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Development and characterization of a novel biodegradable laboratory-made pullulan blended films

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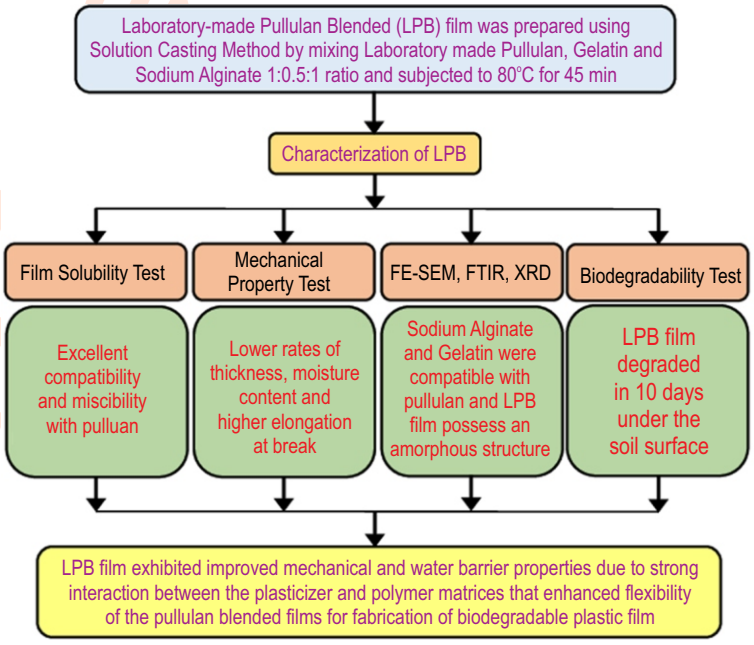
Abstract

Aim: To develop and characterize laboratory-made pullulan blended film using pullulan obtained from yeast-like fungus *Aureobasidium pullulans* mixed with gelatin and sodium alginate.

Methodology: The laboratory-made pullulan blended (LPB) and standard pullulan blend (SPB) films were prepared by the solution casting method. Physical and mechanical properties were performed to examine the moisture content, film thickness, film solubility, tensile strength and elongation at break. Fourier transform infrared spectroscopy, X-ray diffraction, and field emission scanning electron microscope techniques were performed to analyze the interaction between the polymeric blends.

Results: The polymeric blends had excellent miscibility and compatibility with the pullulan. The addition of glycerol to pullulan blended films improved the solubilization time in water at 125 sec. The blending ratio of pullulan, sodium alginate and gelatin led to lower rates of thickness, moisture content and higher elongation at break. Field Emission-SEM and FTIR analyses revealed that both sodium alginate and gelatin were compatible with pullulan. XRD results confirmed that pullulan blended films possess an amorphous structure that readily absorbs the moisture content. The biodegradability test confirmed the potential use of pullulan blended films as biodegradable plastic film.



Interpretation: The laboratory-made pullulan blended films exhibited improved mechanical and water barrier properties due to strong interaction between the plasticizer and polymer matrixes which enhances the flexibility of pullulan blended films for the fabrication of biodegradable plastic film.

Key words: *Aureobasidium pullulans,* Biodegradable plastic film, Microscopic techniques, Pullulan blended films

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Introduction

Overexploitation of plastics in the last decades has made a crucial impact on the environment. Plastics are used in day-to-day life as domestic packages, plastic bottles manufacturing, and packaging industries, etc (Rafey and Siddiqui, 2021). Adversely, 30% these synthetic plastics remain unrecycled in the landfills for thousands of years which causes huge environmental pollution (Manjula *et al.,* 2019). Therefore, an increasing public awareness of plastic pollution, eco-friendly alternatives to synthetic plastics have been developed. Bio-based polymers have become a feasible substitute for conventional plastics. In recent years, the use of biodegradable materials has gained attention in diverse fields such as pharmaceuticals, food packaging, medical and agriculture sectors, etc. (Gadhave *et al.,* 2018). Bioplastics can be produced from biomass, organic waste, natural polysaccharides, renewable raw materials such as agricultural and food industrial waste (Karn and Jenkinson, 2019). These can be degraded by different microorganisms such as bacteria, fungi and algae.

