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Effects of Sodium Alginate and Gelatin on Physical and Thermal Properties of Pullulan Blend Films

Imelda Yi Zi Liew and Ing Hong Ooi[*]

Department of Pharmaceutical Chemistry, School of Pharmacy, IMU University, Kuala Lumpur, Malaysia

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Abstract: In this paper, we discussed blending pullulan with other polysaccharides or proteins as a strategic approach to improve the inferior properties of pullulan films, such as brittleness and low water resistance. In this work, films made from pullulan:sodium alginate, pullulan:gelatin, and pullulan:sodium alginate:gelatin mixture were prepared by the solvent casting method. By adjusting the alginate-to-gelatin ratio in PU:SA:GE blend films, the water content, light transmittance/absorbance, thermal stability, and water solubility of the films can be tailored to achieve desirable performance levels, highlighting their potential for packaging applications. The PU:SA:GE (5:1:3) ternary blend film exhibited the highest light absorbance, good thermal stability, adequate mechanical strength, and moderate water solubility, making it a promising candidate for packaging light-sensitive foods. Compared with PU:SA and PU:GE binary blends, this formulation provides greater opacity and reduced water sensitivity, thereby offering a functional advantage for food packaging applications.

Keywords: Biopolymer blend films, Gelatin, Physical properties, Pullulan, Sodium alginate.

1. Introduction

Blending biopolymers with differing characteristics and preparing the resulting films via solvent casting offers a rapid and convenient approach for evaluating their functional properties, thereby helping to identify suitable applications across various sectors, including pharmaceutical, biomedical and food industries [1-3]. Pullulan, sodium alginate, and gelatin are biopolymers widely used in the food, food packaging, medical, and pharmaceutical industries. Each possesses unique functional properties desirable for specific applications. For example, pullulan offers good moisture retention, inhibits fungal growth, and provides excellent oxygen barrier properties. Sodium alginate exhibits gelling, thickening, and stabilizing abilities, along with good water retention. Gelatin, on the other hand, is valued for its elasticity and clarity. Their biodegradability makes them attractive alternatives to synthetic polymers, particularly in light of growing environmental and safety concerns.

Pullulan, an exopolysaccharide produced through the fermentation of sugar by the yeast-like fungus *Aureobasidium pullulans*, is primarily composed of maltotriose units linked by α-(1,6) glycosidic bonds (Fig. 1). This unique structure imparts its characteristic flexibility and solubility [4,5]. Being odorless, tasteless, non-toxic, and biodegradable [6], pullulan is used as film-forming agent, flocculent agent, and food additive [7,8]. However, its high production cost has limited widespread commercial application. Another challenge associated with pullulan is its brittleness in response to changes in humidity [9,10]. These limitations have been addressed by blending pullulan with other polymers. For example, pullulan-gelatin blend films exhibit improved tensile strength [11]. Blends with other polysaccharides, such as alginate [12], chitosan [13], and starch [14], have also been reported to enhance thermal and mechanical properties.

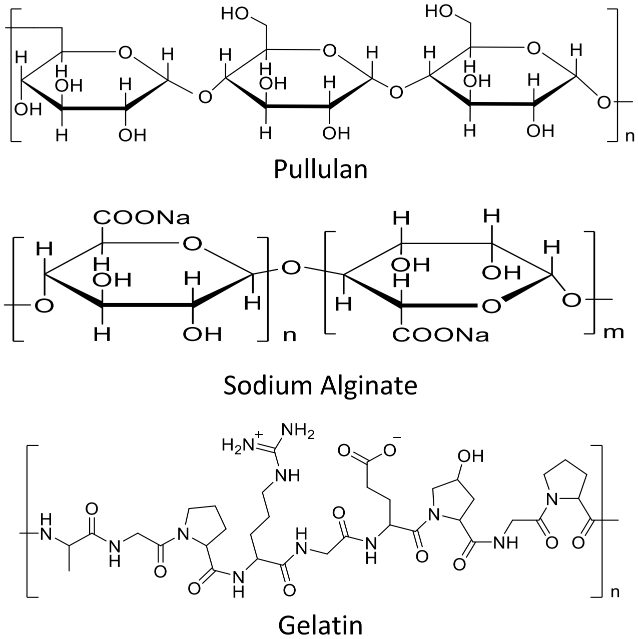

Fig. 1: Chemical structures of pullulan, sodium alginate, and gelatin

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[*] Corresponding author E-mail: inghong_ooi@imu.edu.my

Sodium alginate, a natural polysaccharide extracted from brown seaweed, is composed of α-L-guluronate (G blocks) and β-D-mannuronate (M blocks) linked by 1-4 glycosidic bonds (Fig. 1) [15-17]. It is an anionic, highly mucoadhesive, non-allergenic, non-toxic polymer that is compatible with most water-soluble compounds [18, 19]. Alginate can be processed into various forms such as hydrogels, foams, and microspheres, each suited for different applications [20]. A study done by Xiao Q *et.al*. demonstrated that pullulan-sodium alginate films exhibited increased tensile strength but reduced elongation at break under low water activity conditions [12].

Gelatin (Fig. 1) is a protein derivative obtained from the partial hydrolysis of water-insoluble collagen fibers. Its film-forming properties are influenced by molecular weight, with higher molecular-weight gelatin producing films of superior quality.[21]. A study reported that incorporating gelatin enhanced the performance of pullulan-gelatin films, improving tensile strength and reducing oxygen permeability [11]. These enhancements were attributed to molecular interactions including the formation of glycosylated proteins, increased β-sheet content, and enhanced intermolecular hydrogen bonding. Therefore, both sodium alginate and gelatin are promising candidates for blending with pullulan due to their favorable film-forming abilities and low cost.

As consumer awareness of and demand for healthy food continue to rise, the food industry is increasingly focused on developing innovative solutions such as edible coating and packaging films to enhance food safety and quality. In particular, materials made from biodegradable polymers are gaining attention for their environmental and health benefits. Consequently, bio-based food packaging films have emerged as a key area of research and development, with the potential to produce packaging materials that better align with the needs of health-conscious consumers [22]. This study aimed to investigate the effects of sodium alginate and gelatin on the physical and thermal properties of PU:SA:GE blend films, focusing specifically on water solubility (water resistance) and transparency. The relative proportions of gelatin, sodium alginate, and pullulan (PU:SA:GE = 5:3:1, 5:2:2, and 5:1:3) were selected based on preliminary tensile test results from PU:SA (5:4) and PU:GE (5:4) blend films, which demonstrated significantly better and comparable mechanical strength, respectively, to that of pure pullulan films. The formulation also aimed to address the weak mechanical property of gelatin [23] and the low elongation at break (limited flexibility) characteristic of sodium alginate [12], thereby providing a balanced and improved material for potential food packaging applications.

The ratios 5:1:3, 5:2:2, and 5:3:1 were selected to evaluate the influence of increasing sodium alginate and decreasing gelatin content on the mechanical and physicochemical properties of pullulan-based films. These variations help determine the optimal balance of light absorbance, water sensitivity, flexibility, and structural integrity suitable for use as a packaging material. Other general functional properties of the blend films, including thermal behavior, water content and morphology were also reported.

