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Impact of Pomegranate Peel (*Punica granatum L.)* Extract on Rice Starch-Pectin-Based Films for Maintaining the Organoleptic Properties of Tomatoes

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Abstract— **Pomegranate peel (*Punica granatum L.)* is commonly disregarded as a byproduct in the juice industry despite its rich content of bioactive compounds. This study investigates the impact of incorporating pomegranate peel extract at concentrations of 0%, 1%, 2%, and 3% into rice starch-pectin-based films. Characterization of the films was conducted using Fourier Transform Infrared Spectroscopy (FTIR) and Field Emission Scanning Electron Microscopy (FESEM). Physicochemical properties including thickness, color, opacity, moisture content, water solubility, and water vapor permeability were assessed. The results demonstrate that the incorporation of pomegranate peel extract significantly enhanced the properties of the rice starch-pectin-based films ($p < 0.05$). Moreover, antimicrobial assays revealed substantial inhibition of Escherichia coli by films containing 3% pomegranate peel extract. Furthermore, the efficacy of these films was evaluated using real tomato samples to assess their potential to extend the shelf life of perishable foods. The findings suggest that the inclusion of pomegranate peel extract in the film matrix contributes to natural pigment preservation, mitigates microbial contamination, and maintains the organoleptic attributes of tomatoes, thereby potentially enhancing their shelf life. These results underscore the promising applications of pomegranate peel extract in the development of sustainable and functional food packaging materials.**

Keywords— **composite film, pectin, pomegranate peel extract, rice starch, tomatoes**

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I. INTRODUCTION

The pomegranate (*Punica granatum* L.) is renowned for its rich content of bioactive compounds, including phenolic acids, flavonoids, and tannins, which confer significant antioxidant and antimicrobial properties [1]. Global pomegranate production has reached nearly 3.8 million tons, with pomegranate rinds, comprising over half the fruit's weight, often discarded as waste in the juice industry [2], [3]. However, pomegranate peels possess superior antioxidant and antimicrobial characteristics compared to other parts of the fruit [4]. Recently, there has been growing interest in utilizing pomegranate peel extract as a natural additive in biodegradable films to enhance both film quality and food preservation. The incorporating pomegranate peel extract into food packaging reduces the need for synthetic preservatives, thereby extending the shelf life and quality of packaged food [5]. Additionally, the inclusion of pomegranate peel extract significantly improves the physical, mechanical, chemical, and antibacterial properties of chitosan-based films [6].

Rice starch, derived from rice grains, is widely used in various food formulations due to its versatility. It contains two main polymers, amylose and amylopectin, which play crucial roles in determining the mechanical, gas barrier, and optical properties of rice starch-based films [7]. However, rice starch films face limitations, such as brittleness and high hydrophilicity, which compromise their mechanical properties and barrier efficiency against highly polar compounds [8]. To address these issues, rice starch can be blended with biopolymers like pectin, a water-soluble and biodegradable component extracted from plant cell walls, to enhance film properties [4]. Research by Tarique et al. [9] and Riaz et al. [10] has shown that pectin, when combined with starch and glycerol, can form films with improved mechanical strength and barrier properties. Tomatoes, being climacteric fruits, continue to ripen post-harvest and are prone to quick spoilage, reducing their market quality and shelf life [11]. The deterioration of perishable foods, including tomatoes, is often accelerated by pathogenic microorganisms. The phenolic compounds in pomegranate peel extract can interact with starch polymers, potentially exhibiting high antioxidant activity and enhancing the oxidative stability of food [12].

This study investigates the incorporation of pomegranate peel extract into rice starch-pectin-based films. The novelty of this research lies in leveraging pomegranate peel extract to enhance the functional properties of the films, specifically their physicochemical characteristics and antimicrobial efficacy. While previous studies have primarily focused on the role of pectin in modifying rice starch films, this research emphasizes the impact of pomegranate peel extract on the film matrix. By integrating pomegranate peel extract, we aim to improve the film's thickness, water solubility, and antimicrobial properties, thereby extending the shelf life of perishable foods such as tomatoes. This approach not only addresses the challenge of agricultural waste but also contributes to the development of sustainable and eco-friendly food packaging solutions, meeting the increasing demand for environmentally conscious materials in the food industry.

II. MATERIAL AND METHODS

A. Material

The raw materials used to develop rice starch-pectin films incorporated with pomegranate peel extract such as rice starch and high-methoxyl pectin and pomegranate peel. The pomegranate was purchased from a local Tawau, Sabah, Malaysia supermarket. The rice starch and high-methoxyl pectin are obtained from Sigma Aldrich. Tomatoes were the samples used to study the effect of a film based on rice starch-pectin containing pomegranate peel extract during the storage of tomatoes. Besides, the chemicals used can be found at the Faculty of Food Science and Nutrition Laboratory (FSMP), Universiti Malaysia Sabah, such as the Food Microbiology Laboratory, Biochemistry Laboratory, and Food Analysis Laboratory. Among the chemicals used are ethanol, methanol, Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and sodium carbonate.

B. Methods

Preparation of pomegranate peel extract

The pomegranate peels were cut into small pieces and dried in the universal oven at 55°C for 24 hours [6]. The dried peels were then sieved into a fine powder with a blender. According to Ghorbani et al. [13], the extraction process was done with slight modifications. 25 g of pomegranate peel powder was soaked in 500 mL of 80% ethanol, and the mixture was left for 48 hours at room temperature. The supernatant filtration was performed using Whatman No.1 filter paper after centrifugation at 3500 rpm for 15 minutes. Next, 150 mL of supernatant was fed into a rotary evaporator to evaporate the ethanol in the supernatant. The rotary evaporator was set at a speed of 90 rpm at 50 °C for about 40 minutes. The process of evaporation was completed when no excess condensed vapor was available. All the residual condensation was collected in a waste collector. Finally, the extract was stored in a glass bottle at 0 °C until further use.

