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Formulation of pullulan/plasticizer blended films for their physical and biodegradability studies

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Abstract

The present investigation utilizes the eco-efficient pullulan polysaccharide as film forming biopolymer. Pullulan-based edible films offer good physical, thermal and mechanical properties which enable them to use in shelf-life preservation of fresh produce. Blends of other film forming polysaccharides, plasticizers and an antioxidant with pullulan (Pu) solution were prepared in order to determine physical and optical parameters of those films. The morphological and biodegradable studies were attempted to identify the changes on the films' surfaces. The films made from (only) pullulan (10Pu), pullulan composited with sodium alginate(10Pu_0.5SA), gelatin (10Pu_0.5G), polyethylene glycol (10Pu_0.5PG), calcium chloride and lemon juice (10Pu_1CC_2L) resulted heavier film densities, higher whiteness indexes and lower total color difference values.All the films were tested for their biodegradability in soil, where visual changes were appreciated after 15 days, partial and complete degradation took place at the end of 34 days and 53 days, respectively. Thus, these pullulan blended films could be a better replacement for synthetic films towards environmental problems.

Keywords: Pullulan, Plasticizers, antioxidant, edible film, biodegradable

Introduction

Use of bio-active packaging materials for extending shelf-life of fresh produce replaces the traditional petroleum-based (synthetic) packaging polymers (1,2). Edible films using thin wrapping materials, generally have packaging properties that shield the inner part from outer environment by limiting gas and water vapor transportation and improve the shelf-life of fresh produce by protecting them from physical, chemical and microbiological deteriorations (3). Traditionally, waxes, soymilk proteins, gelatin, sucrose and sugar derivatives were used as edible coatings to extend the storage life of many food items (4). Later, due to increased demand of coatings on food was served by petroleum-based coatings on food package. Further, dramatic increase of petroleum costs led to high packaging costs of fresh food materials. The past three decades, researchers have shown tremendous concern over cheaper edible materials for healthy packaging alternatives that lead to the commercial edible films prepared (5, 6, 7).

Typically, edible film composed of film forming compound, plasticizer, additive, and solvent to formulate a film forming dispersion (8). Characteristics of edible films, generally, affected by composition of film forming material, type and concentration of additives (9). In the present study, film forming materials viz., Pullulan (EPS from

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Aureobasidium pullulans), Gelatin, Sodium Alginate, Agar and Starch; plasticizers like Poly-Ethylene Glycol 4000, Sorbitol, Glycerol and Calcium chloride; and Citric acid (in lemon juice) as an antioxidant additive were used in combinations to prepare variety of edible films. The prepared edible films were analyzed for organoleptic (color, whitening index), physical (opacity, light transparency), topographical properties and biodegradability.

Materials and methods

Materials used : All the chemicals (of analytical grade) were purchased from M/s. Qualigens Biochemicalswere purchased from M/s. HIMEDIA chemicals Ltd. Standard Pullulan of medium molecular weight (viscosity:180 centipoise)was purchased from M/s. Kumar Organics Pvt. Ltd., Bengaluru. Deionized water was used from a Millipore Simplicity system.

Film formation : Pullulan (10% w/v) was dissolved in distilled water and heated by a hot plate stirrer at 80°C, 500 rpm for 60 min; and then solution was cooled down to room temperature. Different mixed concentrations (% w/v) of other film forming materials (Gelatin, Starch, sodium Alginate, Agar), plasticizers (Glycerol, Sorbitol, Calcium chloride, Polyethylene glycol-4000, 6000), and additive (Citric acid) were mixed with 10 (% w/v) Pullulan suspension to form variety of film formulations (Table 1). Pullulan based edible films were formed by the casting method and dried at 60°C and 40% relative humidity (RH) in an environment chamber (S.K. Scientific & Surgical, India), for 24 h prior to characterization.

