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Development of Active Packaging by Extraction of Anthocyanin From Red Cabbage

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Abstract-

Anthocyanins are brilliant cell reinforcement/antimicrobial specialists as well as pH-delicate markers that give new possibilities to encourage imaginative savvy bundling frameworks because of their capacity to further develop food time span of usability and identify physicochemical and organic changes in bundled food. Contrasted and anthocyanins from other normal sources, red cabbage anthocyanins (RCAs) are of extraordinary interest in food bundling since they address a variety range over a wide scope of pH values.There is a rising interest in anthocyanins, as normal food colorants, in food items and furthermore in drug items because of their antioxidative potential. The current review manages extraction and cleaning of anthocyanins from red cabbage. Besides, the utilization of RCAs as colorimetric pH-responsive specialists can dependably screen the subjective properties of the packaged food items in a continuous evaluation. Hence, the improvement of brilliant biodegradable movies utilizing RCAs is a promising way to deal with the possibility of food packaging.

***Keywords*: anthocyanins, Biodegradable packaging, pH-values, red cabbage, antimicrobial**.

1.INTRODUCTION

Active packaging is a packaging framework that is composed to keep up with and even further develop the wellbeing properties, organoleptic properties and nature of the packaged food product, in this way expanding its time frame of realistic usability [1].

Regular packaging plastic innovations or dynamic sachets are bit by bit being traded by active nanocomposites for working on the quality and security of packed food items. active nanocomposite films that

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integrate oxygen scroungers could be utilized for packaging an assortment of oxygen-touchy food items. Additionally, nanocomposites could be applied as antimicrobial packaging to control bothersome microorganisms in food varieties. Because of purchaser inclinations for negligibly handled and normally protected food sources and the food business' revenue in putting resources into item quality and security, active packaging will foster from now on. Further examinations are required in regard to various areas of active packaging frameworks [2].

Figure 1. Red Cabbage

2.1.2 Corn Starch

Corn starch, a biopolymer derived from corn, possesses unique properties ideal for food packaging films. Its biodegradability and renewable sourcing make it environmentally friendly. Corn starch films exhibit excellent barrier properties against oxygen and moisture, extending the shelf life of packaged foods. Additionally, its film-forming ability and compatibility with other biopolymers enhance its mechanical strength and flexibility, crucial for packaging durability. Furthermore, corn starch-based films are non-toxic, ensuring food safety [4].

2.1.3 Glycerol

Glycerol plays a pivotal role in the production of food packaging films due to its unique properties. As a humectant, it retains moisture, ensuring the flexibility and pliability of the film, thus preventing it from becoming brittle over time. Its hydrophilic nature enhances the film's ability to adhere to various surfaces, improving its sealing properties. Furthermore, glycerol's biodegradability aligns with sustainability goals in packaging [5].

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Red Cabbage

Red cabbage has a high nutritional value since it is high in minerals, vitamins, oligosaccharides, and a variety of bioactive chemicals that are beneficial to human health, such as anthocyanins, flavanols, and glucosinolates [3]. Red cabbage has its own distinct anthocyanin pattern. It contains a substantial amount of anthocyanins, the major structure of which is cyanidin glycosides in most cases. The major anthocyanin compounds in red cabbage are acylated.

2.1.4 Anthocyanin

Anthocyanins are compounds with complicated patterns of hydroxylation, methylation, glycosylation, and acylation. Anthocyanins are water-soluble pigments found in fruits and vegetables that are red, orange, blue, or purple [6]. Anthocyanins are ingested by humans in amounts that may be physiologically relevant as components of plant food products.

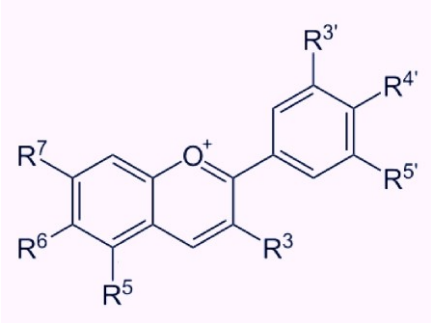

Figure 2. Anthocyanin structure

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2.2 Methods

2.2.1 Extraction of Anthocyanin

Anthocyanins are natural pigments found in many plant sources, including fruits, vegetables, flowers, and grains. These pigments can be extracted from various plant materials using different extraction methods
[7,8].

