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Effects of gelatine, alginate, glycerol, sorbitol, and flavedo extract on biofilms' properties

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Abstract

Edible components incorporated into biofilms have demonstrated numerous benefits and promising potentials for diverse applications in food technology. The present work investigated the impact of various film-forming ingredients, including pectin, alginate, gelatine, glycerol, sorbitol, and flavedo extract of *Citrus maxima* (Burm.) Merr. pomelo on the morphological, mechanical, microstructural, thermogravimetric, antioxidant, and antibacterial properties of films. The PGS 5 film, formulated with 85% pectin solution, 5% gelatine solution, 5% sorbitol, and 5% flavedo extract, exhibited superior morphological characteristics, optimal mechanical properties (tensile strength: 9.87 ± 0.19 MPa; elongation at break: 50 ± 1.39%; and thickness: 0.149 ± 0.003), improved heat resistance, poor optical transmittance, and enhanced scavenging ability for free radicals DPPH (23.76 ± 2.314%) and ABTS (22.71 ± 1.95%). Despite inducing minor structural changes and intermolecular interactions, the supplemented film with 5% flavedo extract demonstrated resistance to various pathogenic bacterial strains. These findings suggested the potential use of the developed PGS 5 film with flavedo extract for food applications.

Introduction

The detrimental impact of synthetic packaging materials, such as polyamide and polyethylene, on the ecological environment and human health, is a pressing concern. Synthetic materials, being typically inedible and resistant to natural decomposition, pose significant challenges. Moreover, these synthetic films harbour deleterious molecules that can potentially cross-contaminate food when in contact with food surfaces (Jridi *et al*., 2020). Recent research efforts have been directed towards the search for natural, biodegradable, and environmentally-friendly polymers, as evidenced by studies conducted. However, films derived from natural polymer materials exhibit drawbacks, such as suboptimal water vapour barrier properties, and lower mechanical strength in comparison to their synthetic counterparts.

Enhancing film properties can be achieved through the synergistic combination of diverse biopolymer materials. Pectin is recognised as a macromolecule with the capacity to undergo hydrogel conversion. It can establish a flexible network of polymer chains (Rodsamran and Sothornvit, 2019). Widely acknowledged for its advantageous characteristics, pectin is non-toxic, economically viable, and abundantly available, rendering it a versatile choice across various industries (Freitas *et al*., 2020). Pectin imparts numerous technical functional properties to products, including facile dissolution in common environments, gel formation in acidic conditions, and proficiency in foaming and film development (González-Saucedo *et al*., 2019). These attributes contribute to the multifaceted utility of pectin in improving the overall performance of biopolymer films. Several studies have used edible pectin-based films to preserve cut cantaloupe, such as the work by Martiñon *et al*. (2014). Similarly, Oms-Oliu *et al*. (2008) combined pectin with

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N-acetylcysteine and glutathione to preserve processed pears, maintain quality, and control spoilage microorganisms for up to 14 days.

Alginate, a prevalent polysaccharide, is widely acknowledged in the literature (Tabassum and Khan, 2020). Sodium alginate, the most prevalent alginate salt, is extensively studied in scientific research (Sharma *et al*., 2012). Notably, films derived from sodium alginate exhibit a spectrum of desirable properties including durability, gloss, tastelessness, odourlessness, flexibility, water solubility, and low permeability to O_2 or oil. Combining glycerol into sodium alginate formulations has been employed to produce coatings for fruit and vegetable preservation. This approach has demonstrated efficacy in retarding spoilage induced by microorganisms (Salvia-Trujillo *et al*., 2015). Sodium alginate films have poor properties for fruit packaging, but adding thymol improves their strength, UV protection, and antimicrobial activity. Thymol/sodium alginate films also better preserve fresh-cut apples by reducing weight loss, and maintaining nutrition and colour, making them a promising packaging material (Chen *et al*., 2021).

The enzymatic breakdown of collagen yields the natural polymer gelatine (Kumosa *et al*., 2018). Bloom index is the quantitative indicator of gelatine durability, and its value is contingent upon the gelatine extraction sequence. Notably, the initial extraction phase yields the highest bloom number, indicative of superior gelation ability, while subsequent stages exhibit lower bloom numbers (Netter *et al*., 2020). Gelatine stands out for its exceptional physical properties, including gel-forming capability, affinity, high dispersibility, low viscosity, dispersion stability, and elevated water retention capacity (Alipal *et al*., 2019). Leveraging its commendable film-forming attributes, gelatine finds extensive application in the edible film and coating materials industry (Ramos *et al*., 2016).

Polyol plasticisers like sorbitol and glycerol reduce interactions between biopolymer chains, enhancing flexibility and stretchability during film formation (Hazrati *et al*., 2021). Glycerol is categorised as a polyfunctional alcohol, and possesses three hydroxyl groups, contributing to its hygroscopic properties and water solubility. It is a colourless and odourless liquid under ambient conditions (Ballesteros-Mártinez *et al*., 2020), and acts as a plasticiser in various biopolymers, including tapioca starch films, chitosan films, and alginate films. Sorbitol, a common polyol in fruits, is interesting in its roles as a humectant, texturiser, and emollient in food processing (Silveira and Jonas, 2002). This compound disperses between polymer chains, breaking hydrogen bonds, and improving the flexibility and thermal properties of films (Su *et al*., 2017).