Color parameters measurement : Color Flex EZ 45/0°color spectrophotometer (Hunterlab, Reston, VA, USA) was used to test the color parameters of the edible films, according to ASTM E308. The CIE lab scale measurements were made, and the instrument was calibrated with a standard white plate (*L'* = 91.83, *a'* = - 0.73, *b'* = 1.52) as film background before the measurements. Results were expressed as *L** (luminosity), *a** (red/green) and *b** (yellow/blue) parameter values of the film samples sample and *L'*, *a'* and *b'* are the color parameter values of the standard white plate. A mean value of five repetitions of film's top and bottom side was recorded.

The total color difference (*ÄE*) was calculated using the following equation (10):

$$\Delta E = \langle [L^* - L']^2 + [a^* - a']^2 + [b^* - b']^2 \rangle^{\frac{1}{2}}$$

The Whitening Index (*WI*) of edible films was calculated as described by Bolin and Huxsoll (1991):

$$WI = 100 - \sqrt{[100 - L^{*2}] + a^{*2} + b^{*2}}$$

Film thickness and density measurement : Film thickness was measured by using Mini Electronic Dial Thickness Indicator (Gauge Tester, Joro). The thickness reading was made of mean value of three experimental tests in ten different positions, each twice per second measuring a minimum, a maximum and an average value, with a resolution of 0.001 mm, at room temperature. The density of each film (in g/cm^3) was determined and expressed as an average of three measurements and standard deviation.

Film transparency measurement : Transparency of pullulan-based films was measured according to the procedure of Han and Floros, 1997 (11) using a spectrophotometer (Biospectrometer® kinetic, Eppendorf, New York, USA), as per ASTM method D1746-92 (12). Edible film's transparency was calculated using following equation (13):

$$\text{Transparency} = \frac{A_{600}}{b} \text{ or } \frac{\log T_{600}}{b}$$

where T_{600} is transmittance at 600 nm, A_{600} is absorbance, and *b* is the length of the light path through the medium (i.e., film thickness).

Morphology evaluation : The films' morphology was determined by using a microscope (Olympus CX31 HD Digital Microscope, Feasterville, PA)

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with a standard light. Film strips were observed in black and white and the images were recorded at a 20X magnification (13).

Biodegradability in soil : Biodegradation of prepared films was tested in soil under controlled laboratory conditions (25ºC, 25% humidity and pH=7). Samples were placed onto the soil located in plastic containers which were covered by aluminium foil in order to keep the films free of dirt. Biodegradation was studied taking photographs at regular time intervals (14).

Results and Discussion

Color related characteristics : The consumer acceptance of any edible film depends, primarily, on film's color that in turn influenced by the ingredient composition by means which it was formulated (15). The different composition of film forming compounds, plasticizers, additive used for pullulan based edible films and film thickness, total color difference (*ÄÅ*), whiteness index (*WI*), and Transparency values were tabulated in the Table 2. The presence of various materials significantly affected the films formation and their characterization. All the pullulan-based films have shown the thickness range of 0.04 to 0.1 mm. Thickness can affect barrier properties, particularly water vapor permeability due to differences between the water vapor pressure below the film and that of the moisture build-up above the film (16). To obtain films with similar thickness, same suspension volumes were used. However, films prepared with plasticizers exhibited higher thickness that those prepared with other polysaccharides. The films' thickness values are in good agreement with previous studies (17, 18).

The Hauntercolor parameters results (L^*, a^*, b^*) were used to calculate the total color difference (*ÄÅ*) and whiteness index (*WI*), the values shows significant variation of color characteristics (p<0.05). The films made from (only) pullulan, pullulan composited with sodium alginate, gelatin, polyethylene glycol, calcium chloride and lemon juice resulted heavier film densities, higher whiteness indexes and lower total color difference values. The similar results were also reported in previous study (15, 18). The transparency (based on absorbance at 600 nm wavelength) values of these films were very high, also attracts the packaging visual characteristics in both product consumer acceptability and food quality, when these are applied on fresh produce. So, these five films were used in the mechanical and thermal characterization. The same trend was also reported by Bertan et al., 2005 (19) andTaqi et al., 2011 (20).