Characterization of physicochemical properties

Thickness

The thickness of composite films was measured using a hand-held micrometer (Wilkens-Anderson Co.) with an accuracy of 0.001 mm [14]. Five random measurements were made at least five different locations on each film. The measurements were made in triplicate. Then, the average thickness of each film was determined.

Color

The color was determined using a colorimeter according to the method of de Almeida Soares et al. [6] with minor adjustments. The color of each film has been expressed in L*, a*, and b* values. L* represents the darkness of black at L=0 and the brightness of white at L=100, while a* indicates the color of red and green, where the negative value signifies green color, and the positive value signifies red color. The value of b* represents the color yellow and blue, where the yellow color is at the positive value and the blue color is at the negative value. These films were measured in triplicate. The color index (ΔE) that identifies the amount of color difference between each sample is determined as the following equation:

$$\Delta E = [(L^* - L^*_{\text{standard}})^2 + (a^* - a^*_{\text{standard}})^2 + (b^* - b^*_{\text{standard}})^2]^{½} \quad (1)$$

L^*_{standard}, a^*_{standard} and b^*_{standard} are the color values of the standard white plate (L* = 51.25, a* = -25.27, b* = 14.79). In contrast, L*, a*, and b* are the color values of the sample films.

Opacity

According to the method used by Vonnie et al. [15], the opacity of each film was determined using the UV-Vis spectrophotometer (PerkinElmer, London, UK) to analyze the absorbance of film at 600 nm. Each film was cut (4 cm x 1.5

cm) and placed in the cell holder of the UV-Vis Spectrophotometer. The measurements were taken in triplicate and the opacity of the film was calculated using the following equation:

$$Opacity = \frac{A_{600}}{x} \quad (2)$$

A_{600} is the absorbance at 600 nm and x is the thickness of film (mm).

Moisture Content
The film's moisture content was measured according to the method by de Almeida Soares et al. [6] with minor modifications. Each film was cut into squares of size 2 cm x 2 cm, and the initial weight was weighed. It was then dried in the universal oven at 105 °C for 24 hours. After drying, the films were cooled to room temperature, and the final weight of the film was recorded as W_1. The moisture content of the film was calculated using equation:

$$Moisture\ content\ (\%) = \frac{(W_0 - W_1)}{W_0} \times 100 \quad (3)$$

Water Solubility
The water solubility of the film was determined by reference to Vonnie et al. [16]. The initial weight of film (2 cm x 2 cm) was weighed and recorded as W_i. The film was then immersed for 24 hours in 50 mL of distilled water at room temperature. After hydration, the wet film was gently wiped using filter paper and dried for 24 hours at 105 °C in the universal oven to remove the free water. The final weight of the dried film was measured and recorded as W_f. The film's water solubility was calculated using the equation:

$$Moisture\ content\ (\%) = \frac{(W_i - W_f)}{W_i} \times 100 \quad (4)$$

Water vapor permeability
The water vapor permeability of the film was determined using the ASTM E96 method. The thickness of the film was measured and the area of the film was also calculated. Each film was placed on a cup containing 30 g of silica gel using cellophane tape and laminated with aluminum. Each cup was then placed in a desiccator containing distilled water at 25 °C. A measure of weight gain was taken by weighing the cup every 24 hours for 5 days. Three replications of the film were prepared and recorded. The gain weight over time slope was calculated by linear regression $(r^2 > 0.99)$. The water vapor transmission rate (WVTR) was determined by following equation:

$$WVTR = \frac{Slope(x)}{A} \quad (5)$$

The water vapor permeability (WVP) was then calculated using following equation below:

$$WVP = WVTR\frac{x}{\Delta P} \quad (6)$$

Where Δm is the gained weight, A is the film exposed area (m^2), Δt is the test time (day), x is the thickness of film (m) and ΔP is the differential of water vapor pressure through the film (Pa). A driving force of 2339 Pa was used as a differential vapor pressure of the water.

Fourier Transform Infrared Spectroscopy (FTIR)
Investigations of the molecular arrangement of films and the interactions between polymers are carried out using infrared spectroscopy. Agilent Technologies Cary 630 FTIR is used at spectral ranges from 4000 to 6000 cm^{-1} spectral ranges. Fourier transform infrared spectra for film samples will be captured by the attenuated total reflection method with a resolution of 4 cm^{-1} [17].

Field Emission Scanning Electron Microscopy (FESEM)
The film samples were analyzed using FESEM (JSM-7900f Schottyky) with a magnification of 1000 for the film surface and 300 for the cross section. The film samples were coated with platinum palladium to FESEM to allow and improve the imaging of the sample [18].

Storage stability
The storage stability of each film was determined by measuring the weight reduction of the film over 21 days [15]. The films with different concentrations of pomegranate peel extract (1 %, 2 % and 3 %) were placed in the container at three different temperatures, namely room temperature (25 °C), incubator (37 °C) and cold room (4 °C). The initial weight of films (W_i) were taken on Day 0. The films were weighed again on Day 7, 14 and 21 to obtain the final weight (W_f). The weight loss of film (W_L) was calculated using equation:

$$\text{Weight reduction } (W_L) = W_i - W_f \quad (7)$$

Analysis of antioxidant and antimicrobial properties
Antioxidant activity.
The antioxidant activity of films was determined by using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and following the method by Homthawornchoo et al. [19]. 1.5 ml of the film extract solution was mixed with 1.5 ml of 0.15 M of DPPH in 95% ethanol, stirred vigorously, and left to stand in the dark for 30 minutes. 95% ethanol was used as the blank solution (control). By using the UV-visible spectrophotometer, the antioxidant activity was determined at the absorbance of 517 nm, and it was calculated by using the following equation:

$$Antioxidant\ activity\ (\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (8)$$

Where A control is the absorbance of the control sample and A_{sample} is the absorbance of the sample.

