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## PHYSICAL AND CHEMICAL CHARACTERISTICS OF COMPOSITE FILMS MADE FROM COMBINATION OF PECTIN AND CHITOSAN

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### ABSTRACT

We are creating a composite film with two components made of pectin and chitosan and loaded with oregano extract. Films made of pectin and chitosan are created using the solution casting technique. Pectin and chitosan were added to improve the film's water absorption, while glutaraldehyde (0.5 ml) was utilised as a crosslinking agent. By using FT-IR and XRD, the compatibility of these two biopolymers was identified. Pectin and chitosan were combined exactly. In daily life, medicinal plants are frequently utilised to treat a variety of illnesses. Oregano extract has been utilised as an antibacterial agent. The composite membrane was found to have the best antibacterial properties, chemical analysis is also carried out such as swelling study. Oregano-loaded pectin/chitosan films were examined for their capacity to combat the *Escherichia coli* (*E. coli*). These findings imply that bio-composites made of pectin and chitosan can be employed as unique materials in the biomedical and food packaging sectors. We analyze various analysis techniques, including SEM, and TGA to examine the properties of the films.

**Keywords:** Pectin, chitosan, antibacterial, SEM, XRD, F T-IR

### 1: INTRODUCTION:

After being discarded, polymer films made from non-renewable materials frequently harm the environment. Limiting the usage of films that aren't biodegradable and aren't

renewable, research is increasingly focusing on substitute materials [1, 2]. Both natural (natural polymer) and synthetic (synthetic polymer) sources contain the building

blocks known as monomers that form up polymers. Contrary to synthetic polymers, which are produced from non-renewable petroleum resources, natural polymers are produced in large quantities from renewable resources like biodegradable polymers.

**1.1. Pectin:** Pectin, which is abundant in the green sections of terrestrial plants, is made up of complex polysaccharides that are found in the major cell walls of plants. [3]. Where it holds cells together is in the middle lamella, pectin is the main structural element. Vesicles produced in the golgi are used to deposit pectin into the cell wall by exocytosis [4]. Pectin is a material that varies across plants, within a plant through time, and in various parts of a plant in terms of amount, structure, and chemical makeup. Pectin is a vital polymer present in cell walls that aids in the growth of plants and the lengthening of their major cell walls [5]. Pectin can differ between plants, over time, and in different regions of the same plant in terms of quantity, structure, and chemical makeup. A vital polymer that supports plant growth and main cell wall elongation is pectin.

**1.2. Chitosan:** It is a linear polysaccharide composed of d-glucosamine and its 14-link n-acetyl derivative, which are scattered at random (acetylated unit). it is created by exposing the chitin shells of crustaceans, such as shrimp, to an alkaline solution, such as sodium hydroxide. There are numerous

industrial and potential biomedical applications for chitosan. It can be used in agriculture as a biopesticide and seed treatment, assisting plants in warding off fungus infestations. It can be used as a fining agent in winemaking, which also helps to ward off rotting. It can be utilised in self-healing polyurethane paint coatings for industrial use. It serves as an antibacterial agent and a valuable component in bandages in medicine. It can also be utilised to facilitate the delivery of medications via the skin. Biodegradable chitosan films have the ability to maintain the firmness of a variety of food products while preventing weight loss from dehydration. Additionally, composite biodegradable films with chitosan and antibacterial additives are being developed as secure substitutes for preserving food item [6].

**1.3. Antibacterial Drug:** In the Mediterranean and Asia, oregano is a common herb that is frequently used. it can extract aromatic oil from its complete grass, which is oregano essential oil (Oeo). Oeo primarily consists of carvacrol and thymol, both of which have potent biological properties including anti-inflammatory, antibacterial, and anti-oxidative effects. oeo contains a wide range of antibacterial activities and has an inhibitory effect on several different types of bacteria. Bacteria may be combated by oregano and the carvacrol it contains [9]. The staphylococcus

aureus bacterium, one of the most common causes of sickness, causes illnesses like food poisoning and skin infections. In one study, the effect of oregano essential oil on the survival of 14 staphylococcus aureus-infected mice was investigated. It was found that the survival rate for mice given oregano essential oil was 43 percent for more than 30 days, which was almost as high as the survival rate for mice given conventional antibiotics, which was 50 percent.