Microstructure of films : Edible films are usually hydrophilic in nature, whereas conventional plastic films are non-polar, as reported by Antarés and Chiralt, 2016 (21). The incorporation of various plasticizers and additives in the film forming

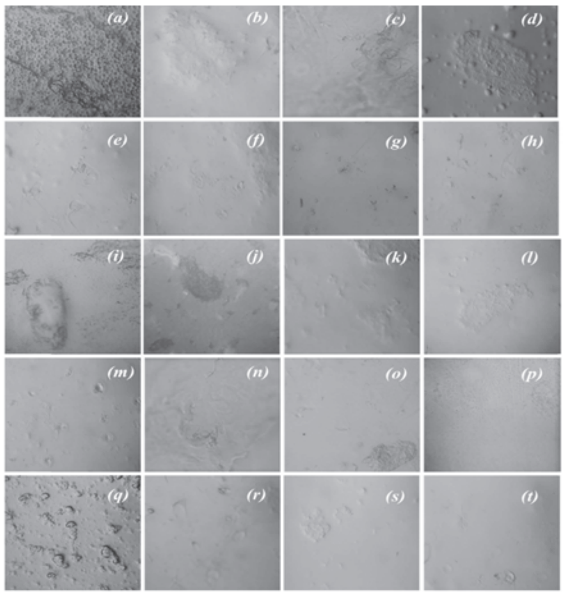

Fig. 1: Surface micrographs of Pullulan based edible films (a)10 Pu, (b) 10 Pu_0.5 SA, (c) 10 Pu_0.5 G, (d) 10 Pu_0.5 St, (e) 10 Pu_0.5 A, (f) 10 Pu_0.5 G*, (g) 10 Pu_0.5 S*, (h) 10 Pu_1 CC, (i) 10 Pu_0.5 PG, (j) 10 Pu_0.5 PG*, (k) 10 Pu_0.5 G*_0.5 S*, (l) 10 Pu_0.5 G_0.5 G*, (m) 10 Pu_0.5 SA_0.5 G, (n) 10 Pu_0.5 SA_ 0.5 G*, (o) 10 Pu_0.5 SA_1 PG, (p) 10 Pu_1 CC_1 PG, (q) 10 Pu_1 PG_2.5St, (r) 10 Pu_1 CC_2 L, (s) 10 Pu_1 CC_2 S*, (t) 10 Pu_2 L

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dispersion is done by homogenization of polymer aqueous solution. When the film is dried, plasticizers and additives embedded into the polymer matrix, as observed by microscopy. The drying time plays an important role in determining the arrangement of the components during the film-forming step, thus the final microstructure of the edible films. Morphological analysis of the films under study was carried out by optical microscopy (20X magnification), to evaluate the homogeneity and the structure of the prepared films. Fig. 1 shows the surface micrographs of all the prepared films. Pullulan composited films presented a more homogeneous and uniform structure than pullulan (alone) ones. Starch containing films (Fig. 1. (*d*), (*q*)) shows a mild change (agglomerations) in the structure of films due to non-uniform distribution of starch. The most irregular structure was observed in Pullulan film (Fig. 1. (*a*)). The morphology of the films supported the tensile results, evidencing that a different structural arrangement of the components in the film forming dispersion significantly influences both mechanical and gas barrier properties. The similar surface micrographs were also observed by many researchers for edible films (13, 14, 18).

Biodegradability tests : Edible films made from biopolymers takes advantage of reducing environmental pollution clean-up (14). The pullulan-based films were visualized using a digital camera. Fig. 2 (a) and (b) shows the surface of pullulan blended films at 0, 4, 8, 15, 27, 34, 41, 46 and 53 days in contact when buried under fertile soil. The visual images outline the films' surface change indicating cracks, holes, color changes and microorganism's appearance, in a progressive manner. Over the time, microorganism's(present