Abudayeh *et al*. (2019) have demonstrated some chemical components and antioxidant activity of pomelo peel extract. Therefore, the purpose of the present work was to evaluate the mechanical, microstructural, thermogravimetric, FTIR spectroscopic, X-ray diffraction, antioxidant, and antibacterial properties of films obtained from film-forming ingredients including pectin, gelatine, and alginate supplemented with pomelo peel extract, thereby making the basis for the application of films in fruit preservation.

Materials and methods

Materials

The Da Xanh pomelo (*Citrus maxima* (Burm.) Merr.) used in the present work was sourced from pomelo-growing households within the designated geographical indication areas of Ben Tre province, Vietnam. The pomelos were selectively harvested from 5- to 8-year-old trees. The bacterial strains employed were *Bacillus cereus* NRRL B-3711, *Staphylococcus aureus* NRRL B-313, *Listeria monocytogenes* CIP 106, *Salmonella* Typhimurium DSM 10506, *Escherichia coli* NRRL B-409, *Shigella boydii* ATCC 8700, *Campylobacter jejuni* DSM 24114, *Vibrio parahaemolyticus* DSM 2172, *Citrobacter freundii* RLS1, and *Proteus mirabilis* ADL-72. These bacterial strains were maintained at the Institute of Applied Technology and Sustainable Development at Nguyen Tat Thanh University.

Chemicals

High methoxyl pectin (HiMedia, India), gelatine with bloom 125 (Germany), sorbitol (China), glycerol 99% (China), Mueller Hinton agar/broth (Himedia, India), NaCl 99.5% (China), $NaNO_2$ 99% (China), $AlCl_3.6H_2O$ 97% (China), NaOH (China), CH_3COOH 99.5% (China), FeCl (China), Folin and Ciocalteu reagent (USA), ABTS 98% (Sigma Aldrich, USA), DPPH (Sigma Aldrich, USA), Na_2CO_3 99.6% (China), $CH_3COONa.3H_2O$ 99% (China), and dimethyl sulfoxide (China) were used.

Flavedo extraction from Citrus maxima (Burm.) Merr.

The flavedo peel underwent heat pump drying at 35°C until its moisture content was reduced to below 10%. Subsequently, the dried pomelo peel was finely ground, and sifted through a 0.5 mm sieve. Following the grinding process, the pomelo peels were subjected to extraction using 90% ethanol, with a material/solvent ratio (w/v) of 10/146 (g/mL). The extraction process occurred over 48 h at room temperature. Once the pomelo peel extract was obtained, it underwent vacuum evaporation, and was further convection dried at 45°C until the moisture content of the extract was reduced to less than 5%. We have previously reported the properties of pomelo peel extract (Nguyen *et al*., 2024).

Film formulation

Pectin solution (1:20 g/mL) and gelatine/alginate solution (1:50 g/mL) were stirred magnetically until completely dissolved. The film-forming components were fixed at 5% pomelo peel extract, 5% glycerol or sorbitol, and variable gelatine (G)/alginate (A) components ranging from 5, 15, 25, 35, 45, to 55%, denoted in the symbol of film-forming formula. The remaining portion comprised pectin (P), to a final composition of 100%. The proportions of the film-forming components are shown in Table 1.

Table 1. Ratios of film-forming components.

Film	Pectin (P) (%)	Gelatine (G) / Alginate (A) (%)	Glycerol (G) / Sorbitol (S) (%)	Extract (%)
PGG 5	5	5	5	85
PGG 15	5	5	15	75
PGG 25	5	5	25	65
PGG 35	5	5	35	55
PGG 45	5	5	45	45
PGG 55	5	5	55	35
PGS 5	5	5	5	85
PGS 15	5	5	15	75
PGS 25	5	5	25	65
PGS 35	5	5	35	55
PGS 45	5	5	45	45
PGS 55	5	5	55	35
PAG 5	5	5	5	85
PAG 15	5	5	15	75
PAG 25	5	5	25	65
PAG 35	5	5	35	55
PAG 45	5	5	45	45
PAG 55	5	5	55	35
PAS 5	5	5	5	85
PAS 15	5	5	15	75
PAS 25	5	5	25	65
PAS 35	5	5	35	55
PAS 45	5	5	45	45
PAS 55	5	5	55	35

Tensile strength of film

The tensile strength was calculated according to Antoniou *et al*. (2015) using Eq. 1:

$$TS = F/(L \times x) \quad \text{(Eq. 1)}$$

where, F = tensile force (N), l = width of the film (mm), and x = thickness (mm).

Elongation at break of film

The elongation at break was calculated according to Antoniou *et al*. (2015) using Eq. 2:

$$\%EB = 100 \times (l - l_1)/l_1 \quad \text{(Eq. 2)}$$

where, l = initial length and l_1 = length of the pomelo at the breakpoint.

Thickness of film

The film thickness was determined according to Shahrampour *et al*. (2020) with some changes, using a thickness gauge (Mitutoyo, Tokyo, Japan) in triplicates. The values were expressed as mean ± SD.

Thermogravimetric analysis

The thermal properties of the films were determined by the thermogravimetry (TG) using the Labsys Evo TG-DSC 1600 C system (Setaram Instrumentation, France) over a temperature range of 30 - 220°C at a rate of 10°C min^{-1}.

Scanning electron microscope

SEM uses a focused electron beam with relatively low energy as an electron probe that is scanned regularly over the specimen, and the device structure is shown in the report of Abdullah and Mohammed (2019). Pomelo samples were measured using an S-4800 field emission SEM (Hitachi, Japan) at a scanning voltage of 10 kV.