Antimicrobial activity
The agar disc diffusion method was used to determine the antimicrobial activity. Each film was tested against *Escherichia coli* (Gram-negative bacteria) and *Lactobacillus* (Gram-

positive bacteria). All strains of bacteria were grown in Mueller-Hinton (MH) broth (Difco, Detroit, MI, USA) and incubated in a shaker at 37°C for 24 hours. The cultured bacteria were streaked on the MH agar plates and cultured at 37°C for another 24 hours to produce a single colony. A sterile brush was used to inoculate the culture on the MH agar plate. Each 5 mm diameter film sample was sterilized with UV light for 30 minutes before being placed on an MH agar plate with an inoculum and incubated at 37°C for 24 hours. Ampicillin (10 g/disc) was used as a positive control. The inhibition zones were used to observe antimicrobial activity [19].

Analysis of the real samples

Film samples were prepared for packaging testing to study the effect of pomegranate peel extract in composite films to extend the storage time of tomatoes. The same quality and size tomatoes were selected to be washed with deionized water and dried at room temperature. Then, the tomato was wrapped in a prepared film sample with different concentrations of pomegranate peel extract (0%, 1%, 2%, and 3%). The unpacked tomato was used as a blank. All tomatoes were stored at room temperature. The weight reduction was measured by weighing the sample differences before and after the storage period, and the following equation calculates the percentage of tomato weight reduction:

$$Weight\ reduction\ (\%) = \frac{(w_i - w_f)}{w_i} \times 100 \qquad (9)$$

Where Wi is the initial sample weight (g), and Wf is the sample weight after storage (g).

Statistical Analysis

The IBM SPSS 29.0 for Windows determines significant differences between films using one-way Analysis of Variance (ANOVA). The data was collected using Duncan's 95% confidence level test to determine the significant difference across the film sample. All the analyses were carried out using three replicates, and the data was recorded using mean and standard deviation.

III. Results and Discussion

Characterization of the Physicochemical Properties

Thickness

Thickness is a crucial parameter in the characterization of films as it directly impacts their mechanical properties and overall performance. The preparation method, including factors such as the drying process and the surface of the container, can also influence the thickness of the films [20]. Table 1 presents the thickness measurements of rice starch-pectin films incorporated with varying concentrations of pomegranate peel extract (PPE). The data in **Table 1** indicate that the control film (0% PPE) exhibited the lowest thickness, measuring 0.071 mm. In contrast, films with PPE concentrations of 1%, 2%, and 3% showed increased thicknesses of 0.081 mm, 0.082 mm, and 0.084 mm, respectively. These differences are statistically significant ($p < 0.05$), underscoring the impact of PPE on film thickness.

The increase in film thickness with higher concentrations of PPE can be attributed to the molecular interactions between the polyphenolic compounds in PPE and the polysaccharide chains of the rice starch-pectin matrix [21]. These interactions, which include hydrogen bonding and other non-covalent bonds, contribute to the formation of a more robust and thicker film structure. Furthermore, the increased thickness of the composite films has implications for their functional properties. Thicker films typically exhibit reduced water vapor permeability, enhancing their barrier properties and making them more suitable for applications in food packaging where moisture control is critical.

TABLE 1
THE THICKNESS OF RICE STARCH-PECTIN FILM INCORPORATED WITH POMEGRANATE PEEL EXTRACT AT DIFFERENT CONCENTRATIONS.

Film	Thickness (mm)
0 % (Control)	0.071 ± 0.006^a
1 %	0.081 ± 0.002^b
2 %	0.082 ± 0.002^b
3 %	0.084 ± 0.004^b

* Mean standard ±deviation (n=3).
* The values having different alphabets on the same column showed significant difference at $p < 0.05$.

Color

Color is a major factor in the acceptance of food products and also an important physical property in film analysis. The color of rice starch-pectin films incorporated with pomegranate peel extract at different concentrations were measured and the results obtained were indicated in **Table 2**. Next, the color values found have been expressed as L* is brightness or darkness, a* is green or red color and b* is blue or yellow, while ΔE indicates the amount of color difference. The standard value of L* was 51.25, a* is -25.27 and b * is 14.79.

The control film (0 %) showed the highest L* value of 63.71 where the film has a bright film compared to other films. The brightness of the film decreases with increasing concentration of pomegranate peel extract. The 3% film has the lowest L* value of 36.68 which indicates that the film is the darkest. In addition, the control film (0 %) recorded an a* value of negative while the a* values for films containing pomegranate peel extract of 1%, 2% and 3% were positive, showing a red color. This is because the content of phenolics in pomegranate peel extract has a color pigment, namely anthocyanin, which affects the value of a* and red color in the film [6].Similarly, the b* value increased when the concentration of pomegranate peel extract was added to the film from 7.14 (film 0%) to 37.58 (film 3%).

TABLE 2
THE COLOR VALUE OF RICE STARCH-PECTIN FILM INCORPORATED WITH POMEGRANATE PEEL EXTRACT AT DIFFERENT CONCENTRATIONS.