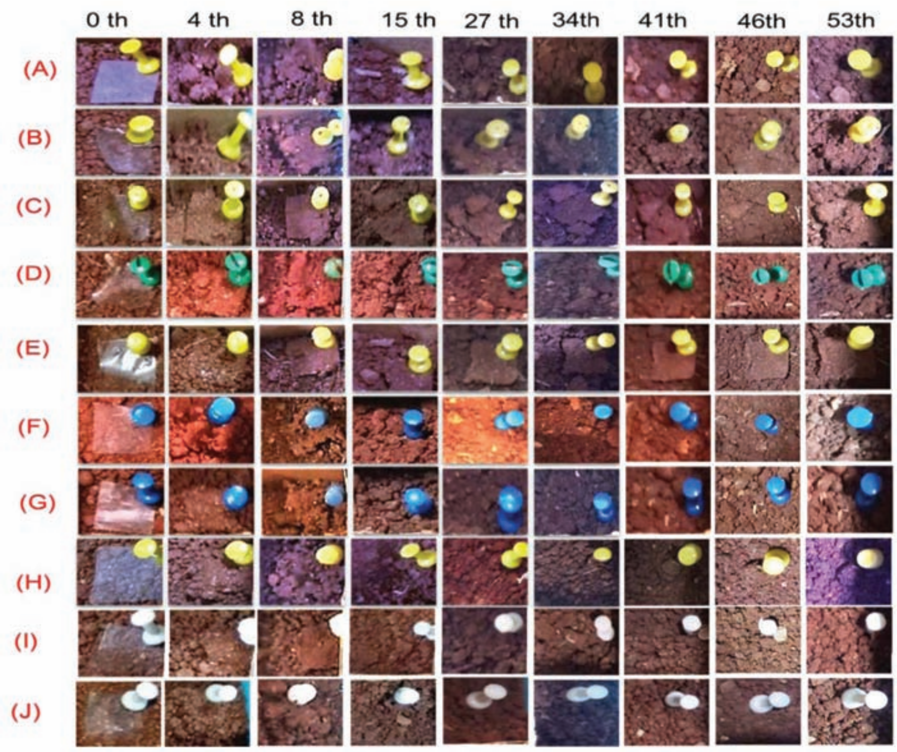

Fig. 2 (a). Pullulan based edible films degraded in soil after different (0, 4, 8, 15, 27, 34, 41, 46 and 53) days: (A)10 Pu, (B) 10 Pu_0.5 SA, (C) 10 Pu_0.5 G, (D) 10 Pu_0.5 St, (E) 10 Pu_0.5 A, (F) 10 Pu_0.5 G*, (G) 10 Pu_0.5 S*, (H) 10 Pu_1 CC, (I) 10 Pu_0.5 PG, (J) 10 Pu_0.5 PG*

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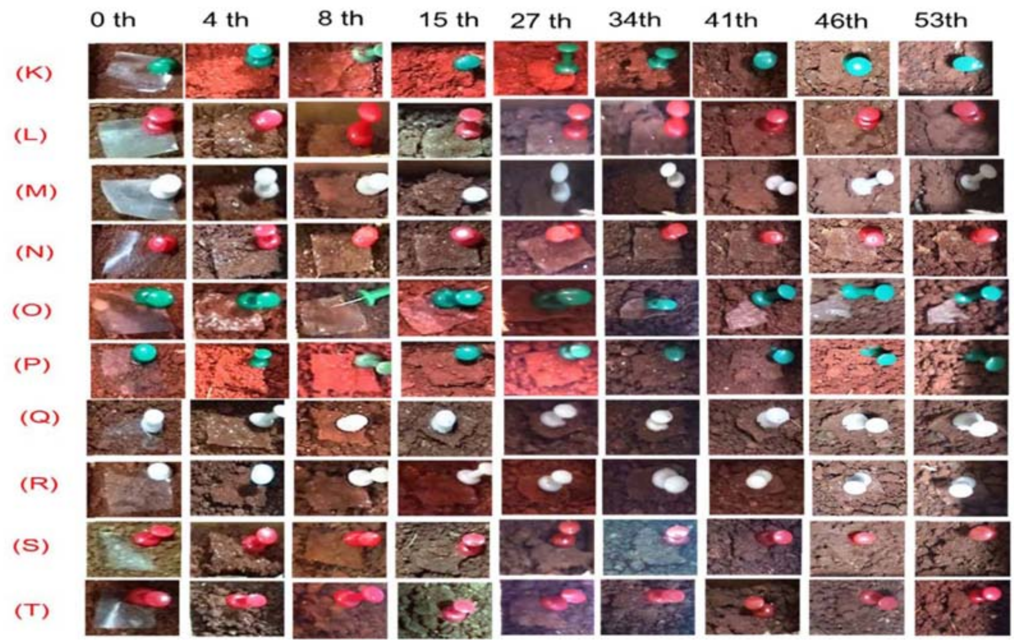