2. Materials and Methods

2.1 Materials

Pullulan (Viscosity of 10% solution at 30°C - 100.09 mm^2 s^{-1}), sodium alginate (15-25 cP, 1% in H_2O), and gelatin (Type A, ~300 g Bloom, made from porcine skin) were purchased from Kumar Organic Products LTD (India), Sigma Life Science (USA) and Sigma Life Science (UK), respectively. Glycerol (≥ 95%) was purchased from Sigma-Aldrich (Germany).

2.2 Film preparation

Table 1: Composition of pullulan (PU), sodium alginate (SA), and gelatin (GE) of the pullulan-based blend films

Formulation		Component weight (g)		
		Pullulan	Sodium alginate	Gelatin
A	(PU)	4.5	-	-
B	(PU:SA=5:4)	2.5	2.0	-
C	(PU:SA:GE=5:3:1)	2.5	1.5	0.5
D	(PU:SA:GE=5:2:2)	2.5	1.0	1.0
E	(PU:SA:GE=5:1:3)	2.5	0.5	1.5
F	(PU:GE=5:4)	2.5	-	2.0

Pullulan, sodium alginate, and gelatin powders were mixed in varying proportions and dissolved in 100 mL of deionized water, as detailed in Table 1. A glycerol concentration of 20% (w/w) has been reported to enhance the flexibility of pullulan-based films, making them suitable for packaging applications that require stretchability [24]. Therefore, glycerol was added at 20% (w/w) relative to the total amount of the three-component biopolymers, with stirring at room temperature. The mixture was then heated at 95°C with stirring at 160 rpm for 15 minutes, and centrifuged at 1700 g for 5 minutes to remove air bubbles. A 10-mL mixture was poured evenly into a plastic petri dish and dried for 48 hours at room temperature. All the films were peeled and stored in a humidity chamber (Binder, Germany) maintained at 55 ± 5% relative humidity (RH) and 25 ± 1°C until further use.

2.3 Film thickness

The film thickness was measured using a digital caliper (Mitutoyo Corporation, Japan) with an accuracy of 0.01 mm. For each film sample, measurements were taken at five random positions, and the mean thickness with its standard deviation was calculated.

2.4 Film transmittance

Films of 1 cm x 3 cm were cut and placed into a plastic cuvette, and the absorbance at 600 nm was determined using a UV-Vis spectrometer (Lambda 25, Perkin Elmer, USA). The measurement was conducted in triplicate for each sample. The film transmittance was then calculated using the following equation:

$$\text{Transmittance (\%)} = \text{Antilog } (2 - \text{Absorbance}) \quad (1)$$

2.5 Mechanical properties

Films measuring 0.5 cm x 5 cm were mounted on the pneumatic specimen grips of a universal testing machine (Instron 5566, USA). The load cell capacity was 1.0 KN, and the crosshead speed was set at 10 mm min^{-1}, in accordance with ASTM D882. The Bluehill software was used to determine the tensile strength (TS), which measures the maximum stress a material can withstand before breaking, and the fracture strain (FS), which represents the elongation at break or film flexibility. All measurements were conducted in six replicates (n=6).

2.6 Moisture content

The film sample (3 cm × 3 cm) was weighed (W_0) and then dried in an oven at 105˚C until a constant weight (W_1) was obtained. The moisture content was calculated using the following equation:

$$\text{Moisture content (\%)} = [(W_0 - W_1)/W_0] \times 100\% \quad (2)$$

The measurement was conducted in triplicate for each sample.

2.7 Water solubility

Film samples measuring 3 cm by 3 cm were immersed in a beaker containing 50 mL of deionized water at 25°C and stirred for 24 hours. The dried weights of the samples before (W_i), after the test, and after drying at 105°C (W_f), were recorded. The water solubility was calculated using the following formula:

$$\text{Water solubility (\%)} = [(W_i - W_f)/W_i] \times 100\% \quad (3)$$

2.8 Film solubilizing time

Film solubilization time was measured by placing 3 cm x 3 cm film samples in a beaker containing 50 mL of deionized water, which was agitated continuously with a magnetic stirrer. The experiment was conducted at room temperature for formulations A and B. For the other formulations, which were not soluble at room temperature, the procedure was carried out at 37 ± 1°C. All measurements were performed in triplicate.

2.9 Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

FTIR spectra of the films were recorded using an ATR-FTIR spectrophotometer (IRAffinity-1S, Shimadzu, Japan) by scanning from 400 cm^{-1} to 4000 cm^{-1} at a resolution of 2 cm^{-1} for 20 scans.

2.10 Differential Scanning Calorimetry (DSC)

Film samples of approximately 5 mg were placed in aluminum pans and heated from -10˚C to 250˚C at a rate of 10˚C min^{-1}, under a nitrogen flow of 20 mL min^{-1} using a differential scanning calorimeter (DSC823e; Mettler Toledo, USA).

2.11 Thermogravimetric Analysis (TGA)

Film samples of approximately 8 mg in aluminum pans were heated from 25˚C to 600˚C at a rate of 10˚C min^{-1} under a nitrogen flow of 60 mL min^{-1}, using a thermogravimetric analyzer (TGA-DSC HT 3; Mettler Toledo, USA).

2.12 Scanning Electron Microscopy (SEM)

A small piece of film sample was mounted on a specimen holder and coated with a layer of gold. The surface morphology of the films was examined using a TM 3000 SEM (Hitachi, Japan), at an accelerating voltage of 5 kV and a magnification of 1000x, while the film cross-section was imaged at 500x magnification.

2.13 Statistical analysis

All data are presented as mean ± standard deviation. Significant differences among means were determined using one-way analysis of variance (ANOVA), followed by a post-hoc Tukey HSD test, with $P<0.05$ considered statistically significant.