Different extraction procedures have been investigated, including regular techniques like dissolvable extraction (utilizing ethanol, methanol, or water), as well as present day approaches like ultrasound-helped extraction (UAE) and supercritical liquid extraction (SFE) [9,10].

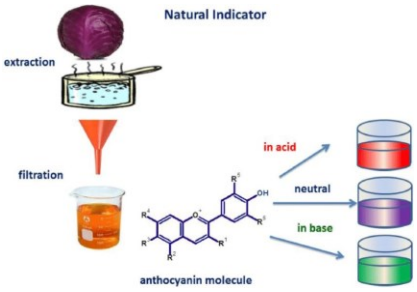

Figure 3. Extraction of anthocyanin

2.2.2 Confirmation of Anthocyanin

Anthocyanins can be confirmed using NaOH and HCl tests. These tests are because anthocyanins are pH sensitive and undergo a color change when exposed to alkaline or acidic solutions [11].

NaOH Test: In this test, a small amount of the sample containing anthocyanins is mixed with a few drops of sodium hydroxide (NaOH) solution. The mixture is then observed for any color change. If anthocyanins are present, the mixture will turn blue or green [12].

The reaction is as follows:
Anthocyanin + NaOH → Blue green color

HCl Test: In this test, a small amount of the sample containing anthocyanins is mixed with a few drops of hydrochloric acid (HCl) solution. The mixture is then observed for any color change. If anthocyanins are present, the mixture will turn red or pink [13].

The reaction is as follows:
Anthocyanin + HCl → Red color

Both tests can be used as preliminary tests to confirm the presence of anthocyanins in a sample. However, they do not provide any information about the identity or concentration of the anthocyanins present. Further analysis, such as high-performance liquid chromatography (HPLC), may be required to identify and quantify the specific anthocyanins present in a sample [14].

2.2.3 Total Anthocyanin Content (TAC)

The total anthocyanin content was estimated by utilizing the pH-differential method, where the TAC is determined as cyanidin-3-glucoside. Anthocyanin extricates were liquefied in a KCl buffer (7ml of pH 1.0 buffer) and Sodium acetic acid buffer (7ml of pH 4.5 support). The distinction between the two cradles at 500 Nm furthermore, 680Nm is proportional to the anthocyanin material [15]. Measurements were made in UV-Vis plate reader or Colorimeter. The TAC is determined utilizing the equation:

$$\text{Anthocyanin concentration} = \frac{\Delta A \cdot MW\ \text{average} \cdot FD \cdot 1000}{\varepsilon \cdot L},$$

(1)

where,

A= diluted sample absorbance

MW= molecular weight (cyanidin-3-glucoside: 449.2g/Mol)
DF=dilution factor (10)

€= molar absorptivity (€=26,900)

L= width of the cuvette (1cm), 1000 is the change calculated from grams to milliequivalent taken 3 times.

2.2.4 Preparation of Film

Measure 10 ml of red cabbage extract using a graduated cylinder and pour it into a clean beaker. Add 0.5 g of cornstarch to the red cabbage extract in the beaker. Add glycerol to the mixture in the beaker. Mix the contents of the beaker thoroughly using a stirring rod until the corn starch is completely dissolved and the mixture is homogeneous. Place the beaker containing the mixture on a hot plate set to 100 degrees Celsius. Heat the mixture in the beaker on the hot plate until the gelatinization of the corn starch is observed [16]. This is indicated by the formation of a gel-like consistency in the mixture. Once gelatinization is achieved, carefully transfer 3 ml of the gel mixture into a clean petri plate. Spread the gel evenly across the surface of the petri plate using a sterile spatula or the back of a pipette tip. Allow the gel in the petri plate to

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dry for 24 hours at room temperature, undisturbed. After 24 hours, the red cabbage indicator gel in the petri plate is ready for further experimentation or analysis.