Polysaccharide-based polymers such as epoxy, polyesters, starch and soy-based polymers, cellulose, levan have been by fermentation process (Reddy *et al.,* 2013). These polymers can be used in food packaging industries due to their excellent biodegradability, thermal properties, nutritional qualities, oxygen and water barrier properties and lower cost (Arayaphan *et al.,* 2020). The most commonly used biopolymers are polylactic acid, polyhydroxyalkanoates, xanthan, sodium alginate, dextran, pullulan, chitosan, gelatin, etc. Pullulan (PUL) is an extracellular linear polysaccharide produced extracellularly by the yeast-like fungus *Aureobasidium pullulans*. It is mainly formed by the repeating maltotriose units connected by α-1→6 glycosidic bond linkages (Haghighatpanah *et al.,* 2020). The alternative pattern of α-(1→4) and α-(1→6) bonds improves the structural flexibility and solubility nature. Pullulan produces films that have excellent film-forming properties, tasteless, colorless, transparent and impermeable to oxygen. The electrical properties of polymer film include dielectric constant, dielectric strength, volume and surface resistivity (ASTM D2305-18, 2018). Although, it has been restricted to its application in pure form due to its poor mechanical properties and high cost.

Pullulan is generally regarded safe as a food-grade pullulan. However, pullulan is quite an extensive biopolymer. This hamperes the usage of pullulan in a wide range of applications. To produce pullulan at low cost, nutritional-rich carbon sources can be exploited for pullulan production by maintaining the growth of *A. pullulans*. One such approach is using of agricultural wastes like sugarcane bagasse, corn steep liquor , and cassava bagasse for the production of pullulan by *A. pullulans* (Zhu *et al.,* 2018). To improve the pullulan film-forming properties as well as to reduce its cost it can be blended with other compatible biopolymers which have high mechanical properties (Priyadarshi *et al.,* 2021). Ganduri *et al.* (2020) formulated edible thin films using pullulan polysaccharide blended with sodium alginate, gelatin, agar and starch. Pullulan polysaccharide blended with additives results in films with higher densities and lower value of color difference. Thus biodegradability of pullulan blended films can be a replacement for the plastic films. Gelatin (GEL), a water-soluble polymer obtained by partial hydrolysis of collagen, is widely used for manufacturing edible packages (Liu *et al.,* 2013). Its structure is mainly arranged by rigid fibrous protein molecules inter-connected by covalent bonds. Due to the hygroscopic nature of gelatin, they exhibit excellent tensile strength and film-forming properties which can be compatible with the materials such as starch, water-soluble polysaccharides, chitosan, sodium alginate, and methylcellulose (Susmitha *et al.,* 2021).

Also, pullulan-gelatin blended films might accomplish improved tensile strength, lower oxygen permeability, and be applied in a wide range of applications (Shen *et al.,* 2021). The incorporation of gelatin into pullulan-based films effectively increases the mechanical properties. Sodium alginate (SA) is a water-soluble, anionic and non-toxic polysaccharide that possesses excellent film-forming properties. It is mainly isolated from marine brown algae and used in the food, pharmaceutical industry and coating applications (Verdi *et al.,* 2021). Tong *et al.* (2008) developed composite edible films by blending pullulan, alginate and carboxymethyl cellulose using solvent casting method. Addition of glycerol as a plasticizer, enhances water solubilization, reduces tensile strength with increased elongation at break. Thus, alginate and CMC may reduce the cost of pullulan based edible films. In light of the above, this study focused on the development and characterization of laboratory-made pullulan blended film using pullulan obtained from yeast-like fungus *Aureobasidium pullulans* mixed with gelatin and sodium alginate. Further, the physical and mechanical properties of developed pullulan blended films were investigated. In our study, attempts were made on the development of pullulan blended films using laboratory-made pullulan produced by mutant *A. pullulans* employing cassava waste as a carbon source instead of commercial pullulan reported in the literature.

Materials and Methods

Production and preparation of laboratory-made pullulan film: Pullulan was obtained from the mutant culture of *Aureobasidium pullulans* UV9 by solid-state fermentation (Jafari *et al*., 2017). The laboratory-made pullulan blended (LPB) films were prepared using a solution casting method. PUL: SA: GEL were mixed in 1:0.5:1 ratio with 100 ml distilled water and kept in a water bath at 80°C for 45 min. Upon continuous stirring, 1 g of glycerol was added to the prepared dispersions to obtained coarse emulsion (Chu *et al.,* 2020). After cooling, the prepared film-forming dispersions were cast on transparent plastic sheets and dried at 50°C in an oven for 8 hr. The dried films were carefully peeled and conditioned at 25°C by maintaining 55% relative humidity in a humidity chamber for further studies. The standard pullulan blend (SPB) film dispersions were also prepared according to Chu *et al.* (2020).