3. Results and Discussion

3.1 Film thickness and transmittance

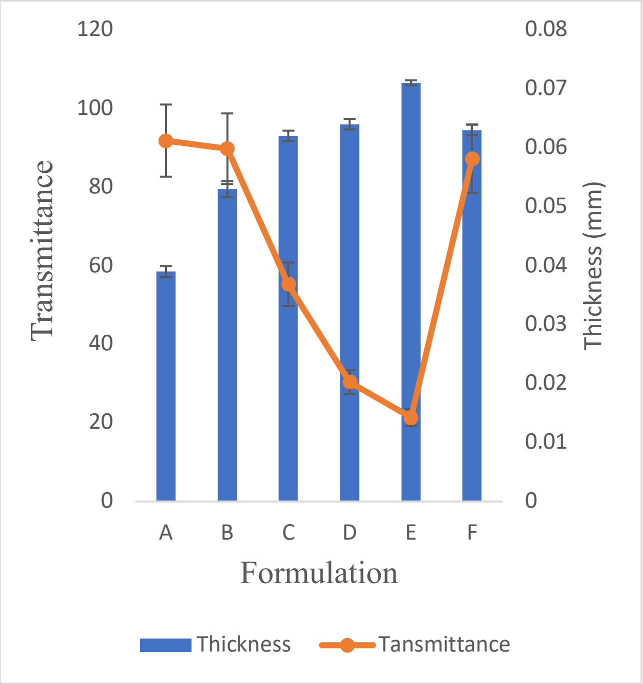


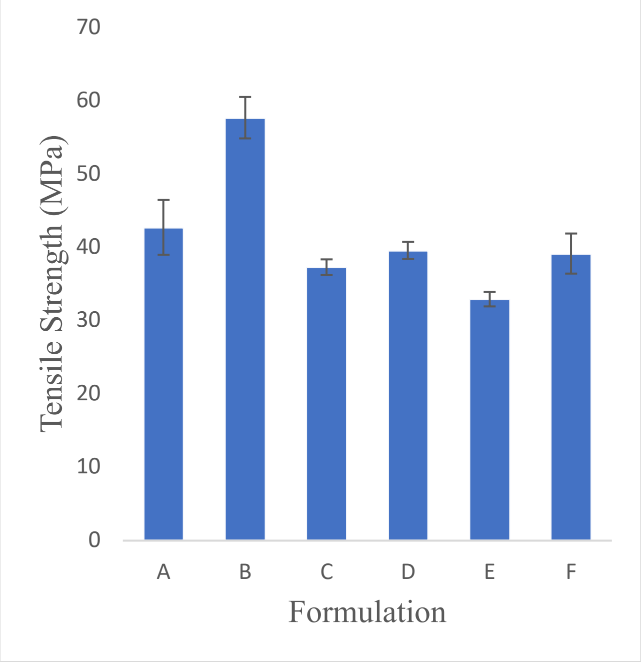
Fig. 2: The film thickness (bar) and transmittance (line) of A (PU), B (PU:SA=5:4), C (PU:SA:GE=5:3:1), D (PU:SA:GE=5:2:2), E (PU:SA:GE=5:1:3), and F (PU:GE=5:4) blend films. Bars labeled with different letters indicate significant differences in film thickness ($P < 0.05$). The transmittance of blend films C, D, and E is significantly different ($p<0.01$) from A.

All the blend films could be peeled off easily from the petri dish, except for the pullulan film, which was somewhat brittle. Figure 2 shows the results of the thickness and transmittance of the pullulan (PU) and its blend films. The PU:SA:GE (5:1:3) blend film exhibited the highest thickness and the lowest transmittance, with the latter being significantly different ($p<0.01$) from the other films. It was also noted that the film thickness increased with the increasing gelatin-to-sodium alginate ratio in the PU:SA:GE films, likely due to swelling caused by intermolecular hydrogen bonds and ionic interactions between sodium alginate and gelatin [25].

The PU, PU:SA (5:4) and PU:GE (5:4) blend films were highly transparent, while the PU:SA:GE (5:3:1), PU:SA:GE (5:2:2), and PU:SA:GE (5:1:3) blend films were opaque. However, unlike translucent films, the light absorbance or transmittance of highly transparent films is reported to be independent of film thickness [26]. Therefore, for meaningful comparison, the absorbance/thickness ratio (AU mm^{-1}) of the opaque PU:SA:GE (5:3:1, 5:2:2 and 5:1:3) blend films was determined and compared. The results showed that their absorbance/thicknesses were 4.15 AU mm^{-1}, 8.08 AU mm^{-1}, and 9.43 AU mm^{-1}, respectively, indicating the relative effect of sodium alginate and gelatin in absorbing incident light. Notably, increasing the gelatin-to-sodium alginate ratio caused a significant decrease in light transmission, which is consistent with the observation in alginate-gelatin mixed gel, where this effect was attributed to the formation of soluble non-stoichiometric sodium alginate-gelatin polyelectrolyte complexes [27]. Therefore, the addition of sodium alginate and gelatin increased the opacity of pullulan films, thereby enhancing their light transmission barrier, an important property for food packaging to protect food quality against light exposure [28].

3.2 Mechanical properties

i)



ii)

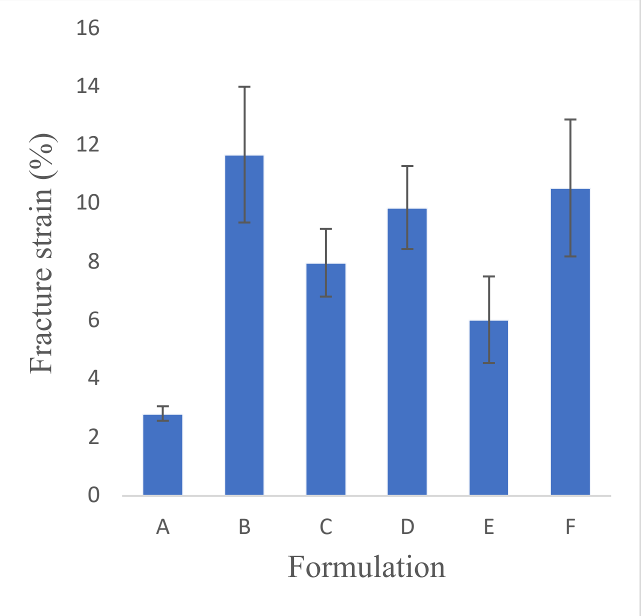


Fig. 3: Tensile strength (i) and fracture strain (ii) of A (PU), B (PU:SA=5:4), C (PU:SA:GE=5:3:1), D (PU:SA:GE=5:2:2), E (PU:SA:GE=5:1:3), and F (PU:GE=5:4) blend films. Bars labeled with different letters indicate significant differences ($P<0.01$) for i) and ($p<0.05$) for ii)

Tensile strength (TS) is an indicator of film strength, while fracture strain (FS) is a measure of film flexibility. Figure 3 shows the TS and FS of the blend films. The PU:SA (5:4) film exhibited significantly enhanced TS and FS compared to the PU film ($p< 0.01$), consistent with the same effect reported previously [12]. In contrast, the TS and FS of the PU:SA:GE films were not significantly different from each other or from the pullulan film. Nonetheless, the average FS of the PU:SA:GE films was 7.96%, which is higher than that of the pullulan film (2.82%), indicating that blending pullulan with both sodium alginate and gelatin can improve the film's stretchability. Compared to TS of the PU:SA (5:4) film (59 MPa), the substantially lower TS (38 MPa) of the PU:SA:GE films could be attributed to SA:GE microgel formation [27], which phase-separated from the PU matric and consequently weakened the polymer chain interactions. The uneven morphology observed in the SEM image of the PU:SA:GE (5:1:3) blend film (Fig. 9 ii) supports this possible phase separation, likely caused by aggregation of the SA/GE complex.

3.3 Moisture content

Figure 4 shows the moisture content of pullulan blend films in water at 37°C. The pullulan blend film exhibited varying moisture content, ranging from 8.20% for PU to 12.73% for PU:SA (5:4). This result could be attributed to the higher hydrophilicity of sodium alginate compared to pullulan [12, 29].

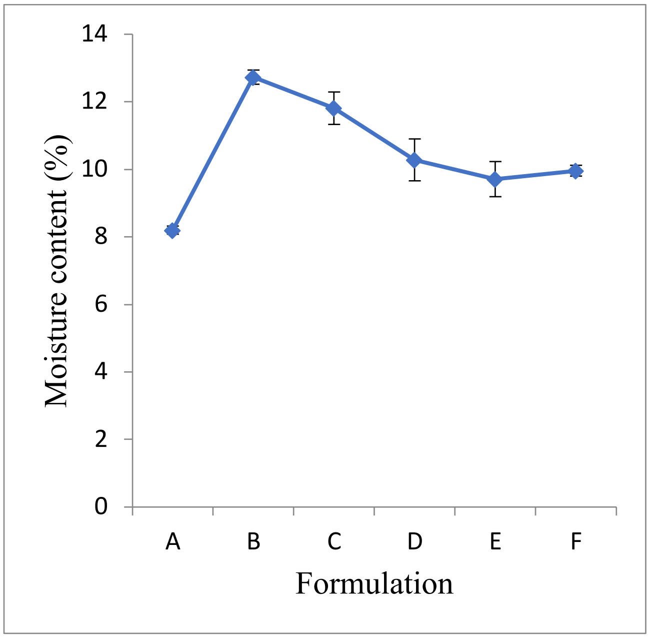


Fig. 4: Moisture content of A (PU), B (PU:SA=5:4), C (PU:SA:GE=5:3:1), D (PU:SA:GE=5:2:2), E (PU:SA:GE=5:1:3), and F (PU:GE=5:4) blend films.