Fourier-transform infrared spectroscopy

Fourier-transform infrared spectroscopy (FT-IR) with a Frontier NIR/MIR system (PerkinElmer, USA) was used to analyse the functional groups of the film-forming components. Measurements were performed in the wavelength range of 4000 - 450 cm^{-1}.

X-ray diffraction

The crystal structure of the pomelo was studied by X-ray diffraction (XRD) using an Empyrean Diffractometer (PANalytical, Netherlands).

Light transmittance

The transmittance of the film was quantified as a percentage using a UV-Vis spectrophotometer (UV-VIS 6850, Jenway, England) within the wavelength range of 200 - 800 nm, maintaining an accuracy of 0.1 nm. Three replicates of each film were subjected to testing.

DPPH free radical scavenging activity

The antioxidant potential of the extract was evaluated by the modified 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, as described by Thaipong *et al*. (2006). A stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol, and the solution was allowed to stand for 24 h. Subsequently, a test solution was formulated by combining 10 mL of the DPPH stock solution with 45 mL of methanol to attain an absorbance of 1,170.02 units at 510 nm, as measured using a spectrophotometer. In the antioxidant assay, 0.15 mL of the extract was aspirated, and 2.85 mL of the prepared test solution was added. After a 30-min incubation period under light protection, the absorbance was measured at 515 nm. Methanol was employed as the negative control in this assessment.

ABTS cation radical scavenging activity

The free radical scavenging activity of the extract was assessed through the $ABTS^{+}$, 2.2'-azino-bis-(3-ethylebenzothiazoline-6-sulfonate), decolourisation method, as outlined by Thaipong *et al*. (2006). Stock solutions were prepared by combining $K_2S_2O_8$ (2.6 mM) and ABTS (7.4 mM) in a 1:1 ratio. The resulting stock solution was further diluted 1:6 with methanol, and its absorbance was adjusted to 1.1 with a precision of 0.02. For the assay, 0.15 mL of the sample solution was introduced into 2.85 mL of the prepared test ABTS. After a 30-min incubation period, the absorbance of the reaction mixture was determined using a UV-Vis spectrophotometer set to 734 nm. Methanol was utilised as the negative control.

Determination of antibacterial ring diameter of film supplemented with flavedo extract

The film samples with pectin, gelatine/alginate, and glycerol/sorbitol as fixed components were supplemented with pomelo peel extract at concentrations of 0, 3, 5, and 7%. The mixture was homogenised, cast into moulds at a solution/substrate ratio of 7 g solution/63.5 cm^2, and dried at 37°C for 48 h. The antimicrobial activity of the formed films was evaluated following the methodology outlined by Homthawornchoo *et al*. (2022) with appropriate modifications. The film samples were cut into discs with a diameter of 6 mm. Pathogenic bacterial strains were activated in Mueller-Hinton broth (MHB) for 24 h at 37°C, and adjusted to a cell density equivalent to McFarland 0.5 using 0.85% NaCl solution. A volume of 50 µL of the diluted bacterial solution was spread evenly on Muller-Hinton agar (MHA) plates using a swab. After allowing the agar surface to dry for 10 min, the film discs were placed on the agar surface. The plates were then incubated at 37°C for 24 h, and the diameter of

the bacterial inhibition zone was measured and recorded in triplicates. The results were presented as the mean values ± standard deviation (SD).

Statistical analysis

Each trial was replicated three times. Statistical analysis was conducted using SPSS version 22 software (IBM Corp., USA), employing One-way ANOVA to assess significant differences among samples at the 5% significance level. *Post-hoc* testing, specifically the Tukey's test, was then applied to compare the significant differences between the mean values of the samples.

Results and discussion

Effect of film-forming ingredients on properties of pectin films supplemented with flavedo extract

The analysis was performed by mixing the film-forming ingredients, which were pectin solution (1:20 g/mL) and gelatine or alginate solution (1:50 g/mL), in which glycerol or sorbitol and pomelo peel extract were added to the ratio of film-forming ingredients. The film was dried at 37°C for 24 h with an air humidity of 55 - 60%. The dried film properties after removal from the mould were recorded as shown in Figure 1. Films with low pectin and high gelatine or alginate content (PGG 35/45/55; PGS 35/45/55; PAG 35/45/55; and PAS 35/45/55) had the common characteristics that were low thickness but high flexibility, being very sticky, and being easy to tear when removed from the mould. In contrast, films with high pectin and low gelatine or alginate content (PGG 5/15/25; PGS 5/15/25; PAG 5/15/25; and PAS 5/15/25) all have characteristics such as high thickness, moderate flexibility, elasticity, less stickiness, and easy release from the mould. This was due to the properties of the film-forming ingredients. The higher the pectin content of the film, the higher its thickness and tensile strength (Chambi and Grosso, 2011). Therefore, selected films (PGG 5/15/25; PGS 5/15/25; PAG 5/15/25; and PAS 5/15/25) were selected for further evaluation of mechanical properties such as thickness, tensile strength, and elongation at break.