#### **MATERIALS:**

All chemical used were of analytical grade. pectin and chitosan were purchased from HI Media Laboratories Pvt. Ltd.-Mumbai (India). Antibacterial drug (oregano) is purchased from the general shop in the market which is mfg, by R.B. patel House, Dwarkesh Nager Soc., jaspur Road, Padra, Vadodara, Gujarat, India.

#### **METHODS:**

##### **Complex Film Preparation:**

pectin was dissolved in distilled water, while chitosan was dissolved in 5 weight percent acetic acid. the pectin aqueous solution was gradually mixed with the chitosan solution. After adding all of the chitosan, the mixture was stirred for 0.5 hours while the reaction took place. Following the filtering of the combination, the filter cake was washed with 5 weight percent acetic acid to get rid of the free chitosan and then with warm water to make the filtrate neutral. The complex was dissolved in formic acid, placed onto a Teflon frame model and kept at room temperature for formation after being dried at 70 to 80 °C.

Different Ratios were made as follows:

**Table 1: Composition in different ratio films**

Ratio	Pectin	Chitosan
75:25	2.5	0.5
60:40	1.8	1.2

The complex film formed was then soaked in distilled water for 1 day and dried. From the start of the distillation, when the first drop of essential oil was deposited, through the conclusion of the distillation, when the heating was switched off, these DTs were measured. The composition of the samples are listed in **Table 1**.

#### **1. Extraction of the antibacterial drug:**

Steam distillation is used to extract oregano essential oil from either the leaves or the shoots. researchers and industries in various countries reported different distillation times (dts) for oregano. The following DTs were examined in three replicates: 1.25 min, 2.5 min, 5 min, 10 min, 20 min, 40 min, 80 min, 160 min, 240 min, and 360 min. The Florentine vessel (a separator) was removed

from the device when the vapor pressure was lowered. The oil was measured using an analytical balance and stored at a temperature of  $-5^{\circ}\text{C}$  for analysis. Grams of oil per 100 g of dried oregano leaves was used to determine the oil yield (content).

## **2. Preparation of antibacterial drug loaded pectin/chitosan composite film:**

pectin was dissolved in distilled water, while chitosan was dissolved in 5 weight percent acetic acid. the pectin aqueous solution was gradually mixed with the chitosan solution. After adding all of the chitosan, the mixture was stirred for 0.5 hours while the reaction took place. Following the filtering of the combination, the filter cake was washed with 5 weight percent acetic acid to get rid of the free chitosan and then with warm water to make the filtrate neutral. The complex was dissolved in formic acid, placed onto a Teflon frame model, and kept at room temperature for formation after being dried at  $70$  to  $80^{\circ}\text{C}$ . and add 2ml oregano extract oil.

## **CHARACTERIZATION:**

**1. Swelling study:** physiological fluid was used to conduct the composite films' swelling research (pf). 8.307 g of sodium chloride (NaCl) and 0.367 g of calcium chloride ( $\text{CaCl}_2$ ) were dissolved in 1 litre of distilled water to create this fluid. Then, both of these were mixed to make the pf solution. All ratio films were now divided into little pieces and individually weighed. The films

were dipped into the PF solution and retrieved at various times to be dried on filter paper and weighed. At 24 hours, this technique was repeated numerous times.

## **2. FTIR:**

The films' infrared (FT-IR) Fourier transform spectra were captured by an IR spectrometer (perkin-elmer, model 2000, usa). An aliquot of the film (3.0 mg) was co-ground with KBR (90 mg), dried at  $105^{\circ}\text{C}$  for two hours, and made into a pellet before being measured. The films were first turned to powder using nitrogen liquid. In the frequency range of  $4000$ - $400\text{ cm}^{-1}$  with a resolution of  $4\text{ cm}^{-1}$ , the measurements were carried out at room temperature [11].