* Significantly different ($p < 0.05$) from pullulan film

For the PU:SA:GE blend films, the moisture content decreased as the proportion of gelatin increased. This may be related to water absorbability, which has been reported to decrease with increasing amounts of GE in SA:GE films [25]. This trend could also be explained by the increasing amounts of hydrogen bonding interactions among the three biopolymers, leading to a deduction in the availability of free hydroxyl group for interaction with water molecules [30,27]. Hence, the results demonstrate that varying the relative proportions of sodium alginate and gelatin can modify the water-holding properties of the films.

3.4 Film water solubility and solubilization time

Table 2: Water solubility of blend films at 25°C over 24 hours

Formulation		Water Solubility
		(Mean ± Standard deviation)
A	(PU)	*Fully soluble*
B	(PU:SA=5:4)	*Fully soluble*
C	(PU:SA:GE=5:3:1)	*Fully soluble*
D	(PU:SA:GE=5:2:2)	81.4 ± 1.49^{a}
E	(PU:SA:GE=5:1:3)	79.3 ± 3.33^{a}
F	(PU:GE=5:4)	65.8 ± 1.82^{b}

Different superscript letters indicate statistical differences between groups ($p < 0.05$)

The water solubility test of PU:SA:GE blend films, conducted at 25°C for 24 hours, showed significantly decreased water solubility compared to that of pullulan film. The PU film, PU:SA (5:4) and PU:SA:GE (5:3:1) blend films were fully soluble, while the PU:SA:GE (5:2:2), PU:SA:GE (5:1:3), and PU:GE (5:4) blend films exhibited reduced solubility (Table 2). These results indicate that the higher the proportion of gelatin, the lower the solubility of the blend film. Notably, the PU:SA (5:4) film was even more soluble than pullulan, which could be attributed to the higher hydrophilicity of sodium alginate [31].

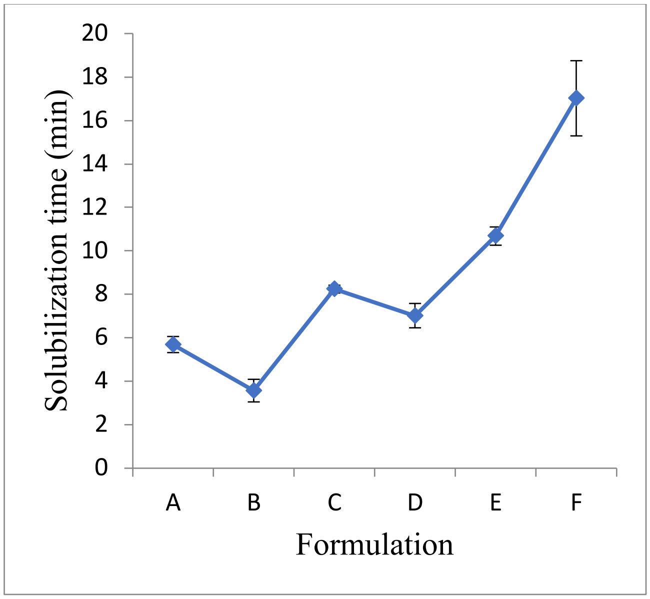


Fig. 5: Solubilization time of A (PU), B (PU:SA=5:4), C (PU:SA:GE=5:3:1), D (PU:SA:GE=5:2:2), E (PU:SA:GE=5:1:3), and F (PU:GE=5:4) blend films.

*Significantly different ($p < 0.05$) from pullulan film; ** Significantly different ($p < 0.01$) from pullulan film

Figure 5 shows the solubilization times of pullulan and pullulan blend films, conducted at 37°C. The solubilization times of PU:SA:GE (5:3:1; 5:1:3) and PU:GE (5:4) blend films were significantly longer compared to that of pullulan film, with the PU:GE (5:4) blend film being the least soluble. The prolonged dissolution time can be attributed to the presence of gelatin, as pure gelatin is readily soluble only at temperature above 40°C [32].

3.5 FTIR study

Figure 6 shows the FTIR spectra of all the blend film samples. The hydroxyl stretching vibration bands between 3000-3600 cm^{-1} were broad, strong, and present in the spectra of all films, indicating intense intra- and intermolecular hydrogen bonding interactions within and between pullulan, sodium alginate, and gelatin chains [27, 33]. This is expected. as gelatin forms interchain hydrogen bonds at temperatures below 35°C [34], as does sodium alginate, which forms both inter- and intramolecular hydrogen bonds [35].

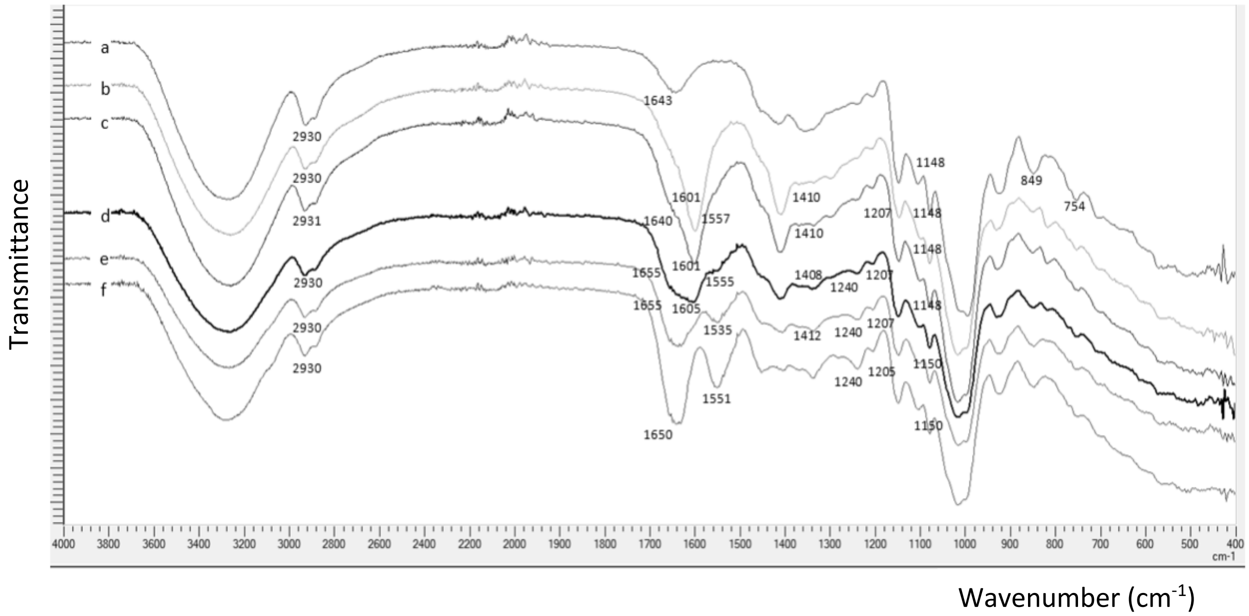


Fig. 6: ATR-FTIR spectra of (a) Pure PU, (b) PU:SA (5:4), (c) PU:SA: GE (5:3:1), (d) PU:SA:GE (5:2:2), (e) PU:SA:GE (5:1:3), and (f) PU:GE (5:4) blend films