2.2.4 Characterization of Film

(i) Water solubility

The water solubility test of film was conducted based on its adjustment of size of film samples. The samples were cut into square film pieces and immersed in 20ml (about 0.68 oz) of distilled water at room temperature for 24 hours. After 24 hours, the samples were taken and immediately weighed (M_0). The remaining film was dried for 24 hours, and the final film mass measured (W_1).

$$WS = \frac{(M_0 - W_1)}{M_0} \times 100 \quad (2)$$

(ii) Swelling Index

The swelling index test of film was conducted based on its adjustment of volume of the distilled water. The sample was cut into circles with a diameter of 1.5cm (SI_0) and immersed in 20ml (about 0.68 oz) of distilled water at room temperature for 24 hours. After 24 hours, the film size (SI_1) was measured immediately.

$$SI = \frac{(S_1 - S_0)}{S_0} \times 100 \quad (3)$$

(iii) Film Moisture Content

The moisture content of the film is measured by weighing its initial weight (W) and kept at 150^0C in a hot air oven for 24 hours. After 24 hours measuring the final weight (D). For calculating the total moisture content present in the film, the equation as follows,

$$MC = \frac{(W - D)}{W} \times 100 \quad (4)$$

(iv) pH Sensitivity

The pH sensitivity of packaging film is an important consideration in certain applications, especially when packaging products that are sensitive to changes in acidity or alkalinity. The pH of a substance is a measure of its acidity or basicity and is expressed on a scale of 0 to 14, with 7 being neutral. Substances with a pH below 7 are acidic, while those with a pH above 7 are basic [17]

The color and spectra of anthocyanins at various pH levels:

· Red Color (Low pH):

- At lower pH levels (acidic conditions), anthocyanins typically exhibit a red color. This is because, in an acidic environment, the anthocyanin molecule is in its flavylium cation form, which appears red.
- The absorption spectrum of anthocyanins in the red form shows a peak around 520-550 nanometers.

· Purple Color (Intermediate pH):

- As the pH increases from acidic to slightly neutral, anthocyanins may shift to a purple color. This transition is associated with the conversion of the flavylium cation to the quinoidal base form.
- The absorption spectrum in the purple form shows a peak at a slightly higher wavelength than in the red form.

· Blue Color (High pH):

- Under alkaline conditions (higher pH), anthocyanins can exhibit a blue color. In this environment, the anthocyanin molecule is in its anionic form.
- The absorption spectrum of anthocyanins in the blue form shows a peak at a longer wavelength, often in the blue range.

(v) Storage Stability

The stability of the film was accomplished for 28 days (about 4 weeks) utilizing a gravimetric strategy.
Five film reproduces were put in one compartment under three distinct circumstances,
· Room temperature (25^0C),
· Incubator (37^0C), and
· Cold room (4^0C)
In the wake of gauging the underlying mass on Day 0, the films were burdened Days 7, 14, 21, and 28 to get the last mass.

(vi) Antimicrobial Test

Suspend 28g of nutrient agar powder in 1L of distilled water. Mix and dissolve them completely. Sterilize by autoclaving at 121°C for 15 minutes with a Petri plate, l- rod, tip. Pour the liquid into the petri dish and wait for the medium to solidify in a dark room overnight set. Cut into a piece of film and dip it into the water and place it on an agar plate.

- Red cabbage film

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Chemicals used for testing. The 4 basic chemicals used for Petri plate

- E. coli
- Bacillus
- klebsiella
- Staphylococcus

2.2.5 Optimization of Film

Anthocyanins can be sensitive to light, heat, and oxygen. Research may focus on stabilizing these compounds through various methods, including the addition of stabilizing agents or encapsulation within a protective matrix [18]. Studies may explore different biodegradable polymers or other materials suitable for film formation. The compatibility of these materials with anthocyanins and their impact on film properties (mechanical strength, flexibility, barrier properties) are crucial considerations [19].

Comprehensive reviews might discuss the analytical techniques used to characterize the developed films, such as Scanning Electron Microscope (SEM), Fourier-transform infrared spectroscopy (FTIR) [20]. Evaluating the performance of anthocyanin-based packaging films is essential. This includes assessing their ability to act as barriers against moisture and oxygen, their mechanical properties, and their overall suitability for food or other product packaging [21].

(i) Scanning Electron Microscope (SEM)

SEM offers high-resolution imaging, allowing for the visualization of surface morphology, microstructure, and defects in packaging films at a nanometer scale. SEM can reveal the surface topography, roughness, and uniformity of packaging films, which are crucial for assessing barrier properties and ensuring the integrity of the packaging. SEM enables cross-sectional imaging of packaging films, providing insights into layer thickness, adhesion between layers, and the presence of defects or delamination. SEM can be used to identify and analyze particles or additives embedded within the packaging film matrix, such as fillers, reinforcements, or contaminants. Energy-dispersive X-ray spectroscopy (EDS) coupled with SEM allows for elemental analysis and mapping, helping to identify the distribution of elements within the packaging film.