Analyses of pullulan blend films

Moisture content: The moisture content of pullulan blend films was determined by drying the film samples in a hot air oven for every 3 hr at 80°C until steady-state was attained and weighed in an analytical balance (M/s. Shimadzu Corporation, Japan) according to ASTM D570-98, 2018.

Film solubility: To determine the film solubility, the film samples were cut into 5 × 5 cm and placed into a beaker containing 100 ml of distilled water and stirred continuously using a magnetic stirrer. Simultaneously, the stopwatch was started and the presence of film was monitored visually. The time taken for film to dissolve completely was reported as solubilization time (ASTM D882-18, 2018).

Film thickness: Before testing, the film thickness was measured using a 0-25 mm manual handheld micrometer screw gauge (M/s. The National Scientific Apparatus Works, India). The overall film thickness was expressed as an average of ten randomly selected locations of film sample (ASTM D6988-08, 2008). The average thickness of film sample was noted as the cross-sectional area of the sample.

Tensile strength and elongation at break: Tensile strength and elongation at break were measured using a tensile tester (Z010, Zwick Roell). Films were cut into rectangular strips (1 × 30 mm) and conditioned at 20 ± 1° C before testing. Initial grip separation and crosshead speed were positioned at 50 mm and 125 mm min^{-1}, respectively (ASTM D882-00, 2000).

Characterization of pullulan blended films

Fourier transform infrared spectroscopy analysis: Structural characteristics of pullulan blended films were performed to obtain the infrared spectra using a FTIR spectrophotometer (M/s. Bruker Optik GmbH, Germany). FTIR spectra analysis was performed in the infrared region with the wave number ranging from 4000 – 400 cm^{-1} with the spatial resolution of 64 scans at 4 cm^{-1} (ASTM E168-16, 2016).

X-ray diffraction analysis: X-ray diffraction (XRD) patterns were acquired using XPERT–3 X-ray diffractometer to obtain the information on the crystalline structure of pullulan blend films. XRD patterns were recorded in the range 2θ = 5-90° with Cu Kα radiation at 1.54Å and operated at 30mA and 45kV. The X-ray patterns were recorded with a 2θ step of 0.01° at a scanning rate of 2°θ / s ((ASTM D3906-19, 2019). Before analysis, the film sample was conditioned at 20 ± 1° C in a desiccator.

Field emission scanning electron microscope analysis: The morphological view of the film surface was examined using a field emission scanning electron microscope (FE-SEM) (Sigma with Gemini Column, Carl Zeiss, USA). Before analysis, films were conditioned in a desiccator at 37 ± 1°C. Film samples were fixed on the specimen holder using adhesive tapes. Further, the film samples were coated with a 10µm thick gold under vacuum. The gold-coated film samples were scanned with an accelerating beam voltage of 10 kV (ASTM E986-04, 2017).

Biodegradability test: Biodegradability test of developed pullulan blended films were performed using soil buried method. The developed pullulan blended films were cut into 5 x 5 cm and buried 10 cm from the surface in a container containing garden soil and observed for 0-25 days (ASTM D5988-18, 2018).

Statistical analyses: The experimental data were analyzed using Microsoft Excel. Moisture content, water-solubility and film thickness were determined in triplicates and the results were expressed as mean ± S.D. The significant difference between the mean values was evaluated using a One-way analysis of variance at a significance level $p<0.05$.