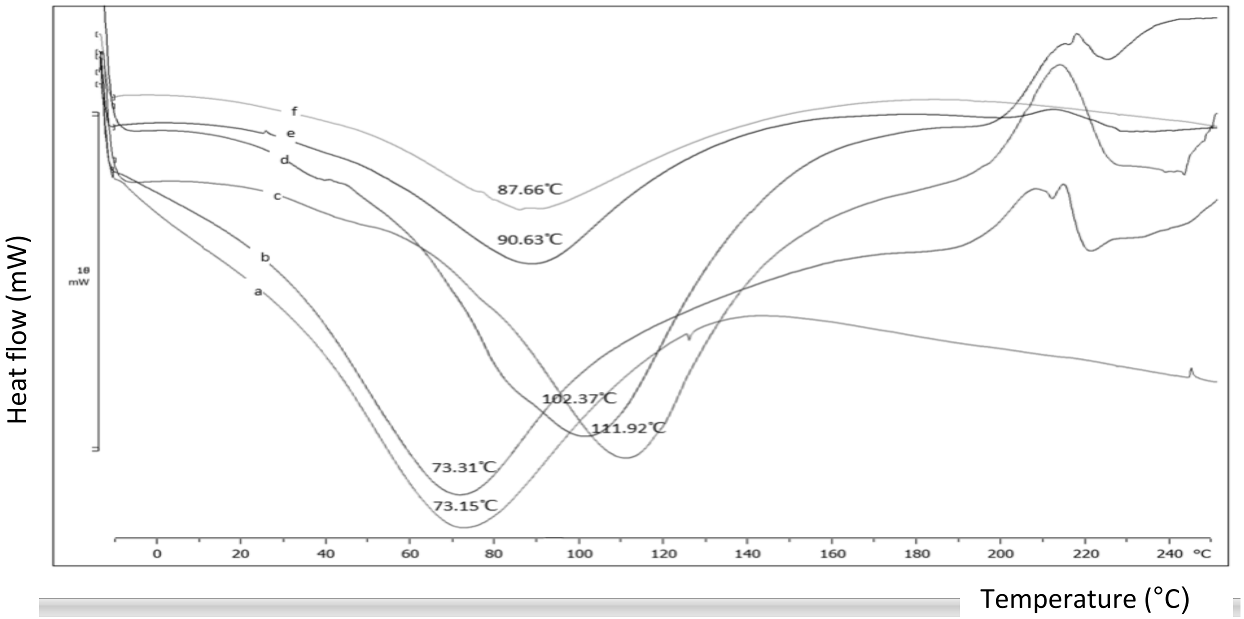
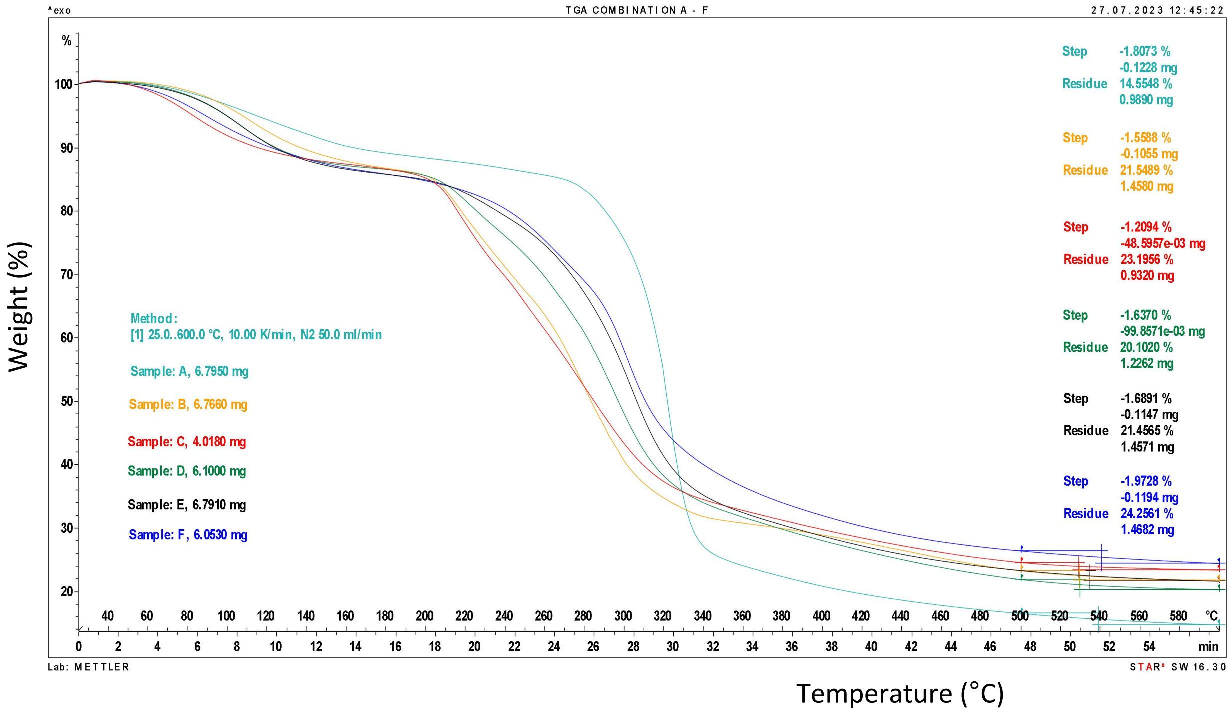


Fig. 7: DSC thermograms of (a) Pure PU, (b) PU:SA (5:4), (c) PU:SA: GE (5:3:1), (d) PU:SA:GE (5:2:2), (e) PU:SA:GE (5:1:3), and (f) PU:GE (5:4) blend films

In addition, the typical stretching vibration band of O–C–O group at 1636 cm^{-1} in pullulan was also strong. The PU:SA:GE films exhibited additional stretching vibration bands at around 1601 cm^{-1} and 1410 cm^{-1}, corresponding to asymmetric and symmetric COO^{-} stretching vibrations arising from sodium alginate [36]. However, these absorption bands gradually weakened as the proportion of sodium alginate in the blend decreased and gelatin content increased (Fig 6: b to e). This was accompanied by a shift from 1410 cm^{-1} to 1412 cm^{-1}, which may indicate ionic association between the carboxylate groups of sodium alginate and the amino group of gelatins.