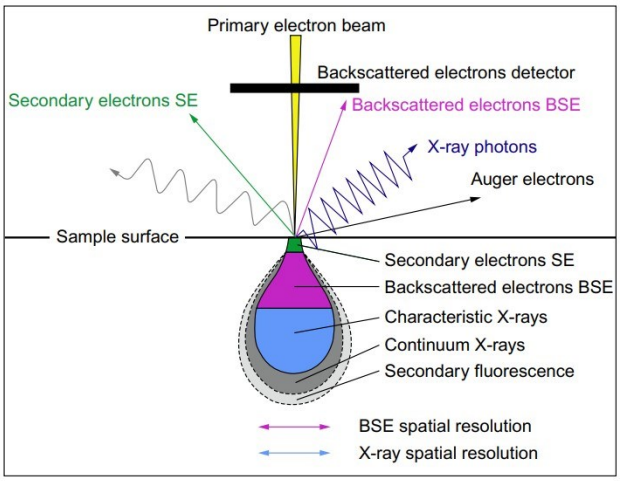

Figure 4. Working of SEM

Fix the anthocyanin film onto SEM stubs using double-sided conductive carbon tape. Optionally, sputter-coat the sample with a thin layer of conductive material (e.g., gold, platinum) to enhance conductivity and reduce charging effects. Place the sample into the SEM chamber and evacuate the chamber to create a vacuum.Use an appropriate electron beam voltage and working distance for imaging (usually in the range of 5-30 kV). Capture SEM images at various magnifications to observe the surface morphology and microstructure of the anthocyanin film. SEM images can be analyzed to characterize the surface morphology, particle size, and distribution of the anthocyanin film.

(ii) FTIR (Fourier-transform infrared spectroscopy)

FTIR provides information about the chemical composition and molecular structure of packaging films by measuring the absorption of infrared radiation by functional groups present in the material. FTIR is widely used to identify the types of polymers used in packaging films, including polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), and others, based on characteristic absorption bands. FTIR can be employed for quality control purposes, detecting variations in polymer composition, additives, or processing conditions that may affect the performance or safety of packaging films. FTIR analysis can assess the presence and distribution of barrier coatings or additives in packaging films designed to enhance properties such as oxygen, moisture, or light barrier. FTIR can be used to investigate the compatibility between different layers in multilayer packaging films, aiding in the optimization of film formulations for specific packaging applications.

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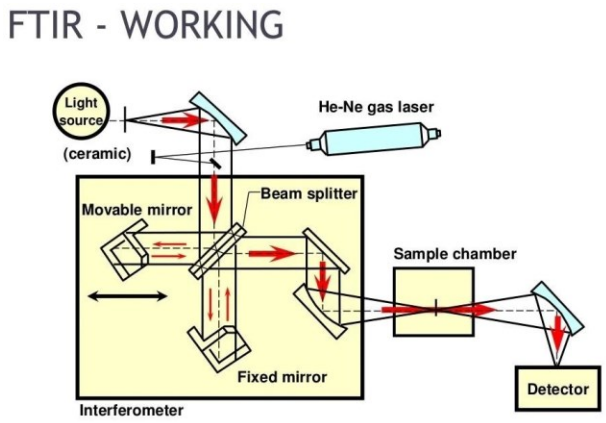

Figure 5. Working of FTIR

Cut the anthocyanin film into small pieces or prepare a KBr pellet containing the film for FTIR analysis. Place the sample onto the FTIR spectrometer's sample holder. Collect the FTIR spectra in the desired spectral range (typically 4000–400 cm^-1). Analyze the obtained spectra to identify functional groups present in the anthocyanin film and to detect any characteristic peaks associated with anthocyanins. FTIR spectra can be compared with reference spectra of known compounds to identify functional groups and chemical bonds present in the anthocyanin film [22].