Results and Discussion

Interaction between water and polymer molecules was determined using moisture sorption. This measurement for selecting appropriate storage conditions and packaging applications (Anukiruthika *et al.,* 2020). Generally, pullulan has poor water barrier properties and high hydrophilicity (Ramakrishnan *et al.,* 2018). The moisture absorption rate of laboratory-made pullulan blended (LPB) film increased drastically and decreased gradually when the absorption time exceeded 12th hr. The results (Fig. 1a) suggested that the LPB films had significantly ($p<0.05$) higher moisture content (13 %) at 12th hr. The moisture content of films is related to the thickness, size and distribution of porosity (Ezati and Rhim, 2020). Moisture absorption may hinder the formation hydrogen bond between polymers because water molecules compete with polymers for hydrogen bonding, thereby resulting in the deformation of film. The water solubility of films was measured to establish the performance of film thereby paving a way for possible application in the food and packaging industries (Nawab *et al.,* 2016). Generally, film dissolution has been correlated with the dissociation of hydrogen bonds and relaxation of polymeric chains (Pella *et al.,* 2020). The results (Fig. 1b) indicated that both LPB and SPB films completely dissolved in water at ~125 sec were significant ($p < 0.05$) which indicated that pullulan films had higher hydrophilicity due to the presence of hydroxyl molecules present in pullulan polymer chains and amorphous structures (Trovatti *et al.,* 2012).

Also, the addition of glycerol to the pullulan films which is highly water-soluble can result in the formation of open pores and facilitate water diffusion into the film, thereby enhancing the film solubilization (Niu *et al.,* 2021). The results suggested that pullulan blended films offer higher solubility, hence, can be used for food packaging applications. The thickness of the film affects the drying phenomenon, film structure and also cause differences in the mechanical properties (Hu *et al.,* 2020). The thickness of pullulan blended films ranged from 0.13 to 0.14 mm which was not