## **3. XRD:**

The x-ray diffraction patterns of each film were captured using an ultima iv x-ray diffractometer in the grazing incidence diffraction mode. The film powder was dried as per FT-IR measurement and then crushed to pass a 100-mesh sieve. With a scanning speed of  $0.02^{\circ}/\text{min}$  and a scanning range of  $3$  to  $80^{\circ}$ , the equipment was used to operate  $\text{CuK}\alpha$  radiation produced at 40 kv and 40 ma. A quantitative calculation of the relative crystallinity was done using the debye-scherrer equation, which goes like this:  $l = (k)/(\cos)$ , where  $l$  is the crystallinity size (nm), x-ray wavelength ( $1.54060\text{ nm}$  for  $\text{CuK}\alpha$ ), is the peak width of the diffraction peak profile at half maximum height due to small crystal size (in radians), and  $k$  is a

constant linked to crystallite shape, which is typically [7].

#### 4. SEM (Scanning Electron Microscope):

An electron microscope called a scanning electron microscope (SEM) scans a surface with a concentrated beam of electrons to produce images of the material. The surface topography and chemical makeup of the sample are revealed by the various signals produced as the electrons contact with the atoms in the sample [8]. Using a scanning electron microscope, the surface morphology of gelatin was examined (fei quanta 200 sem). using a sputter coater in a vacuum, gold was applied to the surface of dried gelatin, and then it was photographed.

#### 5.TGA (Thermogravimetric analysis):

TGA calculates the amount and rate (velocity) of change in a sample's mass across time or space in a controlled setting. The measurements are mostly used to determine the compositional properties of the materials as well as their thermal and/or oxidative stabilities. As a result of oxidation, breakdown, or volatiles loss, the approach can assess materials that experience mass loss or gain (such as moisture). Specifically, it is useful for studying polymeric materials such films, fibres, thermosets, elastomers, composites, coatings, and paints [10].

**6. Antibacterial Activity:** Oregano was used to test the antibacterial activity of loaded pectin/chitosan composite film. In a flask, dissolve 2.9 gm of nutrient agar in

100ml of distilled water. Clean the flask and petri dish. Allow the media to cool to room temperature before spreading the test organism (*E. coli*) into the solidified agar solution with a spreader. For 24 hours, a piece of the composite film is placed over an agar plate and incubated at 36° c. The following day, look for the zone of inhibition.

### RESULT AND DISCUSSION:

#### 4.1. Swelling studies:

Swelling is defined as a rise in the volume of a solid or gel due to the absorption of a liquid or gas. The swelling study was carried out according to the process. A PF solution is made by mixing 1 liter of solution containing 8.307 g of NaCl and 1 liter of solution containing 0.367 g of CaCl<sub>2</sub>. A ready-to-use solution for swelling studies.

Swelling can be defined as increase in volume of solid or gel with the uptake of liquid or gas. Swelling is the polymeric network in polymer chain. Pectin/chitosan film were soaked in pf solution for 30 min and then and then take out form PF solution and dried it. And then checked its actual weight. Then start same process 3 or 4 time **(Table 2, Figure 1)**.

$SR = (M_t - M_o) / M_o$  g/g where  $M_o$  = initial mass and  $M_t$  = mass at different time intervals.

#### 4.2. FTIR:

These FTIR tests were performed at PNP Analytical solution, Vadodara (Gujarat,

India) with full precision. The technique of Fourier transform infrared spectroscopy (FT-IR) is used to find chemical and inorganic components that might taint or damage products. As a result, FTIR is widely used to identify the earliest stage of any material. As a result, FTIR spectra were obtained for all composite films with different ratios Pectin/Chitosan (60:40), Pectin/chitosan (75:25) (**Figure 2**).