2.2.6 Film Testing with Fruits

The initial and final observation of testing of film in fruits under room and cold temperature for 7 days. As fruits like Grapes (pH 3.0 and 4.5), Plums (pH 3.5-5.5), Strawberries (3-3.5) are wrapped with the red cabbage anthocyanin film to test the pH sensitivity which the color changes after contamination of the fruit. During edible coating of film in fruits the deterioration period is so long that it could not be easily contaminated when wrapped [23].

3. RESULTS AND DISCUSSION

3.1 Preliminary Identification of Anthocyanin

NaOH Test: In this test, a small amount of the sample containing anthocyanins is mixed with a few drops of sodium hydroxide (NaOH) solution. The anthocyanins are present, the mixture turns to blue-green.

The reaction is as follows:

Anthocyanin + NaOH → Blue Green color

HCl Test: In this test, a small amount of the sample containing anthocyanins is mixed with a few drops of hydrochloric acid (HCl) solution. The anthocyanins are present, the mixture turns to red color .

The reaction is as follows:

Anthocyanin + HCl → Red color

NaOH Test and HCl: In this test, a small amount of the sample containing anthocyanins is mixed with a few drops of sodium hydroxide and hydrochloric test solution (NaOH&HCl). The anthocyanins are present, the mixture turns to blue violet.

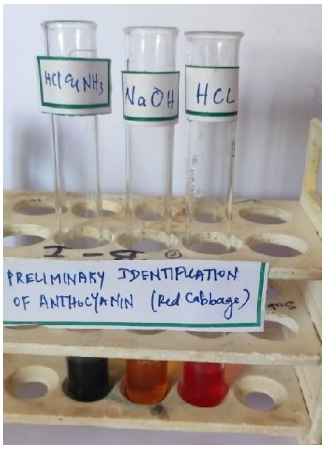

Figure 6. Preliminary Identification tests

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The reaction is as follows:

NaOH + HCl → Blue Violet color

Three tests are used as preliminary tests and the confirmation of anthocyanins in a sample is tested and the results are positive.

S.no	Added solution	Colour	Result
1.	2N HCl	Red colour	Positive
2.	2N NaOH	Blue green	Positive
3.	2N HCl & NH3	Blue violet	Positive

Table 1. Presence of Anthocyanin test

3.2 Total Anthocyanin Content

The distinction between the two cradles at 500 Nm furthermore, 680Nm is proportional to the anthocyanin material. Measurements were made in UV-Vis plate reader or Colorimeter.

Constant values:M.W. OF CYANIDIN 3-GLUCOSIDE = 449.2, Σ=26900

Characterization using UV spectrophotometer analysis

Table 2. Presence of Total Anthocyanin

Colorimeter	500Nm	680Nm
KCl	0.72	0.03
Sodium Acetate	0.18	0.04

ΔA1=Potassium Chloride=**0.72-0.03=0.69,** ΔA2=Sodium acetate = **0.18-0.04=0.14**

ΔA1-ΔA2=0.69-0.14 = **0.55**

ΔA = 0.55

Anthocyanin concentration =

$$= {}_0^{\mathbf{91.84}}.\,55\ X^{\mathbf{\%}}\ 449.\,2\ X\ 10X\ 1000/26900\ X\ 1$$

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Figure 7. Total Anthocyanin Content

3.3 Characterization of Film

3.3.1 Water solubility

The water solubility test of film was conducted based on its adjustment of size of film samples. The samples were cut into square film pieces and immersed in 20ml (about 0.68 oz) of distilled water at room temperature for 24 hours. After 24 hours, the samples were taken and immediately weighed (M_0). The remaining film was dried for 24 hours, and the final film mass measured (W_1) and the result found to be, $^{WS =} 0.59 - 0.24 / 0.59\ X\ 100$

$= 59.3\%$

Figure 8. Water Solubility

3.3.2 Swelling Index

The swelling index test of film was conducted based on its adjustment of volume of the distilled water. The sample was cut into circles with a diameter of 1.5cm (SI_0) and immersed in 20ml (about 0.68 oz) of distilled water at room temperature for 24 hours. After 24 hours, the film size (SI_1) was measured immediately and the result was recorded as,

$^{SI =} 0.59 - 0.24 / 0.28\ X\ 100$

= 42.85%

3.3.3 Film Moisture Content

The moisture content of the film is measured by weighing its initial weight (W) and kept at 150^0C in a hot air oven for 24 hours. After 24 hours measuring the final weight (D). By calculating the initial and final weight of the film, the result found to be,