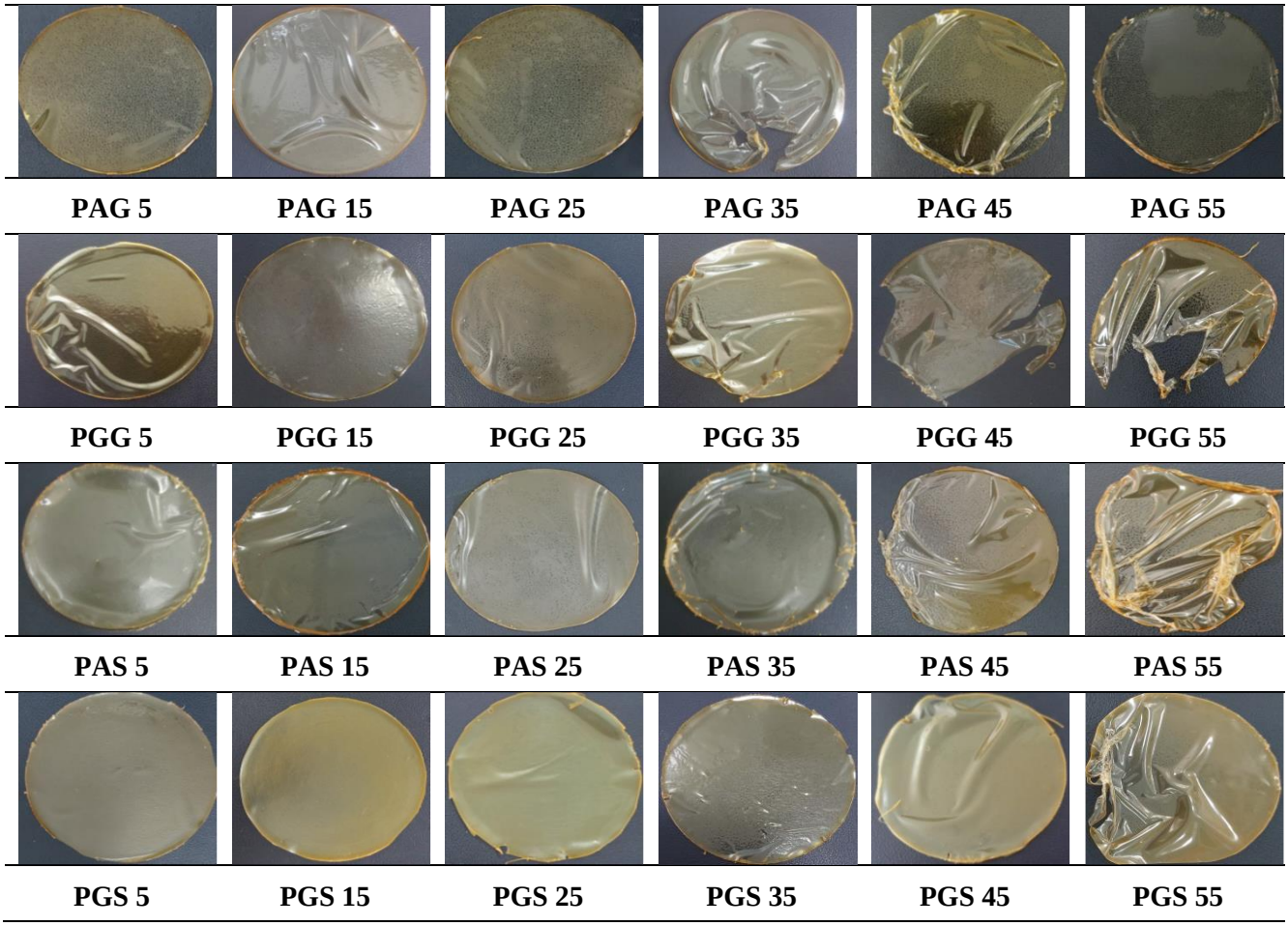

Figure 1. Images of pectin films supplemented with flavedo extract.

Tensile strength, elongation at break, and thickness of pectin film supplemented with flavedo extract

Tensile strength, elongation at break, and thickness of films (PGG 5/15/25; PGS 5/15/25; PAG 5/15/25; and PAS 5/15/25) are presented in Table 2. The results showed that the highest and lowest tensile strength values were shown by PGS 5 and the PAG 25, with a significance level ($p < 0.05$). The highest and lowest elongation values at break were shown by PAG 5 and PGG 5, with a significance level ($p < 0.05$). However, the elongation difference between PAG 5, PGG 15, PGS 25, and PGS 5 film was insignificant. Table 2 also shows that films with the pectin-gelatine (PG) formula constantly had significantly greater thickness than films with the pectin-alginate (PA) formula, specifically the films with the highest and lowest thickness, PGS 5 and PAG 25, respectively.

Mechanical properties such as tensile strength and elongation at break are among the primary properties of packaging films that need to be controlled because they must protect the product from environmental stress outside (Aitboulahsen *et al*., 2020). Tensile strength, elongation at break, and film thickness vary with the film composition; this was due to the different molecular weights, chemical interactions, and compatibility between these ingredients. In general, the pectin-gelatine films plasticised with sorbitol (PGS 5/15/25) exhibited better functional properties than the other films. The carboxylic groups of pectin can interact well with the polymer side chain of gelatine to make the film more durable and less elastic (Chambi and Grosso, 2011). Plasticisers with lower molecular weights can facilitate polymer chain-plasticiser interactions, specifically glycerol (92.09 g/mol), more effectively than sorbitol (182.17 g/mol) in plasticising the film, thus leading to a film with better elasticity. However, as the elasticity increases, the tensile strength significantly decreases, and this harms the mechanical properties of the film. In addition, the plasticiser (sorbitol or glycerol) does not significantly affect the film thickness (Aitboulahsen *et al*., 2020). Films with higher pectin concentrations have significantly higher film thickness and tensile strength (Galus and Kadzińska, 2015). Jridi *et al*. (2020) also demonstrated that when increasing the pectin content in the pectin-gelatine film system, the tensile strength of the film increased, but the elongation at break decreased significantly. Thus, PGS 5 was the film-forming formula chosen for subsequent analysis.