Film	**Color**			
	L*	**a***	**b***	**ΔE**
0 % (Control)	63.71±0.02^{d}	0.16±0.03^{a}	7.14±0.02^{a}	52.43±20.22^{a}
1 %	44.24± 0.06^{c}	10.14± 0.07^{b}	33.40 ±0.06^{b}	56.35±0.06^{a}
2 %	41.11 ± 0.08^{b}	13.55 ±0.02^{c}	36.10 ±0.03^{c}	56.37 ±0.05^{a}
3 %	39.68 ±0.07^{a}	15.56 ±0.05^{d}	37.58 ±0.24^{d}	56.83 ±0.18^{a}

* Mean standard ± deviation (n=3).
* The values having different alphabets on the same column showed significant difference at $p < 0.05$.

Furthermore, the value of ΔE also increased with the increase in the concentration of pomegranate peel extract in rice starch-pectin film from 52.43 to 56.83, as shown in **Figure 1**. The result showed that the film's darkness, red, and yellow color increases with the increase in the concentration of pomegranate peel extract in the film due to the presence of antioxidants and anthocyanin pigments. Thus, the results of this study showed that the combination of pomegranate peel extract in rice starch-pectin film significantly ($p < 0.05$) affects the physical properties of the film's color.

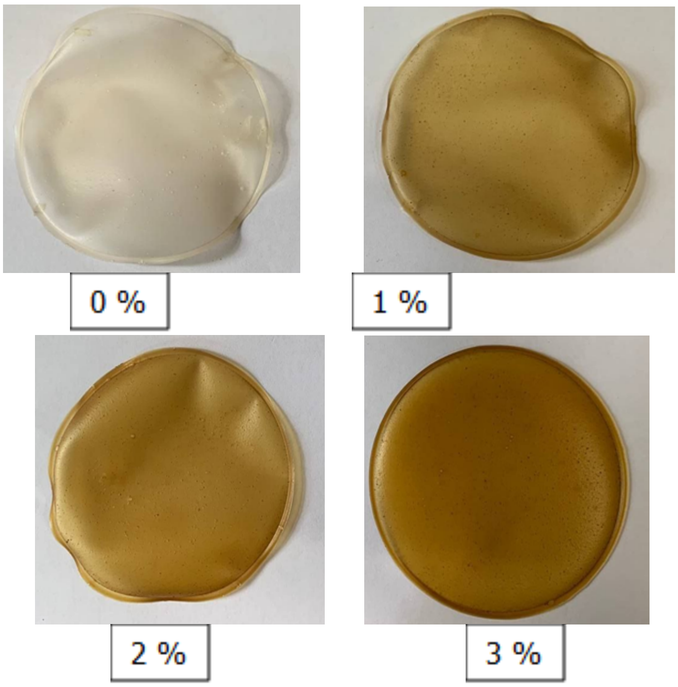

Fig. 1: The color difference between rice starch-pectin film incorporated with pomegranate peel extract at different concentrations.

Opacity

Opacity is an important physical property of packaging film because it affects the translucency or prevention of light transmission. It also directly affects the product's appearance and its acceptance by consumers. **Table 3** shows the results of opacity for rice starch-pectin film incorporated with pomegranate peel extract at different concentrations. The increase in pomegranate peel extract concentration in each film showed a significant difference ($p < 0.05$) which affected the opacity of the film sample. The 2 % film shows the highest opacity which is 16.33 ±1.44 A/mm. There is no significant difference in the high resistance to ultraviolet (UV) radiation. While the lowest opacity is 13.78 ± 0.10 A/mm for the control film (0 %). This was influenced by the presence of phenolic content in pomegranate peel extract that can change the structure pores in the sample film which contributes to increased opacity of rice starch-pectin film [22,23].

TABLE 3
THE OPACITY OF RICE STARCH-PECTIN FILM INCORPORATED WITH POMEGRANATE PEEL EXTRACT AT DIFFERENT CONCENTRATIONS.

Film	**Opacity (A/mm)**
0 %	9.55±0.48^{a}
1 %	11.77±0.92^{b}
2 %	16.33±1.44^{d}
3 %	13.78±0.10^{c}

* Mean standard ± deviation (n=3).
* The values having different alphabets on the same column showed significant differences at $p < 0.05$.

Moisture content

Moisture content is one of the important characteristics in film applications such as food packaging because it affects the stability and efficiency of the film [14]. **Table 4** shows that the film's moisture content decreased when the concentration of pomegranate peel extract was added. The control film has the lowest moisture content of 15.69 ±0.95 % compared to other sample films. The highest percentage of moisture content was recorded by the rice starch-pectin film containing pomegranate peel extract concentration of 3 % which was 20.44±0.22 %, followed by a film sample of 2 % (20.43±0.24 %) and 1 % (17.73 ±0.21 %). Therefore, it can be concluded that the moisture content of the rice starch-pectin film increases as the concentration of pomegranate peel extract increases. This was because pomegranate peel extract has hygroscopic properties that cause intermolecular interactions for the rice starch-pectin film and these polyphenolic compounds are formed in competition with water molecules. This has indirectly increased the ability of the matrix to bind water molecules and promote the absorption of water molecules. According to the study by Homthawornchoo et al. [19], the improvement of green tea extract has led to a decrease in the film moisture percentage of rice starch-pectin film. Furthermore, damage or defects on the surface of the film either cracks or has pores also affect the rate of water absorption when the concentration of pomegranate peel extract increases [20].

TABLE 4
THE MOISTURE CONTENT AND FILM SOLUBILITY OF RICE STARCH-PECTIN FILM INCORPORATED WITH POMEGRANATE PEEL EXTRACT AT DIFFERENT CONCENTRATIONS.

Film	**Moisture content (%)**	**Water solubility (%)**
0 %	15.69±0.95^{a}	27.29±10.14^{a}
1 %	17.73 ±0.21^{b}	53.25±0.46^{b}
2 %	20.43±0.24^{c}	39.11±8.79^{ab}
3 %	20.44 ±0.22^{c}	34.02±17.76^{ab}

* Mean standard ± deviation (n=3).
* The values with different alphabets on the same column showed significant differences at $p < 0.05$.