MC = 11.16 - 11.14 / 11.16 X 100

= 0.18 %

3.3.4 pH Sensitivity

The pH sensitivity of packaging film is an important consideration in certain applications, especially when packaging products that are sensitive to changes in acidity or alkalinity. The pH of a substance is a measure of its acidity or basicity and is expressed on a scale of 0 to 14, with 7 being neutral. Substances with a pH below 7 are acidic, while those with a pH above 7 are basic

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The pH sensitivity test is made with few buffer solutions which shows a major change in color. The buffer solutions taken are pH 4,7,9,14.

S.no	pH Range	Colour	Result
1.	4	Pink	Positive
2.	7	Light Blue	Positive
3.	9	Light Violet	Positive
4.	14	Yellow	Positive

Table 3. pH sensitivities test (pH - 4,7,9,14)

The final results are compared and analyzed with the film taken and dipped with various pH levels; it shows an accurate color change in all pH solutions. This defines that film is sensitive to pH and ensures that its change in pH detects food spoilage easily when it releases the ethylene gas.

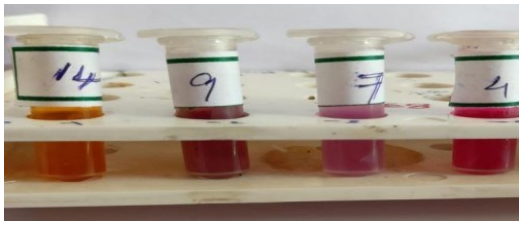

Figure 9. pH Sensitivities

3.3.5 Storage Stability

The stability of the film was accomplished for 28 days (about 4 weeks) utilizing a gravimetric strategy, in the wake of gauging the underlying mass on Day 0, the films were burdened Days 7, 14, 21, and 28 to get the last mass. Compared to all storage temperatures, the film storage stability is more stable in the cold room temperature than incubator and room temperature. So, that the film is in equilibrium state only at cold room temperature.

Table 4. Storage Stability (28 days)

TEMPERATURE	DAY 7		DAY 14		DAY 21		DAY 28	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Room Temperature	0.05g	0.05g	0.06g	0.05g	0.06g	0.04g	0.06g	0.04g
Cold Room Temperature	0.04g	0.04g	0.04g	0.04g	0.04g	0.04g	**0.04g**	**0.04g**
Incubator	0.05g	0.07g	0.07g	0.06g	0.07g	0.06g	0.08g	0.07g

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The studies have been taken 28 days alternatively to note the antimicrobial activity of the film in different temperatures such as Room temperature, Incubator, Cold room temperature the weight was checked and the antimicrobial activity of the film remains stable in Cold room compared to other two. The loss of biologically active anthocyanins can be slowed down by storing them at lower temperatures.

3.3.6 Antimicrobial Test

Cut into a piece of film and dip it into the water and place it on an agar plate. - Red cabbage film Chemicals used for testing. The 4 basic chemicals used for Petri plate

- E. coli
- Bacillus
- klebsiella
- Staphylococcus

After observing it for days, there is no antimicrobial activity in the film because food packaging films act as a barrier to external contaminants, including microorganisms. The structure and composition of the film, particularly if it incorporates anthocyanins, may effectively prevent the entry and growth of microbes, thereby resulting in zero microbial activity.