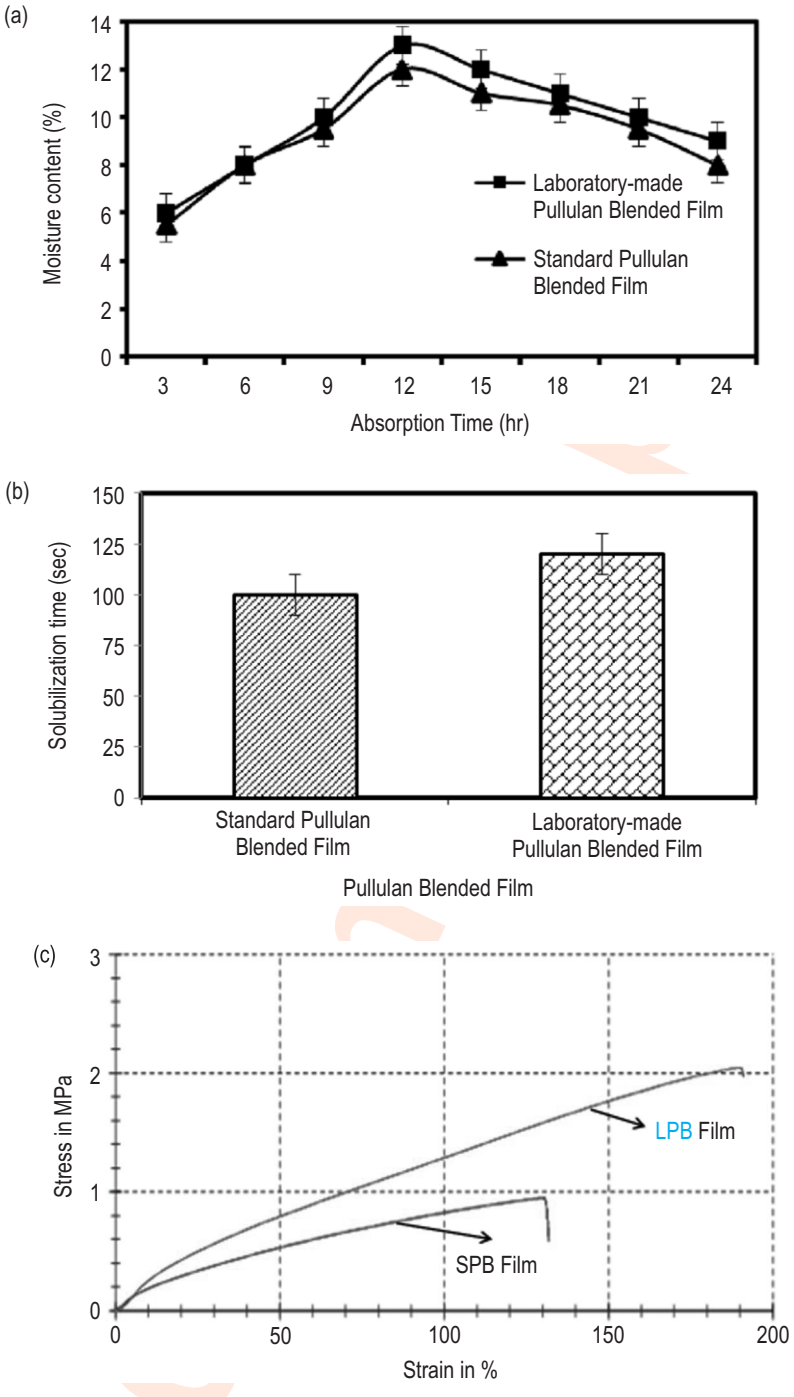

Fig. 1: Effect of (a) moisture content (b) water solubility and (c) stress-strain curves of laboratory-made pullulan blended film.

affected by the addition of sodium alginate and gelatin. Dispersion of polymer blends indicated that uniform thickness was obtained from the prepared film (Shen *et al.,* 2021). Also, the addition of glycerol to PUL: SA: GEL may increase the thickness of film-forming solution. However, glycerol addition did not significantly affect the thickness of pullulan blended films. Casariego *et al.* (2009) reported that the thickness of film can affect the properties of film. Thus, the study showed that no significant difference was observed between the pullulan blended films ($p>0.05$) to examine their properties. Structural strength is one of the important criteria for the evaluation of industrial biodegradable film. Tensile strength is the ability of prepared film to withstand tear upon stretching