Table 2. Tensile strength, elongation at break, and film thickness of films.

Film	Tensile strength (MPa)	Elongation at break (%)	Thickness (mm)
PAG 25	2.06 ± 0.08^{a}	53.00 ± 2.20^{hi}	0.111 ± 0.002^{a}
PAG 15	3.38 ± 0.29^{bc}	47.10 ± 1.77^{cdf}	0.117 ± 0.003^{ab}
PAG 5	3.8 ± 0.14^{c}	55.50 ± 1.68^{i}	0.124 ± 0.004^{c}
PGG 25	3.4 ± 0.36^{bc}	41.10 ± 1.78^{c}	0.131 ± 0.001^{d}
PGG 15	6.02 ± 0.18^{e}	52.00 ± 1.34^{ghi}	0.136 ± 0.002^{d}
PGG 5	3.39 ± 0.20^{bc}	29.50 ± 2.11^{a}	0.144 ± 0.002^{e}
PAS 25	2.46 ± 0.20^{a}	45.90 ± 2.44^{cde}	0.112 ± 0.003^{a}
PAS 15	3.01 ± 0.31^{b}	44.20 ± 2.29^{cd}	0.120 ± 0.003^{bc}
PAS 5	6.17 ± 0.25^{c}	37.30 ± 1.47^{b}	0.125 ± 0.002^{c}
PGS 25	3.55 ± 0.10^{bc}	50.90 ± 1.19^{fghi}	0.132 ± 0.002^{d}
PGS 15	4.88 ± 0.11^{d}	47.50 ± 1.19^{defg}	0.137 ± 0.001^{d}
PGS 5	9.87 ± 0.19^{f}	50.00 ± 1.39^{efghi}	0.149 ± 0.003^{f}

Means in similar column with different lowercase superscripts are significantly different at the 5% significance level using Tukey's test.

Thermogravimetric analysis

Details about intermolecular structural variation brought on by temperature variations are necessary to assess the packaging material's thermal tolerance. The thermal stability of the PGS 5 film was studied by thermogravimetric analysis (TGA). Figure 2A shows the three main mass loss stages of the PGS 5 film in TGA analysis. The first stage of mass loss is due to water evaporation, and the second stage is due to the thermal oxidation of the film. The third stage is

the carbonisation stage of the materials in the film component. In particular, in the temperature range of 50 - 90°C, transpiration and the release of volatile compounds took place (Mendes *et al*., 2019). The depolymerisation of pectin chains took place in a temperature range of 200 - 230°C. Similar degradation of pectin in pectin/cellulose films and pectin films/sage leaf extract was also reported by Bátori *et al*. (2017) and Han and Song (2020). The gelatine network is thermally decomposed at a temperature range of 400 - 450°C. Similar degradation of gelatine in pectin/gelatine films has also been reported by the study of Mishra *et al*. (2011). Figure 2A shows the three main mass loss stages of the control PGS 5 film and the PGS 5 film supplemented with flavedo pomelo extract in TGA analysis. The first stage of mass loss is due to water evaporation. In particular, in the temperature range of 50 - 90°C, transpiration and the release of volatile compounds take place (Mendes *et al*., 2019). The second stage (190 - 400°C) showed a large loss of water due to polysaccharide decomposition, extensive thermal degradation followed by decarboxylation of the carbon ring, the evolution of numerous gaseous products, and finally, the formation of solid char (Sharma *et al*., 2012). At this stage, the film supplemented with pomelo extract retained its mass better than the control film. The third stage, from 400 to 700°C, depicts weight loss due to the formation of partly destroyed solid char stacks (Wang *et al*., 2016). In the third stage, the film supplemented with extract lost mass faster than the control film. After 700°C, various gases are released, and weight loss occurs mainly due to the decomposition of the samples (Sharma *et al*., 2012). Thus, TGA showed that the weight loss in the control film and the film with extract was different. This could have been due to the difference in film composition, as well as the chemical bonds present in the film. Besides, there were decomposition of and chemical change in film-forming components.

FTIR

FTIR spectroscopic analysis was performed to understand the intermolecular interactions and structural changes in the films at the molecular level. The FTIR spectrum of the PGS 5 film is shown in Figure 2B. The results showed that there were clear differences between some peaks of the control PGS 5 film (CT) and the PGS 5 film supplemented with pomelo flavedo extract (EX). The extract added to the film caused the loss of some peaks, and reduced chemical bonds. The first two peaks of the control film and the additional extracted film were 3292 and 3410 cm^{-1}, respectively, representing -OH bonds. The film supplemented with extract had a peak of 3410 cm^{-1}, and showed fewer hydrogen bonds. The same was demonstrated by Sharma *et al*. (2012). The EX-film without the 2360 cm^{-1} peak (similar to the CT film) showed a loss of the ≡CN bond. Although the extract added to the film shifts some peaks, in general, the film (CT and EX) had some of the following characteristic peak ranges. The remaining bands at peaks 2935 and 2936 cm^{-1} also showed -CH stretching vibrations. In addition, vibrational bands representing free carboxylate groups, amide 1 and amide III, were also shown at peaks 1642 and 1629 cm^{-1} (Nisar *et al*., 2022). Peak 1745 cm^{-1} represents the maximum relaxation of aldehyde (Homthawornchoo *et al*., 2022).