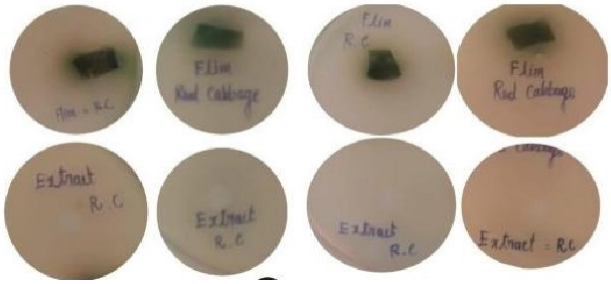

Figure 10. Antimicrobial Test

3.4 Optimization of film

3.4.1 FTIR Characterization

The identification of the characteristic vibrational bands and the various functional groups present in the samples are facilitated by the FTIR analysis. The obtained FTIR spectra for SSN1 and SSN2 are illustrated in Fig.1. The infrared spectrum of the peaks between 3550 cm-1 to 3800 cm-1 refers to the stretching frequency of a free O-H bond is and the peak around 1800 cm-1 were assigned to the vibrational modes of hydroxyl groups, enabling the identification of hydroxyl group in the as-prepared polymer sample. The small and broad peaks of O-H vibrations exist in the range of wavenumber between 3800-3600 cm−1 and the bending mode of hydroxyl group NH stretching absorption in the prepared sample lattice was observed at 1780 cm−1. The absorption peak between 2300 -2400 cm-1 is due to stretching of the C=N bond. In the middle range, the peak at 1770 cm-1 is caused by stretching of C=O in carboxyl and carbonyl groups.

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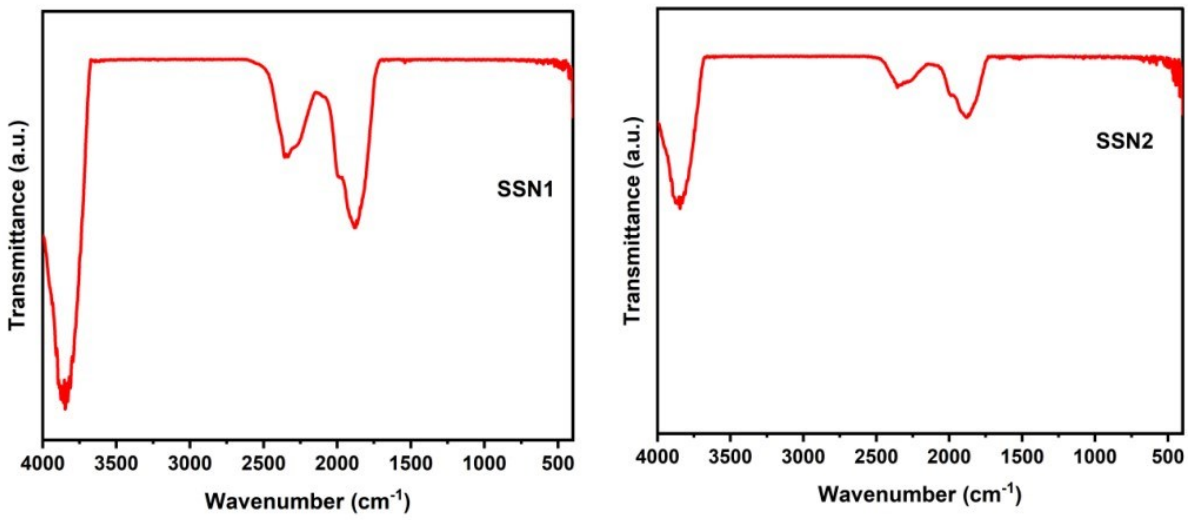

Figure 11. FTIR Characteristics of samples 1 and 2

3.4.1 SEM Characterization

The surface morphology of the as-prepared samples is presented in Fig.2 a-d as revealed by Scanning Electron Microscopy (SEM) images. The prepared samples have surface morphological features with particles that appear to be small sphere-like structures with dense agglomeration. Due to the presence of particle agglomeration, the micrographs of the as prepared polymer materials showed a non-uniform structure with loose aggregates. More micro-size aggregates were also apparent in every sample as seen by the SEM pictures (From the picture values as mentioned between 10 µm to 1 µm). Additionally, non-uniform particle agglomeration and distribution can also cause uneven clustering inside the material as presented in the SEM micrographs. Also, from these micrographs it can be observed that the layer pattern has been observed in higher magnifications (X15,0000) wherein the particle agglomeration are embedded inside.