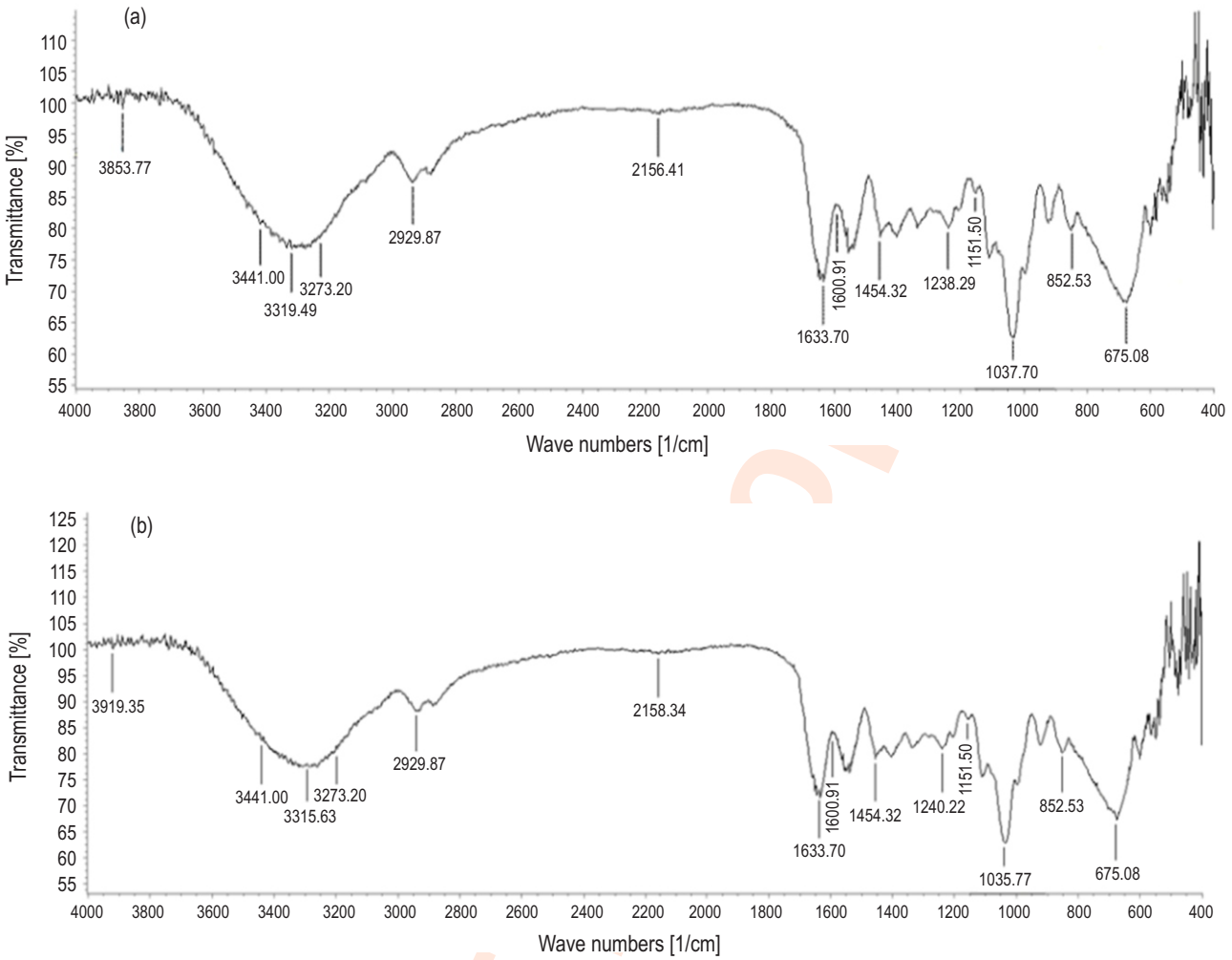

Fig. 2: FTIR spectra of (a) standard pullulan blended film (b) laboratory-made pullulan blended film Fig. 2 (b) Band appeared at 1151 cm^{-1} specifies the pullulan due to α (1-4) glycosidic bond stretching vibration. Broad peak occurs at 1633 cm^{-1} indicates the presence of O-C-O bond and glycosidic linkage. Similar observations were occurred in Fig. 2 (a).

which holds an important property in packaging industries (Marangoni *et al.,* 2021). The stronger intermolecular connections influence the better mechanical resistance. Kanmani and Lim (2013) studied the mechanical properties of whey protein concentrate and pullulan blend films which resulted in the tensile strength and elongation at break percentages between 2-10 MPa and 109 – 199 %, respectively. This result (Fig. 1c) is consistent with the present investigation, where LPB film showed the lowest tensile strength (2.04 MPa) and EAB (190%), which may be due to the incorporation of glycerol in the polysaccharide-based film which improves the mobility of polymeric chains and reduces the tensile strength while enhancing the flexibility of films to fabricate (Prasad *et al.,* 2008).

The addition of sodium alginate and gelatin to pullulan film could efficiently alter the strength and flexibility of pullulan blended films. On the other hand, the standard pullulan blended (SPB) films showed the lowest tensile strength (0.95 MPa) and EAB (130%). Nevertheless, a higher concentration of polymeric blends could favour intermolecular hydrogen bonds resulting in lower tensile strength of the film (Liu *et al.,* 2013). The results revealed that LPB film can be considered as an alternative to SPB film for preparing biodegradable packaging film with improved performance in terms of EAB percentage. Fig. 2) shows the FTIR spectra for standard and laboratory-made pullulan blended films. The broad peak at 1633 cm^{-1} was due to the presence of O-C-O bond and glycosidic linkage (Kazemi *et al.,* 2019). The peak formed around 2937 cm^{-1} was assigned to C-H stretching while the peaks aroused between 1238 and 1037 cm^{-1} corresponded to C-O stretching vibration (Hamidi *et al.,* 2019).

Gelatin and sodium alginate exhibited peaks around 1633 and 1600 cm^{-1}, respectively, due to their symmetric and antisymmetrical COO- groups (Liu *et al.,* 2020). This result