Water solubility

Water solubility is an important characteristic in determining the biodegradability of composite films [6]. Based on **Table 4**, the percentage of water solubility for rice starch-pectin film incorporated with pomegranate peel extract concentration of 2 % was the highest at 53.25 % compared to 3 % (34.02 %) and 1 % (39.11 %) film samples. Meanwhile, the control film (0 %) recorded the lowest percentage, 27.29 %. A high percentage of film solubility indicates that the film has low water resistance. In addition, increasing the amount of bioactive content can interfere with the interaction of polymers and hydrogen bonds in the film, thus contributing to changes in the solubility of the film [24]. The incorporating pomegranate peel extract in chitosan can lead to broken molecular bonds and changes in molecular structure that increase the solubility of the film. Pomegranate peel extract has hydrophilic compounds that contribute to increasing the composite film's hydrophilicity, thereby increasing the polymer's bonding to water molecules [5,6,25].

Water vapor permeability

Water vapor permeability plays an important role in food packaging film. The film with low water vapor permeability, can prevent the food from losing water by reducing the water flow and prolonging the food's shelf life. **Table 5** shows the water vapor permeability values for rice starch-pectin film incorporated with pomegranate peel extract. The 0% film sample showed the water vapor permeability, which was the highest (2.94 ±0.18^{a} $gs^{-1}mPa$) and the weakest caused by the hydrophilicity of starch. Meanwhile, the low water vapor permeability of 1.80±0.56^{a} gs^{-1} mPa was recorded by a 3% film sample. This was because phenolic compounds in pomegranate peel extract can increase hydrophilic behavior and alter the balance of hydrophilic points that promote water absorption [26]. The addition of pomegranate peel extract in the composite film showed a significant difference ($p < 0.05$) in reducing water vapor permeability. This suggests that pomegranate peel extract significantly increases the water barrier capacity for rice starch-pectin films [27].

TABLE 5
THE WATER VAPOR PERMEABILITY OF RICE STARCH-PECTIN FILM INCORPORATED WITH POMEGRANATE PEEL EXTRACT AT DIFFERENT CONCENTRATIONS.

Film	**Water vapor permeability (gs^{-1} mPa)($\times10^{-10}$)**
0 %	2.94±0.18^{a}
1 %	2.37 ±1.22^{a}
2 %	2.65 ±1.08^{a}
3 %	1.80 ±0.56^{a}

* Mean standard ± deviation (n=3).
* The values having different alphabets on the same column showed significant differences at $p < 0.05$.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR analysis was employed to examine the chemical composition of rice starch-pectin films incorporated with different concentrations of PPE. As depicted in **Figure 2**, the FTIR spectra for the composite films containing 1%, 2%, and 3% PPE demonstrated a similar pattern to that of the control film (0% PPE). This indicates that the addition of PPE does not significantly alter the fundamental chemical structure of the rice starch-pectin matrix.

The presence of four distinct peaks was observed: at 3282-3283 cm^{-1} corresponding to the O-H stretch, at 2920-2940 cm^{-1} for the C-H stretch, at 1610-1640 cm^{-1} indicating the C=O stretch, and at 1330-1350 cm^{-1} representing the O-H bending of phenols. These peaks suggest the formation of non-covalent interactions between the rice starch-pectin matrix and the polyphenolic compounds in the PPE. The slight shift in the OH-stretching peak and the reduction in its sharpness and peak area upon the addition of PPE confirm the formation of hydrogen bonds between the rice starch-pectin matrix and the polyphenols [28].

Previous studies have corroborated these findings, highlighting the significant role of phenolic compounds in modifying the structural properties of biopolymer films. For instance, Hamidpour et al. [29] reported that the inclusion of phenolic-rich extracts in biopolymer matrices enhances the antioxidant and antimicrobial properties of the films due to the strong hydrogen bonding between the polymer matrix and the phenolic compounds. Similarly, the study by Hanani et al. [30] on fish gelatin films reinforced with pomegranate peel powder demonstrated improved functional properties, further supporting the potential of PPE in biopolymer film applications. The observed spectral shifts are also associated with the formation of matrix inclusions, which can significantly impact the mechanical properties, solubility, and water vapor permeability of the films. These interactions are crucial for enhancing the film's functional attributes, making them suitable for food packaging applications. Liu et al. [31] emphasized that the inclusion of polyphenolic compounds from natural sources could lead to the development of biopolymer films with superior barrier properties, thereby extending the shelf life of packaged food products.

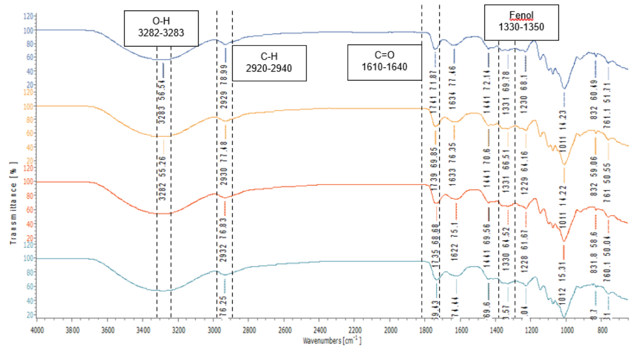

Fig. 2: Fourier Transform Infrared Spectroscopy of rice starch-pectin film incorporated with pomegranate peel extract at different concentrations.