SEM

The microstructure of the PGS 5 film surface was studied through SEM observation (Figure 2C). The control PGS 5 film (Figure 2C - CT) and the film supplemented with flavedo extract (Figure 2C - EX) had uneven surfaces and many wrinkles. However, the PGS 5 film supplemented with extract had a more wrinkled surface structure. In addition, the microstructure of the film at a scale of 20.0 µm shows that the extra-extracted film surface has more obvious cracks than at a scale of 50.0 µm. This was because the extract added to the film caused disruption in the microstructure of the film, and caused loss of film cohesion. Research by Aitboulahsen *et al*. (2020) also demonstrated that *Mentha pulegium* and *Lavandula angustifolia* essential oils added to pectin/gelatine films significantly reduced the uniformity of the film surface. Previous research (Han and Song, 2020) also demonstrated that sage (*Salvia officinalis*) leaf extract added to pectin films caused inhomogeneity on the film surface. However, the extract added to the polymer film also improved the flexibility of the film.

XRD

XRD analysis was used to study the crystal structure of PGS 5. The well-defined peaks at 12.72°, 16.30°, 18.45°, 25.32°, and 40.14° of 2θ are related to the crystallinity of purified pectin (Chaichi *et al*., 2017). The illustrative diffractogram of the PGS 5 film shows good compatibility between the film components because the basic crystalline fraction of

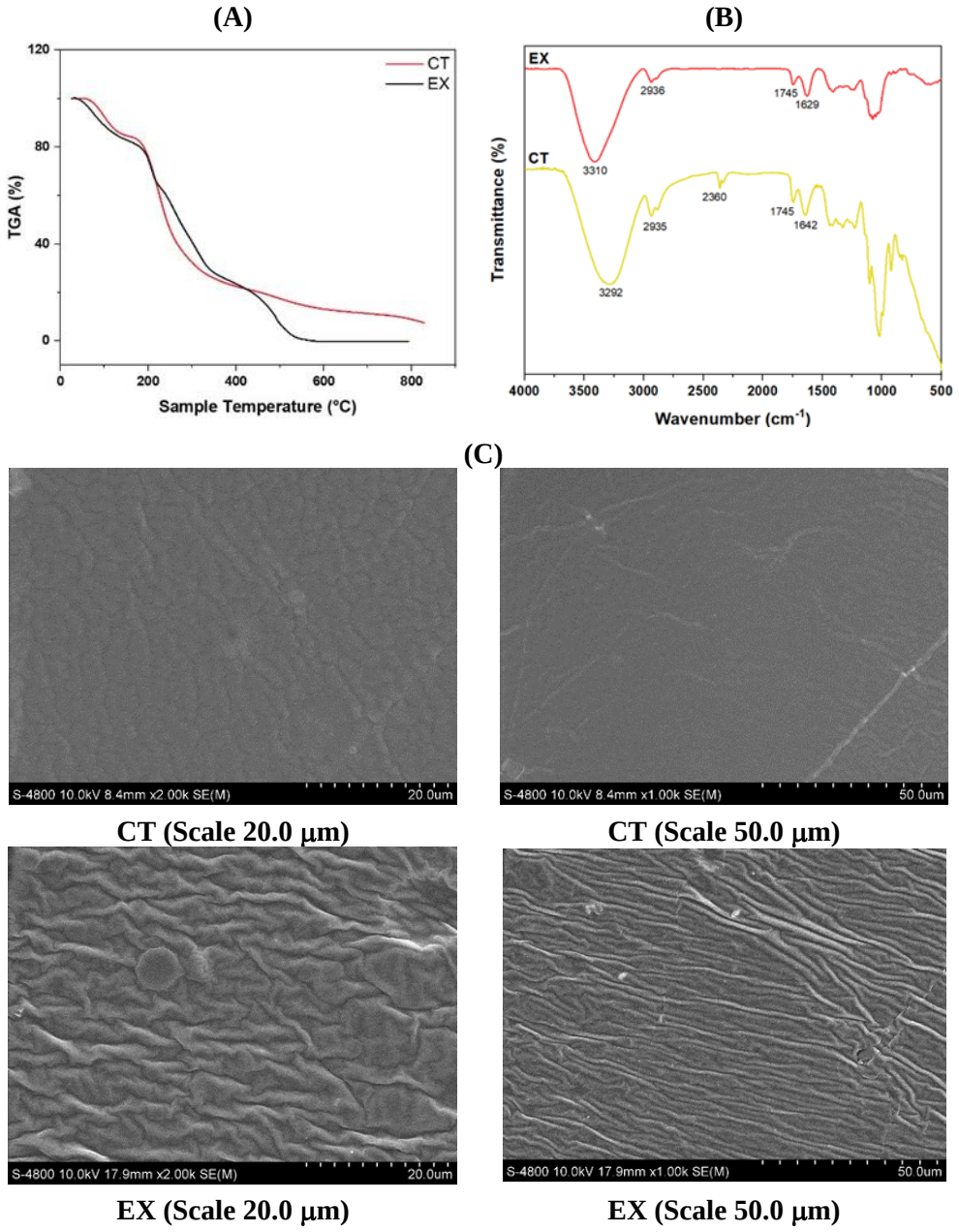

Figure 2. (A) Thermogravimetric analysis chart; **(B)** film transformation infrared spectrum; and **(C)** scanning electron microscope image of film surface (CT: Control PGS 5 film with no extract; and EX: PGS 5 film supplemented with pomelo flavedo extract).

pectin was not reduced in the composite film. The PGS 5 film exhibited sharp peaks, confirming the presence of some crystalline structures (Figure 3A). Chemical interactions of the PGS 5 film-forming components had taken place, as diffraction peaks representing a new crystal structure were discovered.