#### 4.3. XRD:

X-ray diffraction analysis is a technique used in material science to determine a material's crystallographic structure. The sample is subjected to x-ray radiation in this procedure, and measurements are made of the x-ray intensity and scattering as they pass through the material. **Figure 3** shows the XRD spectrum of PECTIN/CHITOSAN composite film. The spectrum shows a hump around  $21.5\text{\AA}$  ( $2\theta = 4.11716$ ) that is consistent with the film's amorphous nature, which was confirmed by an X-ray diffractometer [7].

**4.4. SEM:** A sample is scanned with an electron beam in scanning electron microscopy, which produces a magnified image that can be examined. **Figure 4** shows the electron micrographs of the surface of the Pectin/chitosan composite film. The composite film's particles were found to range in size from 300 to 600 nm. It is evident that the surface morphology does

not contain any rough surfaces or significant agglomerations [8].

**4.5. TGA:** The graph and table of TGA shown above show how stable the gel can be up to what temperature, with the final measurement at  $784.97\text{ }^{\circ}\text{C}$ , its weight remaining only %. The 27.699 % addition of the more stable second component slowed the pyrolysis of the above-shown Gelatin-based formulations. These results could be attributed to the amount of Gelatin dispersion in the blend. As a result, the more stable component should act as a shield, preventing the less thermally stable component from disintegrating (**Figure 5, Table 3**) [10].

**4.6. Antibacterial activity:** *E. coli* was used as the test subject for the antibacterial activity of a film containing oregano extract. In an *E. coli* culture-filled petri dish, one piece of the Pectin/chitosan (75:25) film (labelled as (a) in **Figure 6**) and the Pectin/chitosan (60:40) (labelled as (b) in **Figure 6**) was placed side by side having some distance. This paper's Characterization section discusses the steps involved in creating *E. coli* cultures. The result shows the presence of bioactive compound in pure oregano extract loaded composite film with zone of inhibition measuring diameter 3.7 cm while that of Pectin/chitosan film was measured having diameter 1.6 cm.

Table 2: Different observations of swelling studies.

Time	Pectin/chitosan 75:25	Pectin chitosan 60:40
0 min	0.284	0.221
20 min	0.548	0.606
40 min	0.599	0.630
60 min	0.610	0.635
80 min	0.590	0.587