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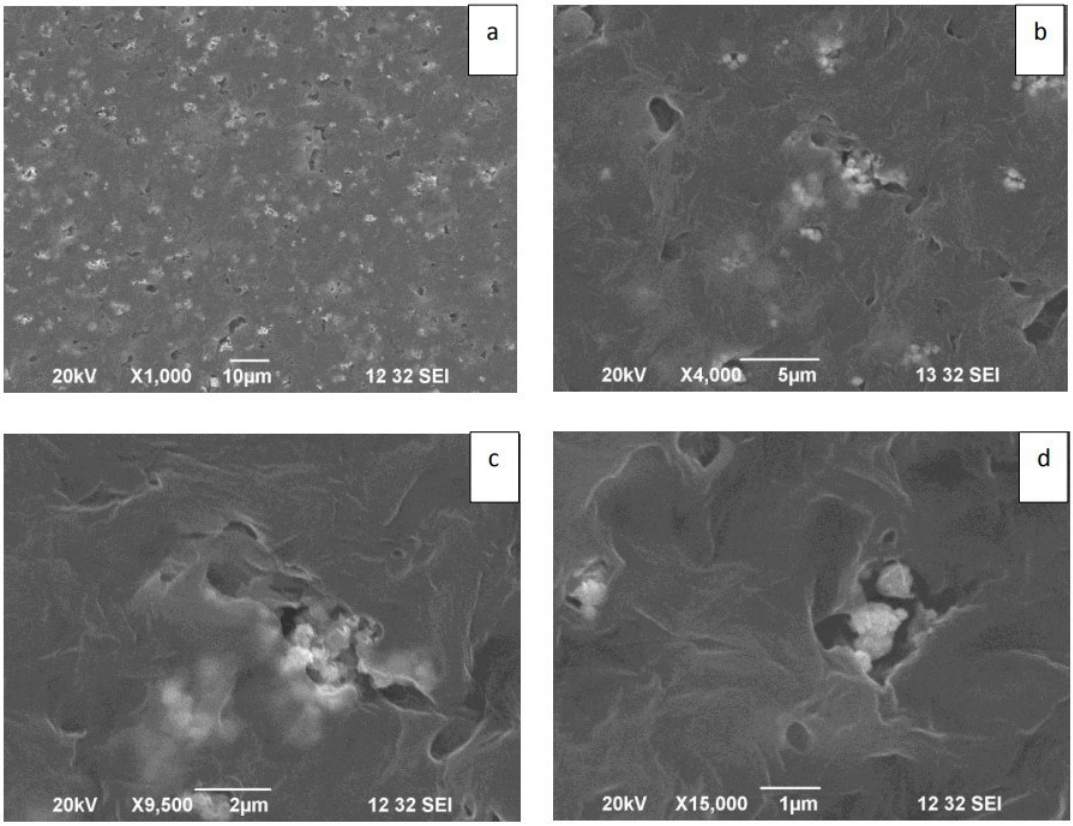

Figure 12. SEM Characteristics of samples a-d

3.5 Film Testing with Fruits

Application tests were conducted on fruit products where films were cut into rectangles and used as indicators to monitor the freshness of the fruit. Grapes, Strawberries, and Plums were used as a sample for the tests.

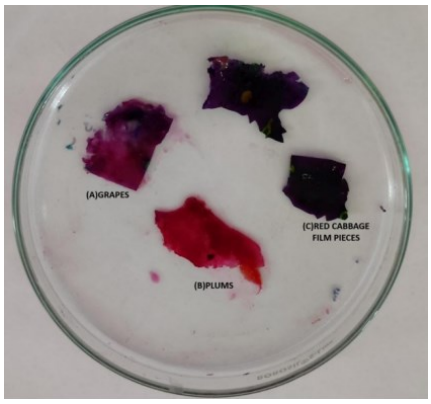

Figure 13. Testing of film with fruits (Grapes, Strawberries, and Plums)

The coated and film strawberries were kept at a cold and room temperature for seven days to observe the color changes and the effect of temperature changes.

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4. CONCLUSION

Active packaging films in light of regular anthocyanins can screen the continuous freshness of food and expand the timeframe of realistic usability of food. This paper explored the extraction, construction, and capability of anthocyanins, and summed up the kinds of active packaging films and their applications in protein-rich food sources. The outcomes showed that anthocyanins could work on the mechanical properties and obstruction properties of the substrate.As a simple to-involve film for food freshness maintenance and checking, active packaging films have extraordinary business likely in the packaging of protein-rich food varieties. Besides, these films' normal anthocyanins are still far from business applications. For instance, profoundly stable anthocyanins with distinct designs should be investigated; film fabrication processes reasonable for business large scale manufacturing are still being scrutinized; and the variety changes brought about by the awareness, security, and reproducibility of packaging films. Consequently, helpful ought to be produced for new cut products of the soil, meat, fish, and milk that lessen food waste and medical issues brought about by food quality and wellbeing.

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