Fig. 2 (b). Pullulan based edible films degraded in soil after different (0, 4, 8, 15, 27, 34, 41, 46 and 53 days: (K) 10 Pu_0.5 G*_0.5 S*, (L) 10 Pu_0.5 G_0.5 G*, (M) 10 Pu_0.5 SA_0.5 G, (N) 10 Pu_0.5 SA_ 0.5 G*, (O) 10 Pu_0.5 SA_1 PG, (P) 10 Pu_1 CC_1 PG, (Q) 10 Pu_1 PG_2.5St, (R) 10 Pu_1 CC_2 L, (S) 10 Pu_1 CC_2 S*, (T) 10 Pu_2 L

in soil) attack on the film's surface was gradually spreaded.The gravimetric biodegradation studies were not performed, due to the microbial degradation of film surface. Almost all the films were slowly degraded after 5 weeks (34 days) in soil but visual changes were only appreciated after the 15 days. By the end of 53 days, all the films were completely degraded by soil microorganisms and disappeared (Fig. 2 (a), (b)). Microorganism proliferation was enhanced by the humidity of films which was favoured by the pullulan content. The similar observations were also reported by Debandi et al., 2016 (13) for films made with Chitosan/ Glycerol.

Conclusion

Use of different polysaccharides, plasticizers and additives were blended to pullulan, edible biopolymer, to formulate the edible thin films was attempted. Film physical, optical, surface and biodegradability properties were evaluated.Out of twenty different pullulan-based edible films, only the films formulated with plasticizers have shown high film thicknesses. High value of density, whiteness index and low value of color difference was observed in 10Pu, 10Pu_0.5SA, 10Pu_0.5G, 10Pu_0.5PG and 10Pu_1CC_2L films. Different morphological changes were also seen in all these pullulan blended films. Biodegradability, of all these films, in soil elucidates the use of these pullulan-based films would be an alternate for plastic films that completely eradicates the environment pollution.

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Table 1. Pullulan blended films classification based on composition

Film symbol	Film Forming Material (% w/v)				Plasticizer (% w/v)						Additive (% w/v)
	Pu	SA	G	St	A	G*	S*	CC	PG	PG*	L
10 Pu	10.0	–	–	–	–	–	–	–	–	–	–
10 Pu/0.5 SA	10.0	0.5	–	–	–	–	–	–	–	–	–
10 Pu/0.5 G	10.0	–	0.5	–	–	–	–	–	–	–	–
10 Pu/0.5 St	10.0	–	–	0.5	–	–	–	–	–	–	–
10 Pu/0.5 A	10.0	–	–	–	0.5	–	–	–	–	–	–
10 Pu/0.5 G*	10.0	–	–	–	–	0.5	–	–	–	–	–
10 Pu/0.5 S*	10.0	–	–	–	–	–	0.5	–	–	–	–
10 Pu/1 CC	10.0	–	–	–	–	–	–	1.0	–	–	–
10 Pu/0.5 PG	10.0	–	–	–	–	–	–	–	0.5	–	–
10 Pu/0.5 PG*	10.0	–	–	–	–	–	–	–	–	0.5	–
10 Pu/0.5 G*/0.5 S*	10.0	–	–	–	–	0.5	0.5	–	–	–	–
10 Pu/0.5 G/0.5 G*	10.0	–	0.5	–	–	0.5	–	–	–	–	–
10 Pu/0.5 SA/0.5 G	10.0	0.5	0.5	–	–	–	–	–	–	–	–
10 Pu/0.5 SA/ 0.5 G*	10.0	0.5	–	–	–	0.5	–	–	–	–	–
10 Pu/0.5 SA/1 PG	10.0	0.5	–	–	–	–	–	–	1.0	–	–
10 Pu/1 CC/1 PG	10.0	–	–	–	–	–	–	1.0	1.0	–	–
10 Pu/1 PG/2.5St	10.0	–	–	2.5	–	–	–	–	1.0	–	–
10 Pu/1 CC/2 L	10.0	–	–	–	–	–	–	1.0	–	–	2.0
10 Pu/1 CC/2 S*	10.0	–	–	–	–	–	2.0	1.0	–	–	–
10 Pu/2 L	10.0	–	–	–	–	–	–	–	–	–	2.0