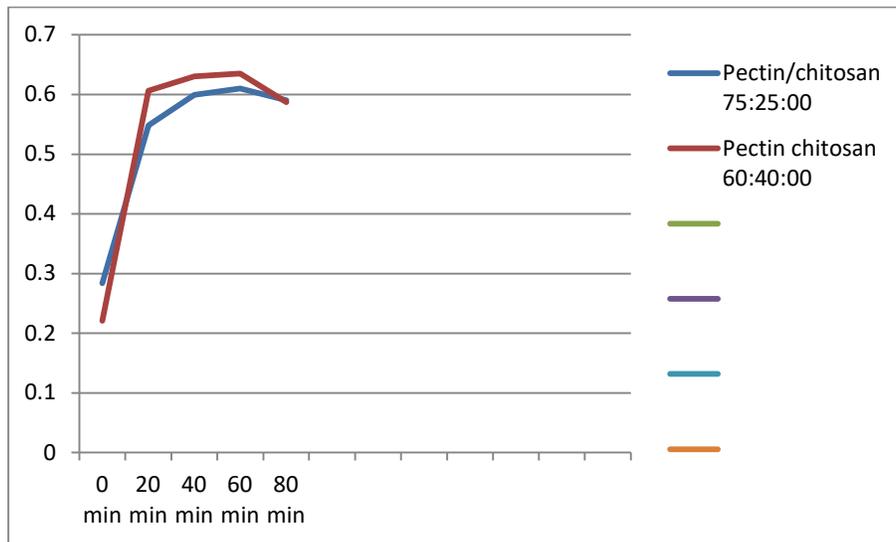
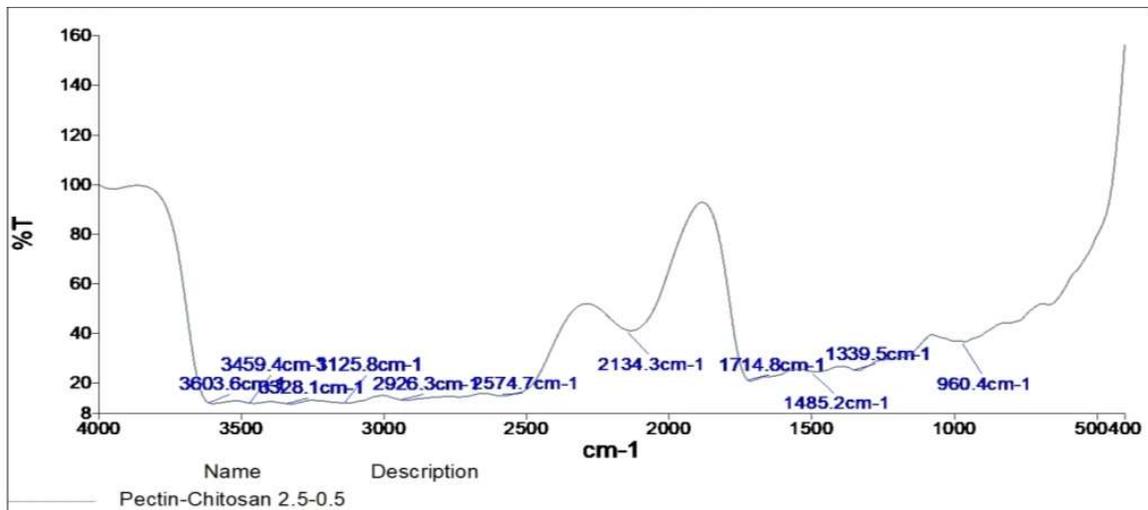
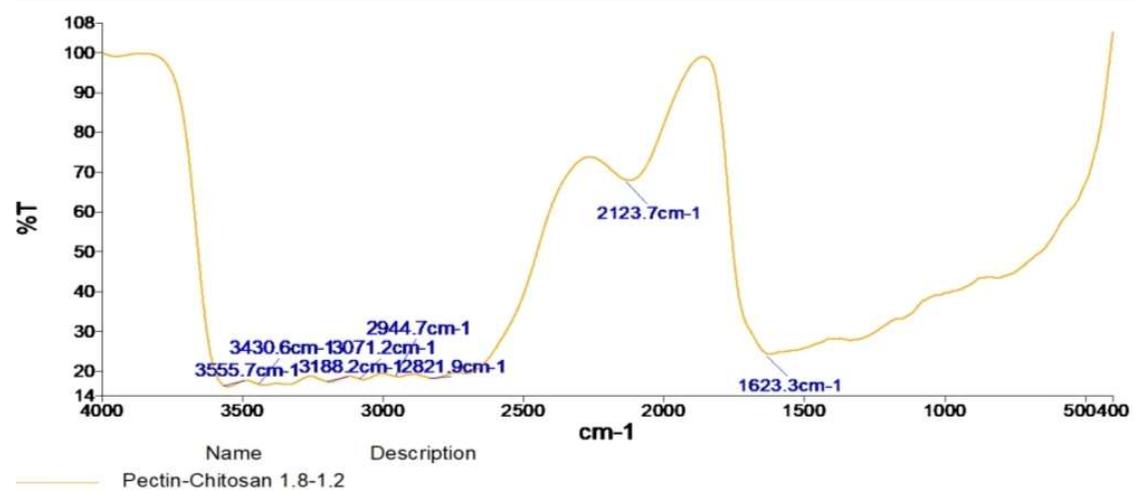


Figure 1: Graph of swelling studies



(a)



(b)  
 Figure 2: FTIR spectrum of pectin/chitosan composite film with different weight ratio:(a)pectin/chitosan =75:25,(b) pectin/chitosan = 60:40.