Field Emission Scanning Electron Microscopy (FESEM)

FESEM was employed to investigate the surface morphology and cross-sectional structure of the rice starch-pectin films incorporated with varying concentrations of PPE. The FESEM images, as shown in **Figure 3**, reveal distinct differences in surface and cross-sectional morphology between the control film (0% PPE) and the films containing PPE. The control film (0% PPE) exhibited a smooth, homogeneous surface and cross-section, devoid of air bubbles, pores, or cracks. This uniformity is likely due to the high amylose content in starch, which forms a stable matrix polymer that contributes to the film's homogenous structure [32]. The smooth morphology suggests a well-formed film with consistent properties, ideal for various applications where uniformity is crucial.

In contrast, films with increased concentrations of PPE showed progressively rougher surfaces and cross-sections, with noticeable particle aggregation. The 2% PPE film, in particular, displayed a rough surface with the formation of some cracks, which can be attributed to clot formation on the surface. The higher content of PPE may introduce stress fields within the film matrix, causing expansion and surface cracking. This phenomenon aligns with findings from Dai et al. [33], who reported that while PPE incorporation did not fundamentally alter the composite film structure, it did influence the surface morphology due to interactions between starch molecules and phenolic compounds. Contradictorily, Farouk [34] revealed that the incorporation of PPE does not significantly alter the film morphology. Besides, the developed film demonstrated a smooth surface without any visible pores or cracks that indicates the compatibility between the matrix and extract.

In this work, the cross-section of the 3% PPE film revealed air pores within the film matrix. These pores likely result from the film-making process, particularly during the pouring of the film solution into the silicone mold. The presence of these air spaces indicates a disruption in the internal structure, leading to significant variations in layer thickness and density. This disruption potentially affects the film's integrity and its functional properties [35]. The rougher surface and internal irregularities observed in higher PPE concentration films highlight the trade-off between adding bioactive compounds for enhanced functionality and maintaining structural integrity. Similar observations were made by Hanani et al. [30], who noted that incorporating natural extracts into biopolymer films can result in surface roughness and structural irregularities due to the interaction between the biopolymer matrix and the active compounds.

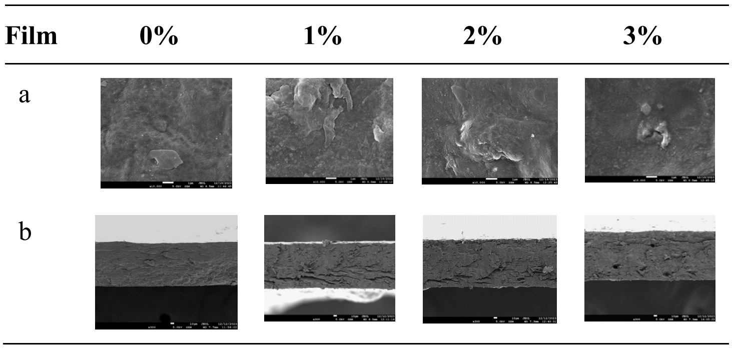

Fig. 3: FESEM image (a) film surface (x10,000) and (b)film cross-section (x300)

Storage stability

The storage stability analysis was conducted to evaluate the quality changes in rice starch-pectin films incorporated with varying concentrations of PPE under different environmental conditions. The results (**Figure 4**) indicated a general trend of weight reduction across all film samples, which began to biodegrade after the seventh day. This weight loss is attributed to the decrease in water-holding capacity and moisture loss of the rice starch-pectin films with PPE [36]. Specifically, the films with 0%, 1%, 2%, and 3% PPE concentrations exhibited varying degrees of weight reduction, with the 3% PPE film showing the highest storage stability across all conditions tested.

The superior performance of the 3% PPE film can be linked to the presence of phenolic compounds in the pomegranate peel extract, which enhance the film's thermal stability and reduce degradation rates [12]. Rathinavel et al. [37] noted that incorporating pomegranate peel extract into biopolymer matrices, such as rice starch-pectin, improves the composite film's thermal stability by reducing the thermal conductivity of the matrix. This is consistent with the findings of Dai et al. [33], who reported that the addition of phenolic compounds from pomegranate peel extract contributes to improved barrier properties and slower degradation rates of biopolymer films.

Moreover, the enhanced thermal stability and reduced degradation rates observed in the 3% PPE films are supported by previous studies, which demonstrate that phenolic compounds play a crucial role in stabilizing biopolymer matrices under various environmental conditions. For instance, Hanani et al. [30] found that gelatin films incorporated with pomegranate peel powder exhibited increased antioxidant and antimicrobial properties, leading to better preservation and longer shelf life of food products.

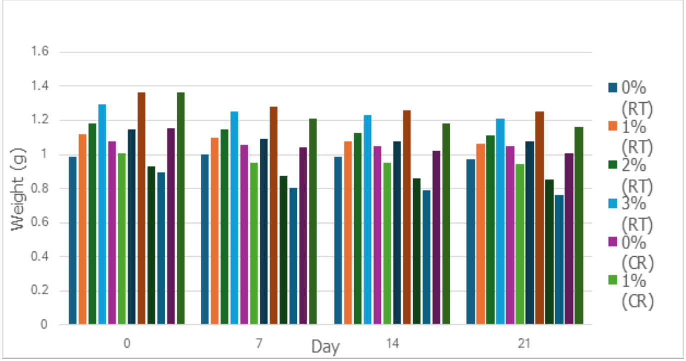

Fig. 4: The storage stability of rice starch-pectin film incorporated with pomegranate peel extract at three different conditions: room temperature (RT), cold room (CR) and incubator (I).