Pu- Pullulan, SA-Sodium Alginate, G- Gelatin, St- Starch, A- Agar, G*-Glycerol, S*- Sorbitol, CC-Calcium chloride, PG- Polyethylene Glycol4000, PG*- Polyethylene Glycol6000, L- Lime juice

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Table 2. Pullulan based edible films thickness, density, color properties and transparency.

Film symbol	Thickness (mm)	Density (g/cc)	Color Difference, E	Whiteness Index, (Abs)	Transparency WI
10 Pu	0.06±0.001§	10.46±0.2¶	13.48±0.55¡	78.39±0.21[¢]	36.13±0.21[¤]
10 Pu/0.5 SA	0.05±0.001	7.95±0.1	16.77±0.47	75.09±0.43	19.08±0.34
10 Pu/0.5 G	0.05±0.002	7.24±0.21	16.47±0.24	75.39±0.34	1.66±0.07
10 Pu/0.5 St	0.05±0.002	0.954±0.09	37.29±0.59	54.56±0.54	2.22±0.12
10 Pu/0.5 A	0.04±0.001	1.450±0.23	29.05±0.24	62.72±0.13	2.35±0.54
10 Pu/0.5 G*	0.07±0.003	0.716±0.08	22.51±0.76	69.45±0.52	2.25±0.27
10 Pu/0.5 S*	0.04±0.001	1.600±0.17	21.38±0.35	70.45±0.31	2.07±0.35
10 Pu/1 CC	0.05±0.002	1.03±0.05	33.46±0.53	58.36±0.28	2.66±0.31
10 Pu/0.5 PG	0.05±0.001	9.10±0.13	16.86±0.37	75.17±0.17	4.30±0.42
10 Pu/0.5 PG*	0.10±0.004	1.463±0.06	35.64±0.45	56.13±0.56	8.42±0.25
10 Pu/0.5 G*/0.5 S*	0.04±0.002	1.075±0.02	39.80±0.55	52.09±0.23	4.95±0.32
10 Pu/0.5 G/0.5 G*	0.04±0.001	1.575±0.01	34.34±0.52	57.45±0.47	4.27±0.38
10 Pu/0.5 SA/0.5 G	0.07±0.001	1.31±0.03	19.73±0.13	72.05±0.87	1.63±0.54
10 Pu/0.5 SA/ 0.5 G*	0.06±0.003	1.233±0.02	27.68±0.65	64.12±0.76	2.21±0.23
10 Pu/0.5 SA/1 PG	0.04±0.001	1.30±0.04	37.75±0.16	54.05±0.58	3.70±0.54
10 Pu/1 CC/1 PG	0.10±0.005	1.854±0.05	36.17±0.56	55.63±0.52	12.02±0.33
10 Pu/1 PG/2.5St	0.06±0.002	1.850±0.21	36.17±0.32	55.63±0.43	2.31±0.26
10 Pu/1 CC/2 L	0.05±0.002	10.35±0.34	19.14±0.44	72.61±0.87	32.62±0.45
10 Pu/1 CC/2 S*	0.04±0.003	1.04±0.02	24.79±0.54	67.56±0.45	4.23±0.28
10 Pu/2 L	0.04±0.001	1.650±0.04	36.47±0.24	55.33±0.37	2.72±0.36

Data shows mean ± standard deviation and different superscript letters (§, ¶, ¡, ¢, ¤) in each column indicated significant differences ($p<0.05$)

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