General

**38.9**

EWS/OBC-NCL

**35.0**

Number of candidates  
appeared for the test paper

**17078**

SC/ST/PwD

**25.9**



*A. Arumuganainar*

*Prof. Chandra Sekhar Seelamantula*

Prof. Chandra Sekhar Seelamantula  
Organising Chairperson, GATE 2024  
On behalf of NCB-GATE  
Ministry of Education (MoE)



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A candidate is considered **qualified** if the marks secured are greater than or equal to the qualifying marks mentioned for the category, for which a valid category certificate, if applicable, must be produced along with this Score Card.

This Score Card is valid  
up to 31<sup>st</sup> March 2027.

### GATE SCORE COMPUTATION

The GATE 2024 score is calculated using the formula

$$\text{GATE Score} = S_q + (S_t - S_q) \frac{(M - M_q)}{(M_t - M_q)}$$

where

M is the marks obtained by the candidate in the paper mentioned on the GATE 2024 Score Card

$M_q$  is the qualifying marks for general category candidates in the paper

$M_t$  is the mean of marks of top 0.1% or top 10 (whichever is larger) of all the candidates who appeared for the test paper

$S_q = 350$ , is the score assigned to  $M_q$

$S_t = 900$ , is the score assigned to  $M_t$

$M_q$  is 25 marks (out of 100) or  $\mu + \sigma$ , whichever is greater. Here,  $\mu$  is the mean and  $\sigma$  is the standard deviation of marks of all the candidates who appeared for the test paper.

Qualifying in GATE 2024 does not guarantee admission to a postgraduate program or scholarship/financial assistance. Admitting institutes may conduct additional tests or interviews for final selection of candidates.

Graduate Aptitude Test in Engineering (GATE) 2024 was organised by Indian Institute of Science, Bengaluru, on behalf of National Coordination Board (NCB) - GATE for the Department of Higher Education, Ministry of Education (MoE), Government of India.

Northeastern University  
College of Science

Dear Durga Gomathi,

Congratulations! It is our pleasure to offer you admission to the Master of Science in Bioinformatics program in the College of Science. We are excited for you to begin your studies in the Fall 2024 term at the Boston campus.

The Admissions Committee was impressed with your academic and personal achievements and believes you will make an excellent contribution to the program. We have confidence in your future success and look forward to embarking on this journey with you.

To secure your place, please visit your [applicant portal](#) to complete your enrollment form and submit your tuition deposit as indicated by **July 15, 2024**.

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We look forward to welcoming you to the Northeastern University community.

Sincerely,



Carla Mattos, Ph.D.  
Associate Dean for Graduate Affairs and Professional Programs  
College of Science