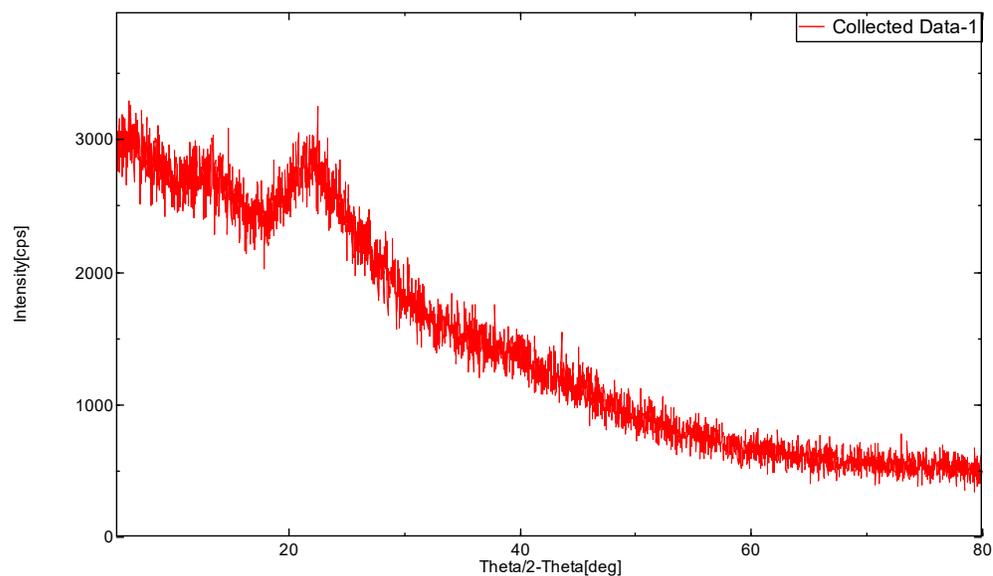
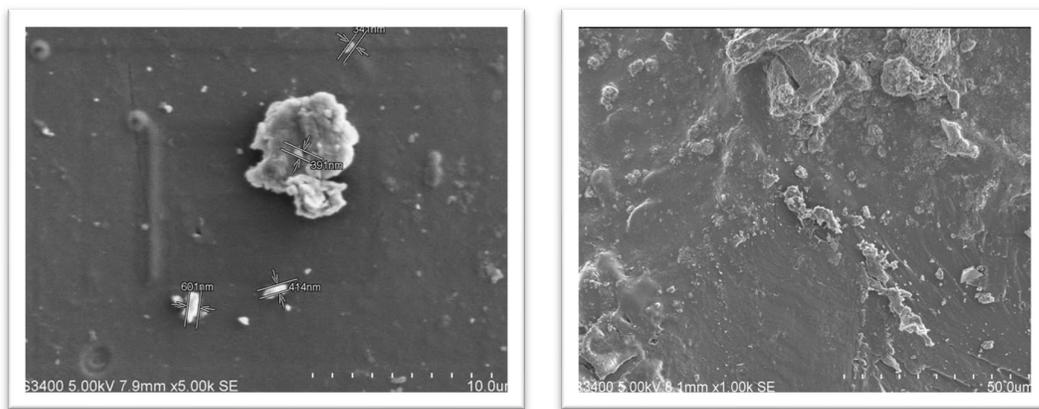