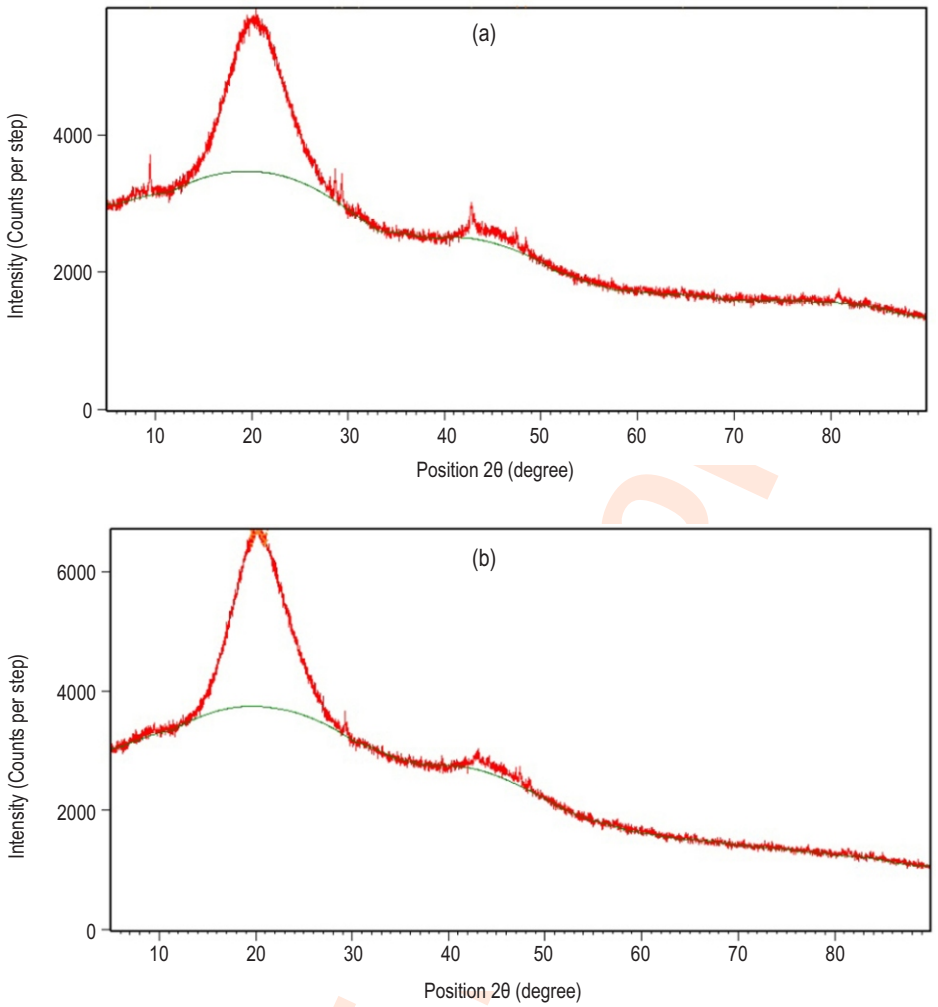

Fig. 3: X-ray diffractograms of (a) standard pullulan blended film; (b) laboratory-made pullulan blended film. (b) The peak at 2θ = 20° confirms that the presence of pullulan polysaccharides is an amorphous structure.

indicated that interactions occurred between the hydroxyl groups that act as cations in the binding site. The O-H stretching for pullulan, sodium alginate and gelatin blended films occurred at 3443, 3273 and 3315 cm^{-1}, respectively. Generally, the peak originated near 1150 cm^{-1} shows the presence of glycosidic linkage in the polysaccharides (Haghighatpanah *et al.,* 2020). This appeared to be consistent with the present observation, as the appearance this band at 1151 cm^{-1} specifies the pullulan due to α (1-4) glycosidic bond stretching vibration. Also, the obtained FTIR spectrum was compared with the standard pullulan blended film and confirmed that the produced pullulan blended film was mainly composed of pullulan (Fig. 2a). Thus, the FTIR result strongly exhibited the interactions between the hydroxyl and amino groups that could pave way for the changes in the properties of pullulan blended films. X-ray diffractograms were measured on pullulan blended films (Fig. 3a,b). Usually, the XRD pattern with sharp signals indicate the crystalline structure whereas the absence of these signals indicate that the polymer has an amorphous structure (Fig. 3b). The XRD diffractograms for pullulan blended films revealed a weak broad peak at around 2θ = 20° (Das *et al.,* 2017). Thus, the results indicated that the presence of pullulan polysaccharides is an amorphous structure. Farris *et al.* (2014) stated that the unique pullulan structure comprising α (1, 6) glycosidic linkages facilitate the formation of an amorphous film structure which permits the moisture to be readily absorbed. Also, the sharp signal appeared at around 2θ = 10° corresponded to a crystalline structure. This observation is consistent with the standard pullulan blended film (Fig 3a).