The absorption bands at 1655-1638 cm^{-1}, 1557-1480 cm^{-1}, and 1240-1206 cm^{-1} were attributed to the amide groups of gelatins [37]. The band at 1148 cm^{-1}, attributed to α-(1-4) glycosidic linkage of pullulan or sodium alginate, was also prominent [38]. Thus, all major FTIR absorption bands associated with pullulan, sodium alginate, and gelatin are recognizable.

i)



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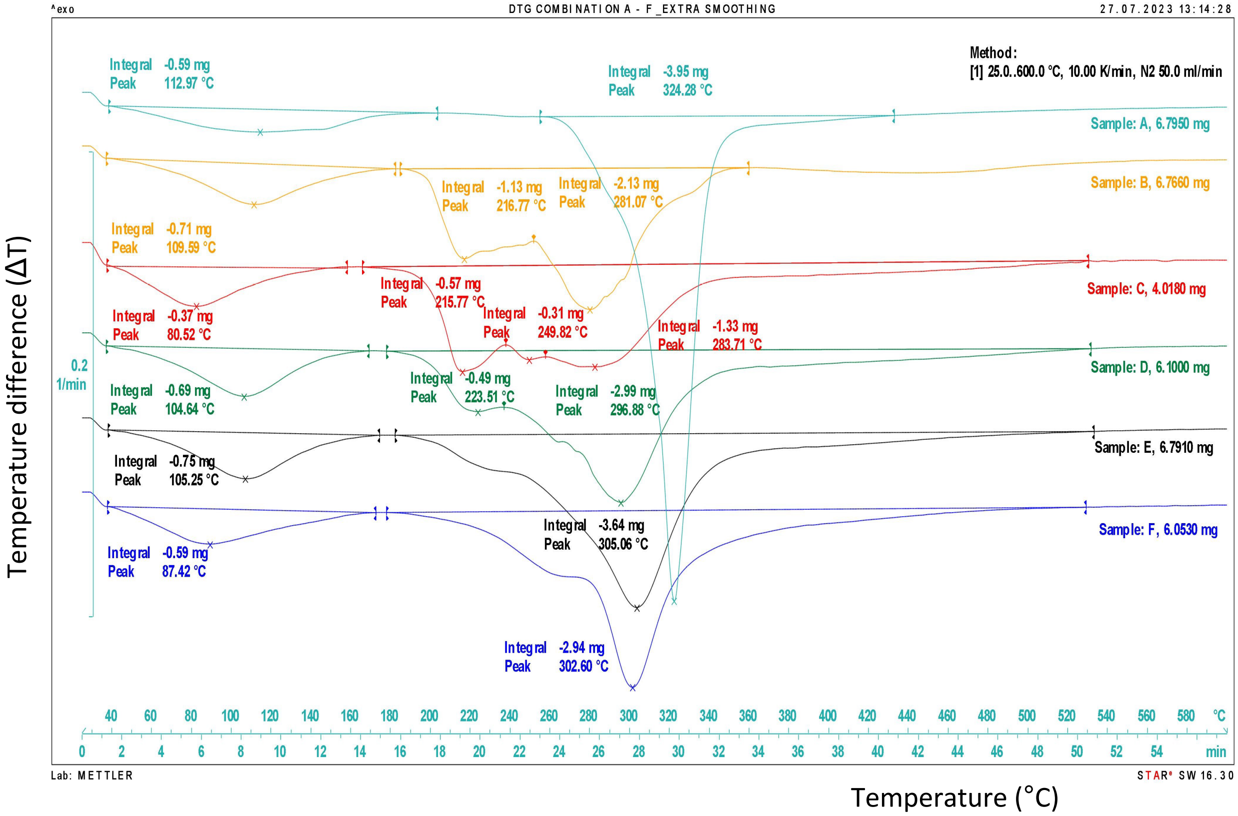

Fig. 8: TGA thermograms (i) and DTA (ii) curves of A (PU), B (PU:SA=5:4), C (PU:SA:GE=5:3:1), D (PU:SA:GE=5:2:2), E (PU:SA:GE=5:1:3), and F (PU:GE=5:4) blend films

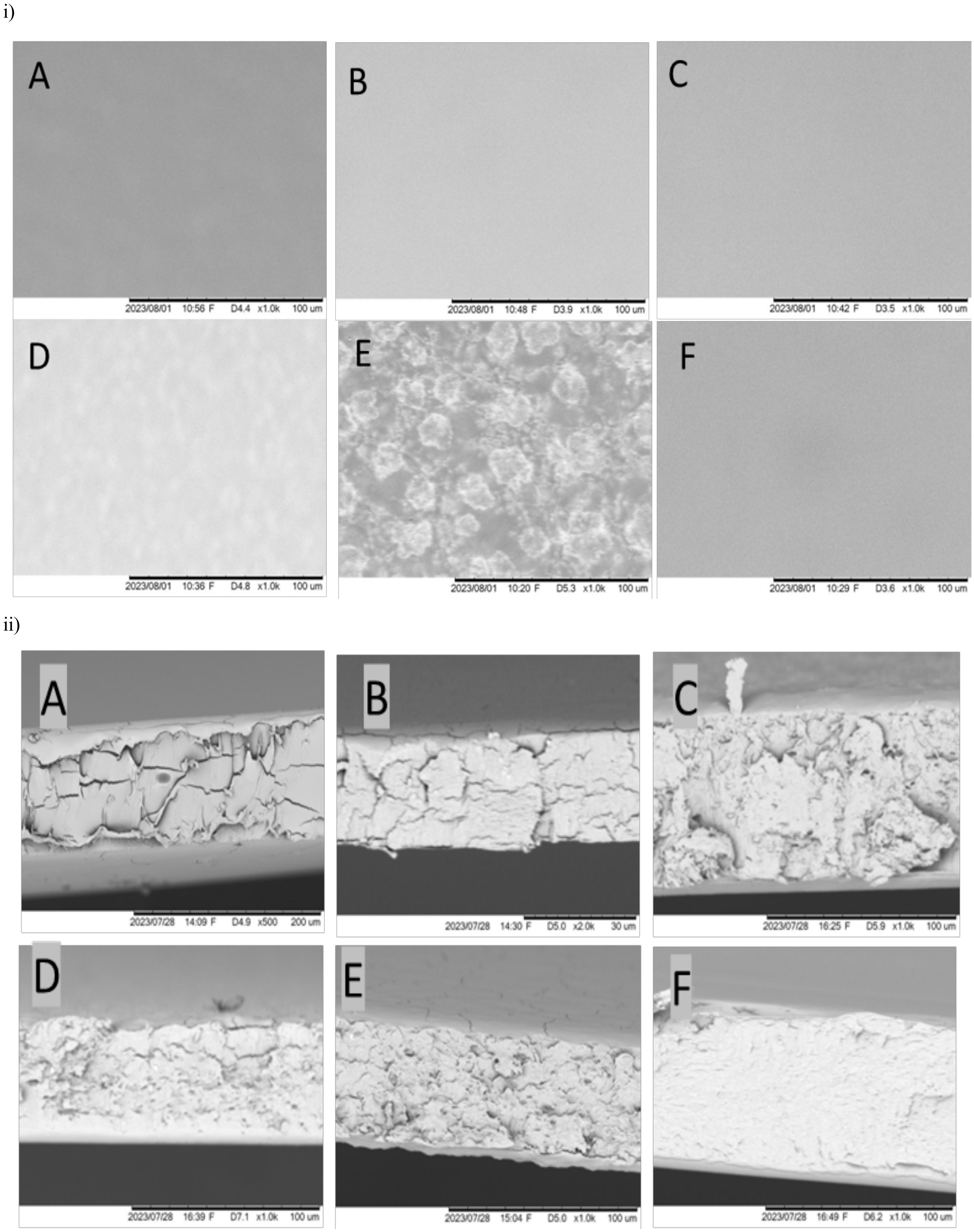

Fig. 9: SEM images of the surface (i) and cross-section (ii) of blend films of A (PU), B (PU:SA=5:4), C (PU:SA:GE=5:3:1), D (PU:SA:GE=5:2:2), E (PU:SA:GE=5:1:3), and F (PU:GE=5:4) at 5kV and 1000x magnification

3.6 Thermal behavior

Figure 7 shows the DSC thermograms of the pullulan blend films. All the films exhibited a broad transition event with varying endothermic peak temperatures below 120°C, most likely due to moisture loss. The PU:SA:GE blend films showed significantly higher endothermic peak temperatures by about 18-39°C, as compared to that of the pullulan film. Among the PU:SA:GE blend films, a decreasing trend in endothermic peak temperature was observed as the gelatin-to-sodium alginate ratio increased, i.e., from 111.92°C (PU:SA:GE= 5:3:1) to 90.63°C (PU:SA:GE=5:1:3). It was also noted that the blend films containing sodium alginate exhibited exothermic transition with an onset around 200°C, attributed to the thermal degradation or oxidation of alginate [39].