Light transmittance

The results of light transmission (Figure 3B) indicated that in the wavelength range of 200 - 400 nm, the transmittance of the film sample was 0%. As the wavelength increased from 400 to 800 nm, the transmittance of the film increased from 0 to 23%. These findings suggested that the light transmission of the PGS 5 film was relatively low. The impact on the light transmission capacity of the film depends on the proportion of the mixed components, particularly sorbitol and high-peel grapefruit extract. A film with low light transmission contributes to hindering light penetration, thereby supporting the fruit preservation process that necessitates light avoidance.

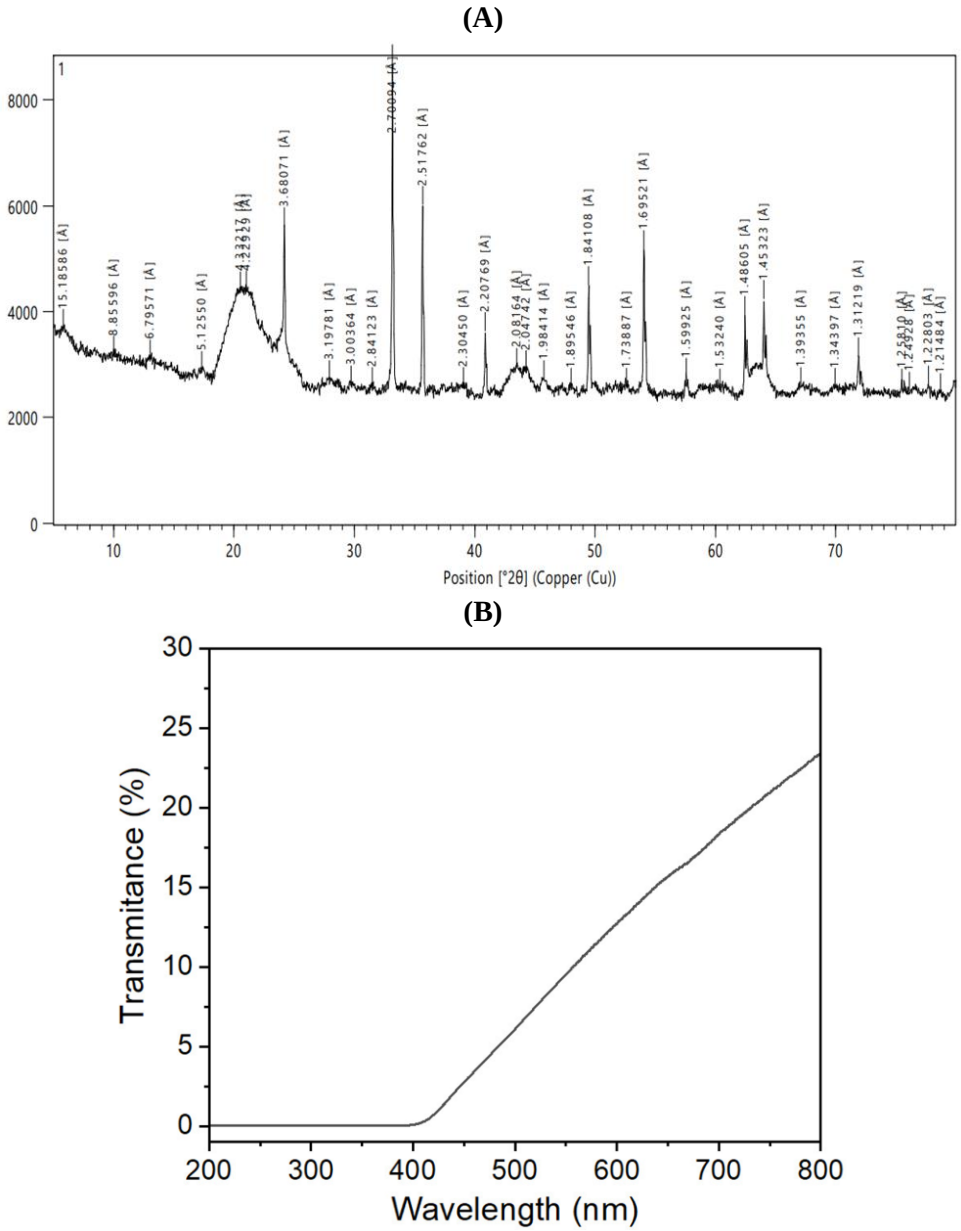

Figure 3. X-ray diffraction pattern **(A)** and light transmittance **(B)** of film PGS 5.

Antioxidant activity of film supplemented with flavedo extract

The PGS 5 film was moulded, dried, and evaluated for antioxidant properties based on its ability to scavenge DPPH and ABTS free radicals. The outcomes derived from the assessment of oxygen resistance in the film, as determined through DPPH and ABTS free radical scavenging assays, reflected the antioxidant efficacy of the film. The film's antioxidative potential, as assessed by its DPPH and ABTS free radical scavenging capabilities, was quantified at 23.76 ± 2.31 and 22.71 ± 1.95%, respectively.

The results showed that PGS 5 film containing 5% pomelo peel extract had DPPH and ABTS free radical scavenging activities. The antioxidant capacity of the film supplemented with extract depends on many factors, such as the composition of the film-forming solution, film drying temperature, extract type, and extract concentration (Roy and Rhim, 2021). Han and Song (2020) also demonstrated that pectin films extracted from tangerine peel supplemented with sage leaf extract (SLE) had antioxidant activity through the ability to scavenge DPPH and ABTS free radicals.