Scanning electron microscopy for the developed pullulan blended films showed a strong interfacial dispersion between the polymeric matrixes which indicates homogeneous distribution without the sign of phase separation. Consequently, SEM study confirmed higher miscibility and compatibility of pullulan with

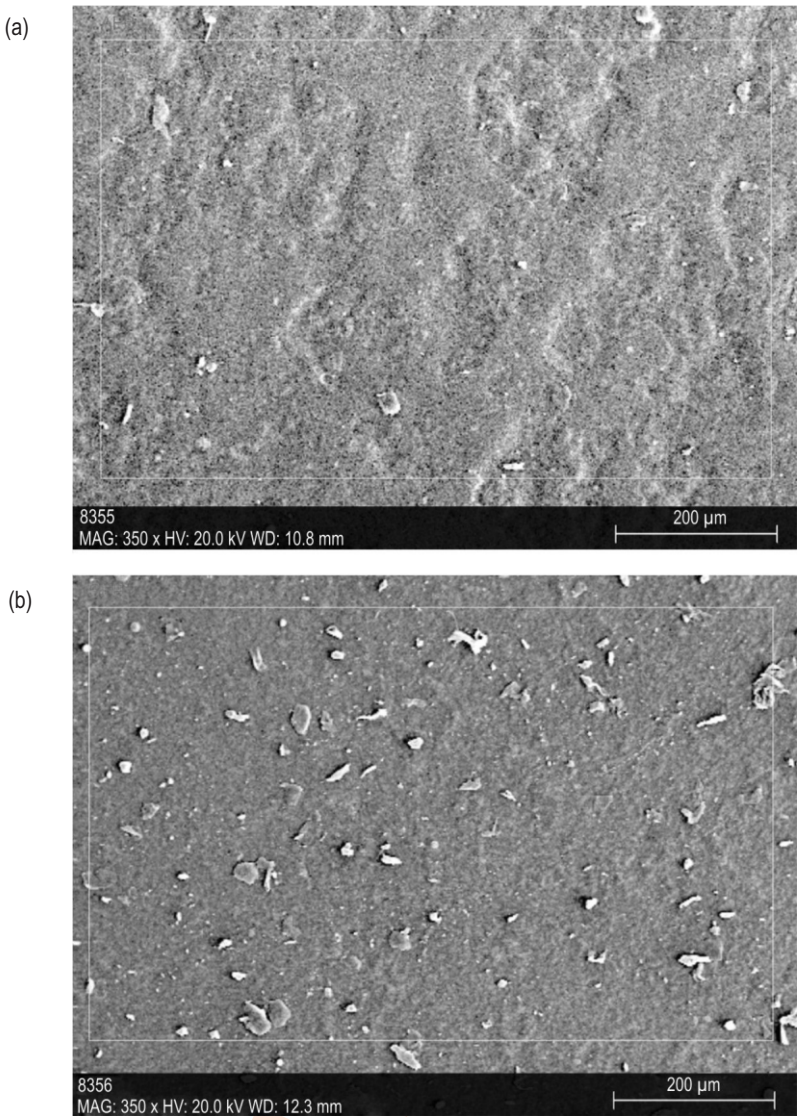

Fig. 4: FE-SEM micrographs of (a) laboratory-made pullulan blended film; (b) standard pullulan blended film.

sodium alginate and gelatin. LPB film clearly showed a good dispersion of polymers with a smooth film surface and formed a dense structure (Fig. 4a). On the other side, SPB film showed a compact structure with occasional pores in the film which may be due to air bubbles (Fig. 4b). Also, pullulan blended films plasticized with glycerol showed higher miscibility, probably glycerol molecules facilitate the interactions through hydrogen bonding. Thus, the results revealed that excellent dispersion between the polysaccharides. The biodegradability of developed pullulan blended films was checked by placing the prepared pullulan blended film under the soil sample. The biodegradability of developed SPB and LPB films was tested and the experiments were repeated thrice. The results showed that SPB and LPB films degraded in 10 days in the soil. Rishi *et al*. (2020) studied the biodegradability analysis of the developed pullulan film by dumping in direct soil pots and observed its degradation for 25 days. They reported that the developed pullulan film degraded in 7 days by soil microflora. Thus, the result suggested that laboratory-made pullulan could be used as a potential biodegradable plastic for food and other applications.

Pullulan blended films have shorter solubilization time in water and higher elongation at break. FE-SEM and FTIR analysis revealed that both sodium alginate and gelatin are compatible with pullulan. PUL: SA: GEL blended films with glycerol as a plasticizer exhibited excellent physical properties that can be exploited for various applications in packaging industries. Thus, the current study paves the way for the production of

biodegradable plastic from laboratory-made pullulan resulting in low cost and eco-friendly products.

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Add-on Information

Authors' contribution: R. Viveka: Experimentation and Manuscript preparation; **E. Nakkeeran:** conception, design of experiment, result analysis and manuscript editing.

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