Figure 8 shows the TGA curves (a) and the corresponding DTA curves (b) of pullulan and its blend films. In general, film mass loss occurred in multiple steps: loss of volatiles such as free and bound water between 80°C to 112°C, decomposition of sodium alginate between 215 to 223°C, decomposition of pullulan from 250°C, and decomposition of gelatin peaking at 302°C. These decomposition temperatures are quite consistent with those reported for the individual components: pullulan (250-280°C) of pullulan [40], sodium alginate (240-260°C) [39], gelatin (296°C) [41], and glycerol (289°C) [42]. Based on the lower weight loss at elevated temperatures (~ 290°C), the order of decreasing thermal stability of the blend films is as follows: PU > PU:SA:GE (5:1:3) > PU:GE (5:4) > PU:SA:GE (5:2:2) > PU:SA:GE (5:3:1) > PU:SA (5:4). Hence, the addition of sodium alginate and gelatin to pullulan only slightly reduced the thermal stability of the blend films. Nonetheless, in the absence of gelatin (PU:SA film), sodium alginate had the greatest destabilizing effect on the pullulan film (Fig 8ii).

3.7 SEM images

Figure 9 shows the SEM images of pullulan blend films. The surface textures of the PU:SA (5:4), PU:SA:GE (5:3:1 and 5:2:2), and PU:GE (5:4) blend films appeared smooth, suggesting good miscibility between pullulan and sodium alginate and/or gelatin. However, the PU:SA:GE (5:2:2 and 5:1:3) blend films exhibited uneven surface morphology. This abrupt change in film texture may be associated with hydrogen bonding and electrostatic interactions between SA and GE, particularly as the GE-to-SA ratio increased, leading to more GE/GE complex aggregations [27]. In contrast, uneven bulk textures were observed in the cross-sectional area of the PU film. Nevertheless, the addition of sodium alginate and/or gelatin to pullulan appeared to smooth the texture (Fig 9 bottom; blend films B to F).

4. Conclusions

The film thickness increased, while the light transmittance decreased with an increasing gelatin-to-sodium alginate ratio in the pullulan-sodium alginate-gelatin (PU:SA:GE) blend films. The film tensile strength and fracture strain of the PU:SA:GE blend films were unaffected by the varying gelatin-to-sodium alginate ratios. Both water solubility and the moisture content decreased as the gelatin-to-sodium alginate ratio increased. SEM images showed that the PU:SA:GE (5:1:3) blend films exhibited an uneven surface texture, while a smooth texture was observed for the PU:SA:GE (5:3:1 & 5:2:2) blend films. FTIR spectra indicated strong hydrogen bonding interactions among the three biopolymers. Among the blend films, PU:SA:GE (5:1:3) exhibited the greatest heat stability and the most effective light transmission barrier, as well as moderate water solubility, properties that may be suitable for use as edible coating or packaging film for light-sensitive foods. In addition, the 5:1:3 ratio reduces the overall reliance on pullulan, making the film more cost-effective, as sodium alginate and gelatin are generally less expensive than pullulan.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] L. Dai, Y. Yuan, Z. Song, Y. Qiu, T. Yue, Preparation and Characterization of Lactobacilli-Loaded Composite Films with Sustaining Antipathogenic Activity and Preservation Effect, *J. Food Sci*., 83(10), 2511-2519 (2018).

[2] J.W. Rhim, Physical-Mechanical Properties of Agar/κ-Carrageenan Blend Film and Derived Clay Nanocomposite Film, *J. Food Sci*., 77(12), N66–N73 (2012).

[3] Y. Wu, C. Weller, F. Hamouz, S. Cuppett, M. Schnepf, Moisture Loss and Lipid Oxidation for Precooked Ground-Beef Patties Packaged in Edible Starch-Alginate-Based Composite Films, *J. Food Sci*., 66(3), 486–493 (2001).

[4] T. Kato, T. Okamoto, T. Tokuya, A. Takahashi, Solution Properties and Chain Flexibility of Pullulan in Aqueous Solution, *Biopolymers*, 21, 1623–1633 (1982).

[5] R.S. Singh, G.K. Saini, J.F. Kennedy, Pullulan: Microbial Sources, Production and Applications, *Carbohyd. Polym*., 73(4), 515-531 (2008).

[6] S. Yuen, Pullulan and Its Applications, *Process*

Biochem., 22, 7-9 (1974).

[7] L. Ghimici, M. Constantin, G. Fundueanu, Novel Biodegradable Flocculating Agents based on Pullulan, *J. Hazard Mater*., 181, 351–358 (2010).

[8] K. Kraśniewska, K. Pobiega, M. Gniewosz, Pullulan – Biopolymer with Potential for Use as Food Packaging, *Int. J. Food Eng*., 15(9), 2019-0030 (2019).

[9] M. Kawahara, K. Mizutani, S. Suzuki, S. Kitamura, H. Fukada, T. Yui, K. Ogawa, Dependence of the Mechanical Properties of a Pullulan Film on the Preparation Temperature, *Biosci. Biotech. Bioch*., 67(4): 893-895 (2003).

[10] S. Farris, I.U. Unalan, L. Introzzi, M. Furntes-Alventosa, C.A. Cozzolino, Pullulan-Based Films and Coating for Food Packaging: Present Applications, Emerging Opportunities, and Future Challenges, *J. Appl. Polym. Sci*., 131(13), 40539 (2014).

[11] C. Zhang, D. Gao, Y. Ma, X. Zhao, Effect of Gelatin Addition on Properties of Pullulan Films, *J. Food Sci*., 78(6), C805–C810 (2013).

[12] Q. Xiao, L.T. Lim, Q. Tong, Properties of Pullulan-based Blend Films as Affected by Alginate Content and Relative Humidity, *Carbohyd. Polym*., 87(1), 227–34 (2012).

[13] J. Wu, F. Zhong, Y. Li, C.F. Shoemaker, W. Xia, Preparation and Characterization of Pullulan–Chitosan and Pullulan–Carboxymethyl Chitosan Blended Films, *Food Hydrocolloid*., 30(1), 82–91 (2013).

[14] J.Y. Kim, Y.G. Choi, S.R.B. Kim, S.T. Lim, Humidity Stability of Tapioca Starch-Pullulan Composite Films, *Food Hydrocolloid*, 41, 140-145 (2014).