Antioxidant activity

The radical scavenging activity of DPPH was utilized to assess the antioxidant potential of rice starch-pectin films incorporated with varying concentrations of PPE. Each film sample was analyzed based on absorbance values measured at a wavelength of 517 nm. The results, presented in **Table 6**, indicate a clear trend in the enhancement of antioxidant activity with increasing concentrations of PPE. The control film, which contained 0% PPE, exhibited negligible antioxidant activity, recording the lowest free radical scavenging activity at -21.73%. This suggests that the rice starch-pectin matrix alone does not contribute significantly to free radical neutralization. However, as the concentration of PPE increased, there was a marked improvement in the radical scavenging activity. The films with 1%, 2%, and 3% PPE showed DPPH radical scavenging activities of 9.27%, 32.72%, and 68.38%, respectively. This indicates that higher concentrations of PPE significantly enhance the antioxidant properties of the films [20,25].

The substantial increase in radical scavenging activity with the addition of PPE can be attributed to the high phenolic content in pomegranate peel [38]. Phenolic compounds are well-documented for their strong antioxidant capabilities, as they can donate hydrogen atoms or electrons to neutralize free radicals, thereby stabilizing them [39]. This is consistent with findings from previous studies, such as those by Hanani et al. [30], which demonstrated that incorporating phenolic-rich pomegranate peel extract into gelatin films significantly boosted their antioxidant and antimicrobial properties. Furthermore, the highest concentration of PPE (3%) yielded the greatest antioxidant activity, suggesting that the phenolic compounds in the PPE are effectively integrated into the rice starch-pectin matrix, enhancing the film's overall oxidative stability. This is supported by research from Dai et al. [33], which highlighted that biopolymer films enriched with plant extracts exhibit improved functional properties, including enhanced antioxidant activity and thermal stability.

TABLE 6
DPPH RADICAL SCAVENGING ACTIVITY OF THE DEVELOPED FILM AT DIFFERENT CONCENTRATIONS.

Film	DPPH Radical scavenging activity (%)
0 %	-
1 %	9.27±6.73^b
2 %	32.72±3.83^c
3 %	68.38±5.74^d

* Mean standard ± deviation (n=3).
* The values with different alphabets on the same column showed significant differences at $p < 0.05$.

Antimicrobial activity

The antimicrobial properties of rice starch-pectin films incorporated with different concentrations of PPE against Gram-positive bacteria (*Lactobacillus aureus*) and Gram-negative bacteria (*Escherichia coli*) were evaluated using the agar disc diffusion method, as shown in **Table 7**. The control film (0% PPE) exhibited no antimicrobial activity against either microorganism. This suggests that the base matrix of rice starch and pectin alone is insufficient to inhibit bacterial growth which is similar to the findings found by Lopes et al. [40]. However, the incorporation of PPE significantly enhanced the antimicrobial activity of the films. The film with 3% PPE demonstrated the highest inhibition zones, measuring 2.80 mm against *Lactobacillus aureus* and 3.00 mm against *Escherichia coli.* This indicates that higher concentrations of PPE are more effective in inhibiting bacterial growth.

Based on **Figure 5**, it is evident that the composite films with PPE were more effective against Gram-negative bacteria than Gram-positive bacteria. At concentrations of PPE below 1%, the antimicrobial compounds present in the film matrix were insufficient to create inhibition zones for either bacterial strain. This may be due to the higher resilience of Gram-positive bacteria which possess thicker peptidoglycan layers in their cell walls compared to Gram-negative bacteria [41]. The effectiveness of PPE in inhibiting bacterial growth can be attributed to its high phenolic content, particularly punicalagins, which are known for their potent antimicrobial properties. These phenolic compounds can disrupt bacterial cell membranes, leading to the inhibition of bacterial growth and ultimately cell death [42,43]. Previous research by Mahmood et al. [44] supports these findings, highlighting the broad-spectrum antimicrobial activity of phenolic compounds found in pomegranate peel extract. Furthermore, phenolic compounds exhibit multiple biological functions, including antioxidant, anti-inflammatory, and anticancer activities [45], which enhance the overall effectiveness of PPE as a multifunctional additive in biopolymer films. This is consistent with the findings of Hanani et al. [30], who demonstrated that incorporating pomegranate peel powder into biopolymer films significantly improved their antimicrobial and antioxidant properties.

TABLE 7
INHIBITION ZONE OF THE DEVELOPED FILM AT DIFFERENT CONCENTRATIONS.

Film	**Inhibition zone (mm)**	
	Lactobacillus **(gram-positive)**	***Escherichia coli*** **(gram negative)**
0 %	-	-
1 %	2.00	2.00
2 %	2.50	2.50
3 %	2.80	3.00

* Mean standard ± deviation (n=3).
* The values having different alphabets on the same column with significant differences at $p < 0.05$.

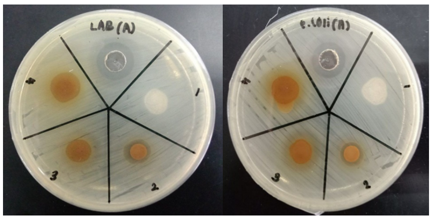

Fig. 5: Inhibition zones of rice starch-pectin film incorporated with pomegranate peel extract at different concentrations tested against LAB and E. coli.

Analysis of the real samples

To evaluate the efficacy of rice starch-pectin films incorporated with various concentrations of PPE, tomatoes were used as real samples. The tomatoes were coated with film samples containing 0%, 1%, 2%, and 3% PPE and stored at room temperature. The weight reduction of the tomatoes was measured over a 15-day period to assess the films' effectiveness in preserving the tomatoes' quality (**Figure 6** and **Figure 7**). Weight loss in fresh produce, such as tomatoes, is a critical indicator of quality deterioration, as it affects both shelf life and marketability. The results revealed a significant difference in weight reduction among the tomatoes wrapped in films with different PPE concentrations. Tomatoes coated with the 0% PPE film exhibited the highest weight reduction at 14.29%, indicating the least effective moisture barrier. This is likely due to the natural respiration process and water loss to the surrounding environment [46], which is exacerbated in the absence of PPE's protective properties.