Antibacterial activity of film supplemented with flavedo extract

Table 3 shows that for the same bacterial strain, between films PGS (pectin, 85%; gelatine, 5%; and sorbitol, 5%) supplemented with different extract concentrations (0, 3, 5, and 7%), the bacterial resistance ring diameters were different; specifically, film with 0% flavedo extract did not present bacterial resistance activity. On the other hand, when comparing the differences between each bacterial strain in the same type of film, it showed that the PGS film supplemented with 3% flavedo extract did not show antibacterial activity (0.00 ± 0.00 mm) against *E. coli*, *S. boydii*, and *V. parahaemolyticus*; most antibacterial ring diameters ranged from 8.00 ± 0.00 to 13.33 ± 2.52 mm.

Next, the PGS film supplemented with 5% extract showed antibacterial ring diameters ranging from 10.67 ± 1.15 to 15.00 ± 1.00 mm. The film supplemented with 5% extract had insignificantly ring diameters of *C. jejuni*, *L. monocytogenes*, and *V. parahaemolyticus*. Similarly, the film supplemented with 7% flavedo extract, showed resistance activity against some pathogens, and the resistance ring diameters ranged from 12.00 ± 1.00 to 23.33 ± 1.15 mm. However, the film's resistance against *B. cereus* and *C. jejuni*; *S. typhimurium* and *V. parahaemolyticus* strains had no significant difference.

In general, films containing larger extract concentrations had better antibacterial properties; films with 7% extract concentration demonstrated better antibacterial properties than films with 5% extract concentration; and films with 5% extract concentration showed better antibacterial activity than films with 3% extract concentration. It can be explained that films with higher concentrations of pomelo peel extract will contain higher levels of active ingredients and antibacterial properties due to the presence of phytochemicals in pomelo peel (Dulta *et al*., 2022) which include phenolics, flavonoids, naringin, hesperidin, tannins, alkaloids, and saponins (El-Baky *et al*., 2021).

The PGS film with flavedo extract concentrations of 3 and 7% was used as a control to compare the antibacterial activity of film with flavedo extract concentration of 5%. The results showed that the PGS film supplemented with 3% flavedo extract did not show antibacterial activity against some pathogens, while the film with 5% extract showed full antibacterial activity against all tested pathogens. This showed that films with 5% extract can be used to help inhibit some strains of pathogens on the surface of fruit peels during the film coating process (Han and Song, 2020; Homthawornchoo *et al*., 2022). The film supplemented with 7% flavedo extract had better antibacterial activity than the film supplemented with 5% flavedo extract. However, as the extract concentration increased, the solubility and mechanical durability (tensile strength and elongation) of the film decreased, thereby reducing the film's ability to effectively preserve fruits (Aitboulahsen *et al*., 2020; Homthawornchoo *et al*., 2022).

Table 3. Antibacterial activity of pectin films supplemented with pomelo peel extract at high concentrations of 0, 3, 5, and 7%.

Pathogen	Inhibition ring diameter of films added with flavedo extract (mm)			
	PGS - 0% extract	PGS - 3% extract	PGS - 5% extract	PGS - 7% extract
B. cereus	–	8.00 ± 1.00^{Ab}	12.00 ± 1.00^{Bab}	12.66 ± 2.08^{Bab}
C. jejuni	–	9.33 ± 1.15^{Abc}	10.67 ± 0.58^{ABa}	12.00 ± 1.00^{Ba}
C. freundii	–	8.00 ± 0.00^{Ab}	15.00 ± 1.00^{Bb}	22.67 ± 0.58^{Cde}
E. coli	–	–	12.33 ± 2.08^{Bab}	16.33 ± 2.31^{Bbc}
L. monocytogenes	–	8.00 ± 0.00^{Ab}	10.67 ± 1.15^{Ba}	14.67 ± 0.58^{Cab}
P. mirabilis	–	11.67 ± 0.58^{Acd}	14.67 ± 0.58^{Bb}	23.33 ± 1.15^{Ce}
S. Typhimurium	–	13.33 ± 2.52^{Ad}	13.67 ± 1.53^{Aab}	15.33 ± 2.52^{Aabc}
S. boydii	–	–	14.00 ± 1.73^{Bab}	19.00 ± 1.00^{Ccd}
S. aureus	–	10.67 ± 0.58^{Ab}	14.33 ± 0.58^{Bb}	21.67 ± 0.58^{Cde}
V. parahaemolyticus	–	–	10.67 ± 1.15^{Ba}	15.33 ± 1.52^{Cabc}

Uppercase superscripts indicate significant difference ($p < 0.05$) between different extract concentrations on similar pathogen; lowercase superscripts indicate significant difference ($p < 0.05$) between different pathogens on similar extract concentration; –: no resistance.

Conclusion

In the present work, a variety of formulations from pectin were prepared; flavedo extracts, alginate or gelatine, and glycerol or sorbitol. It was found that the combination of pectin, gelatine, sorbitol, and flavedo extract improved the mechanical, chemical, and antioxidant properties of the PGS 5 film. Analyses showed that the addition of the flavedo extract of pomelo caused small changes in the morphology and physicochemical properties of the film. Therefore, PGS 5 film containing flavedo extract of *Citrus maxima* (Burm.) Merr. can be used as a potential biodegradable film to slow down the process of deterioration of nutritional value, and extend food shelf life.

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