[15] H. Andriamanantoanina, M. Rinaudo, Characterization of the Alginates from Five Madagascan Brown Algae, *Carbohyd Polym*., 82, 555-560 (2010).

[16] H. Grasdalen, B. Larsen, O. Smidsrød, ^{13}C-NMR Studies of Alginate, *Carbohyd. Res*., 56, C11−C15 (1977).

[17] H. Hecht, S. Srebnik, Structural Characterization of Sodium Alginate and Calcium Alginate, *Biomacromolecules*., 17(6), 2160-2167 (2016).

[18] V.V. Khutoryanskiy, Advances in Mucoadhesion and Mucoadhesive Polymers, *Macromol. Biosci*., 11(6), 748-764 (2011).

[19] Y. Shtenberg, M. Goldfeder, H. Prinz, J. Shainsky, Y. Ghantous, I. Abu El-Naaj, A. Schroeder, H. Bianco-Peled, Mucoadhesive Alginate Pastes with Embedded Liposomes for Local Oral Drug Delivery, *Int. J. Biol. Macromol*., 111, 62-69 (2018).

[20] J. Sun, H. Tan, Alginate-Based Biomaterials for Regenerative Medicine Applications, *Mater (Basel)*., 6(4), 1285-1309 (2013).

[21] M.C. Gomez-Guillen, J. Turnay, M.D. Fernandez-Diaz, N. Ulmo, M.A. Lizarbe, P. Montero, Structural and Physical Properties of Gelatin Extracted from Different Marine Species: Acomparative Study, *Food Hydrocolloid*, 6(1), 25-34 (2002).

[22] L. Kumar, D. Ramakanth, K. Akhila, K.K. Gaikwad, Edible Films and Coatings for Food Packaging Applications: A Review, *Environ. Chem. Lett*., 20, 875–900 (2022).

[23] X. Zhang, M.D. Do, P. Casey, A. Sulistio, G.G. Qiao, L. Lundin, P. Lillford, S. Kosaraju, Chemical Modification of Gelatin by a Natural Phenolic Cross-Linker, Tannic Acid, *J. Agr. Food Chem*., 58(11), 6809–6815 (2010).

[24] P.R. Vuddanda, M. Montenegro-Nicolini, J.O. Morales, S. Velaga, Effect of plasticizers on the physico-mechanical properties of pullulan based pharmaceutical oral films, *Eur. J. Pharm. Sci*., 96, 2017, 290-298 (2017).

[25] C. Xiao, H. Liu, Y. Lu, L. Zhang, Blend Films from Sodium Alginate and Gelatin Solutions, *J. Macrmol. Sci. A*., 38(3), 317-328 (2001).

[26] J. Zhao, Y. Wang, C. Liu, Film Transparency and Opacity Measurements, *Food Anal. Method*., 15, 2840-2846 (2022).

[27] S.R. Derkach, N.G. Voron'ko, N.I. Sokolan, D.S. Kolotova, Y.A. Kuchina, Interactions between Gelatin and Sodium Alginate:UV and FTIR Studies, *J. Disper. Sci. Technol*., 41(5), 690-698 (2020).

[28] J.S. Lee, E.S. Lee, J. Han, Enhancement of the Water-Resistance Properties of an Edible Film Prepared from Mung Bean Starch via the Incorporation of Sunflower Seed Oil, *Sci. Rep*., 10, 13622 (2020). https://doi.org/10.1038/s41598-020-70651-5

[29] Q. Xiao, Q. Tong, Thermodynamic Properties of Moisture Sorption in Pullulan–Sodium Alginate based Edible Films, *Food Res. Int*., 54(2), 1605-1612 (2013).

[30] Y. Yu, T. Tyrikos-Ergas, Y. Zhu, G. Fittolani, V. Bordoni, A. Singhal, R.J. Fair, A. Grafmller, P.H. Seeberger, M. Delbianco, Systematic Hydrogen-Bond Manipulations to Establish Polysaccharide Structure–Property Correlations, *Angew. Chem. Int. Edit*., 58, 13127-13132 (2019).

[31] Q. Tong, Q. Xiao, L.T. Lim, Preparation and Properties of Pullulan-Alginate-

Carboxymethylcellulose Blend Films, *Food Res. Int.*, 41(10), 1007–14 (2008). Available from: http://dx.doi.org/10.1016/j.foodres.2008.08.005

[32] E.J. Lee, K.H. Lim, Hardly Water-soluble Drug-loaded Gelatin Nanoparticles Sustaining a Slow Release: Preparation by Novel Single-Step O/W/O Emulsion Accompanying Solvent Diffusion, *Bioproc. Biosyst. Eng*., 40, 1701–1712 (2017).

[33] Y. Wang, Z. Guo, Y. Qian, Z. Zhang, L. Lyu, Y. Wang, F. Ye, Study on the Electrospinning of Gelatin/Pullulan Composite Nanofibers, *Polymers*., 11(9), 1424 (2019). https://doi.org/10.3390/polym11091424

[34] C. Robinson, *The Hot and Cold Forms of Gelatin*, in Nature and Structure of Collagen, J.T. Randell, Ed., New York: Academic Press Inc., 96-105, (1953).

[35] L. Hou, P. Wu, Exploring the Hydrogen-bond Structures in Sodium Alginate through Two-Dimensional Correlation Infrared Spectroscopy, *Carbohyd. Polym*., 205, 420-426 (2019).

[36] S.H. Rashedy M.S.M. Abd El Hafez, M.A. Dar, J. Cotas, L. Pereira, Evaluation and Characterization of Alginate Extracted from Brown Seaweed Collected in the Red Sea, *Appl. Sci*., 11, 6290 (2021).

[37] J.H. Muyonga, C.G.B. Cole, K.G. Duodu, Fourier Transform Infrared (FTIR) Spectroscopic Study of Acid Soluble Collagen and Gelatin from Skins and Bones of Young and Adult Nile Perch (*Lates Niloticus*), *Food Chem*., 86(3), 325-332 (2004).

[38] N.A. Nikonenko, D.K. Buslov, N.I. Sushoko, R.G. Zhbankov, Investigation of Stretching Vibrations of Glycosidic Linkages in Disaccharides and Polysaccharides with use of IR Spectra Deconvolution, *Biopolymers*, 57(4): 257-262 (2000).

[39] J.P. Soares, J.E. Santos, G.O. Chierice, E.T.G. Cavalheiro, Thermal Behavior of Alginic Acid and its Sodium Salt, *Ecl. Quím*., São Paulo, 29(2), 53-56 (2004).

[40] Y. Tsujisaka, M. Mitsuhashi, *Pullulan*, in *Industrial Gums, Polysaccharides and Their Derivatives*, 3rd ed., R.L. Whistler, J.N. BeMiller, Eds. San Diego: Academic Press, 447-460 (1993).

[41] P.L.M. Barreto, A.T.N. Pires, V. Soldi, Thermal Degradation of Edible Films based on Milk Proteins and Gelatin in Inert Atmosphere, *Polym. Degrad. Stab*., 79, 147-152 (2003).

[42] Q.M. Yu-Wu, E. Weiss-Hortala, R. Barna, H. Boucard, S. Bulza, Glycerol and Bioglycerol Conversion in Supercritical Water for Hydrogen Production, *Environ. Technol*., 33(19), 2245-2255, (2012) DOI: 10.1080/09593330.2012.728738