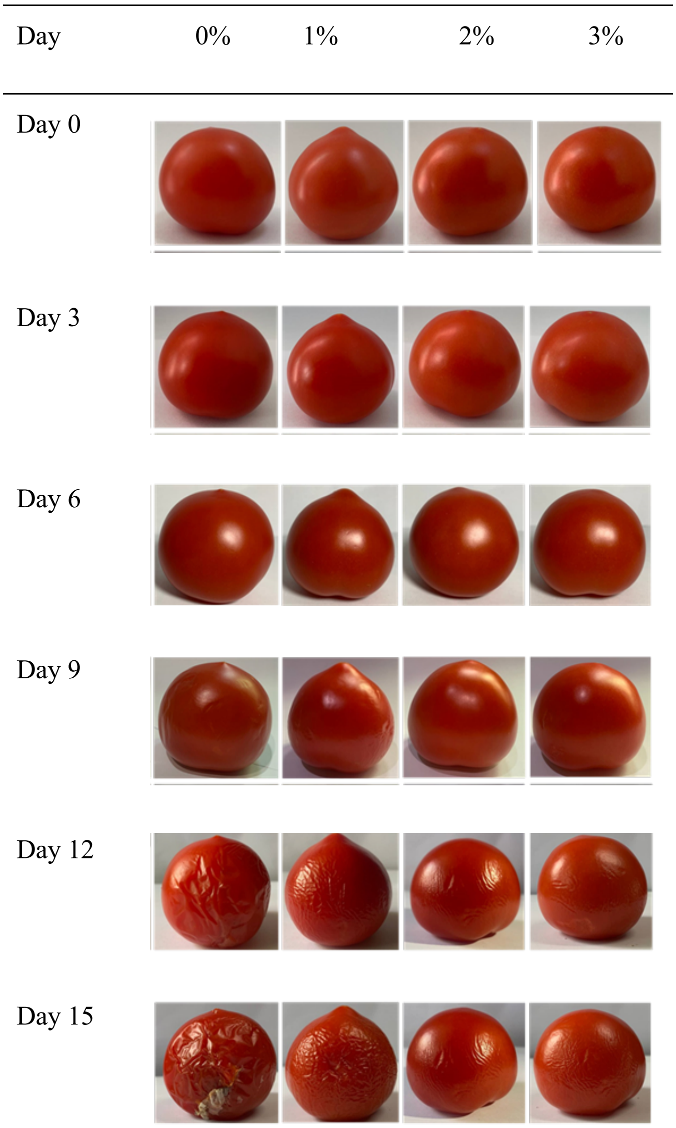

Fig. 6: Image of tomatoes wrapped using film 0 %, 1 %, 2 % and 3 % for 15 days.

Conversely, tomatoes coated with 1%, 2%, and 3% PPE films demonstrated reduced weight loss, with reductions of 12.80%, 12.16%, and 10.67%, respectively. These findings suggest that PPE incorporation enhances the water vapor barrier properties of the rice starch-pectin films, effectively reducing moisture loss [5,25]. The phenolic compounds in PPE are known to create a more robust barrier, minimizing water transfer and thereby preserving the tomatoes' weight and quality [47]. The superior performance of the 3% PPE film, which exhibited the lowest weight reduction, underscores the importance of higher PPE concentrations in improving film efficacy. This aligns with previous studies, such as those by Rathinavel et al. [48] and Dai et al. [33], which have shown that phenolic compounds significantly enhance the barrier properties and thermal stability of biopolymer films. Additionally, after 15 days, tomatoes wrapped in 0% PPE films displayed signs of microbial growth, such as white or moldy spots, indicating compromised quality. In contrast, tomatoes packaged with 1%, 2%, and 3% PPE films maintained better quality, highlighting the antibacterial properties of PPE. This observation is supported by prior research, which demonstrates that phenolic compounds in biopolymer films inhibit microbial growth and extend the shelf life of perishable foods [49,50].

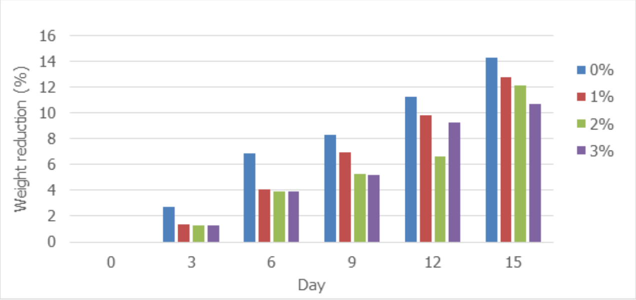

Fig. 7: Percentage reduction in tomato weight for 15 days.

IV. Conclusion

This study demonstrates that incorporating pomegranate peel extract into rice starch-pectin-based films significantly enhances their physicochemical, antioxidant, and antimicrobial properties. Higher concentrations of pomegranate peel extract improve the film's thickness, water solubility, opacity, and storage stability while reducing water vapor permeability. The interaction between the rice starch-pectin film matrix and the phenolic compounds in pomegranate peel extract enhances the composite film's antioxidant and antimicrobial characteristics. Moreover, the rice starch-pectin film with pomegranate peel extract proved effective in food packaging applications, particularly in extending the shelf life of tomatoes for 15 days while preserving their quality. In conclusion, the rice starch-pectin film incorporated with pomegranate peel extract holds potential as a sustainable and functional food packaging material. Future research should aim to further improve the film's physical and mechanical properties and explore its applications in packaging a wider range of perishable foods. This will broaden the scope and utility of these films in the food packaging industry.

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CONFLICT OF INTEREST

Authors declare no conflict of interest to disclose.

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