Figure 3: XRD graph of the composite film



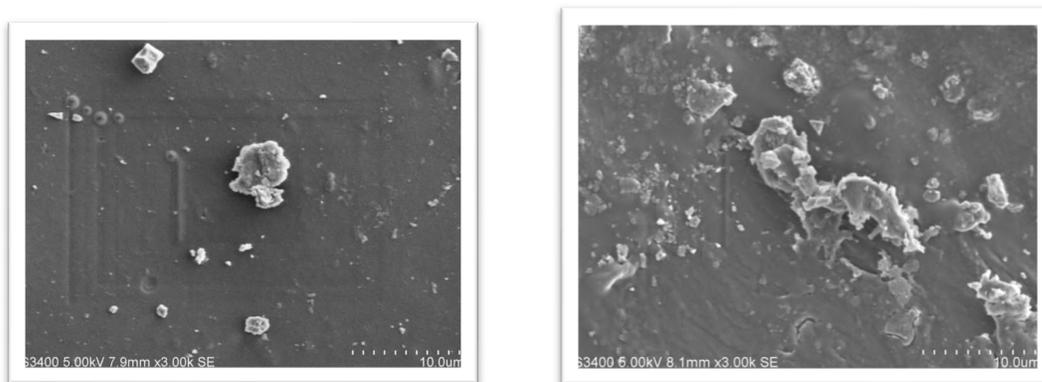


Figure 4: Images of the surface of Pectin/chitosan film

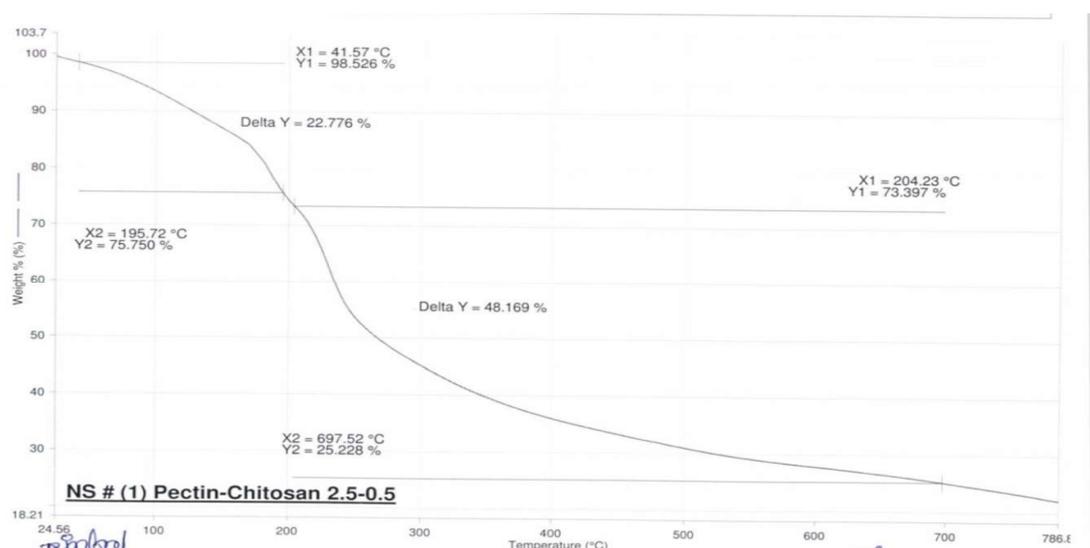


Figure 5: TGA graph

Table 3: Observation of TGA

Temperature	24.56	224.56	324.56	424.56	524.56	624.56	724.56	764.56	784.56
Weight	1.9620	66.040	40.163	34.610	30.025	27.255	24.333	22.974	22.189



Figure 6: Antibacterial activity against *E. coli*

## CONCLUSION

Making biodegradable or consumable packaging materials is a great use of the natural polymers chitosan (Ch) and pectin (Pe). Despite the fact that CH and PE can be used independently, the current study discovered that mixing the two could result in a composite film that performed better than either ingredient utilised separately. More importantly, the blending of CH and PE may have synergistic effects that improve the mechanical and other properties of the resultant films, including transparency. The intermolecular interactions between CH and PE are fundamentally governed by the structure of the involved polymers and the applied environmental conditions. Contextually, given the structural diversity of both CH and PE, more research on this topic should carefully examine the impact of film performance on the structure of the polymer and ambient factors.

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