

Development of Almond gum/alginate composites to enhance the shelf-life of post-harvest *Solanum Lycopersicum* L



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ABSTRACT

Almond Gum/alginate composite (AG/AL) composites namely A1, A2 and A3 were prepared and their physico-chemical properties were evaluated. The measured film thickness, moisture and solubility were indistinguishable among the films whereas water contact angle analysis shows that A2 (83.8 ± 1.65) and A3 (86.2 ± 3.19) film has high hydrophobicity than A1 (58.4 ± 3.21). By increasing plasticizer concentration from 0.4% to 0.66%, elongation was increased from 30 to 53 % whereas tensile strength (3.16 to 1.90 Mpa) and water vapor transmission rate (WVTR) decreased from 212 to 160 $\text{g/m}^2/24\text{hr}$. FT-IR analysis shows that the edible film has O-H, C-H, C=O and -COO functional groups and SEM analysis reveals that the prepared film has even surface due to the strong interaction between polymer and plasticizer. TGA results show major weight loss at 100°C, 190-220°C and 350°C-400°C due to the dehydration/decomposition of water molecules and carbon containing functional groups. Film solution was used as an edible coating in tomatoes and weight loss (WL), titratable acidity (TA), Total soluble solids (TSS), ascorbic acid content (AAC) and anti-microbial activity were determined. The WL analysis shows a significant delay in decay of coated tomatoes over 40 days of storage time whereas control tomatoes decayed on day 25. TA and TSS test confirms that the coating can significantly ($P < 0.05$) prevent the acidity level of tomatoes. Furthermore, the AG/AL coating has great activity against *E. coli*, *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*. Therefore, the present study confirms that AG/AL has superior properties and potential as edible films or coating.

1. Introduction

Contamination of food with the external environment affects the appearance, moisture, aroma, quality and shelf-life of food. Oxidation, microbial spoilage, and metabolism are the main causes of deterioration of many foods during production, transportation, processing, storage and marketing (Han, 2018). Innovation in food packaging systems can assist to improve consumer requirements including food quality, nutrient values and increased shelf-life. More importantly, food packaging is widely used to protect food from external contamination such as odour, temperature, humidity, dust, microorganism, physical and chemical damage (Yildirim, 2018). On the other hand, food packaging materials can account for up to 10 - 20 % of overall total food product cost and it increases the plastic solid wastages (Marsh, 2007). According to Mintel's Global New Products Database (GNPD), 82% of food products used plastics as a primary packaging material. Benson et al., studied that generation of plastic waste was doubled (584 million tons) in

2021 during Covid 19 period. Since, these plastic materials are non-degradable in nature, filled the land fields, creating various environmental impacts. Hence, the development of edible film or biodegradable film using biopolymers might be a better alternative to overcome the difficulties in waste management (Hossain, 2022).

Development of edible film can be an interest to many researchers due to barriers to moisture, oxygen, lipid, gas and solute movement for the food with enhancement in quality and shelf-life of food products (Suresh, 2021). In addition, edible films play an important role in the covering of thermo labile compounds like vitamins, aroma, and flavors, providing an efficient method to preserve their characteristics during food packaging (Bosquez-Molina, 2010). The current trend in packaging is to prepare edible film by blending different biopolymers with natural gums. Natural gums composed of sugars other than glucose and are capable of forming gel like material with high viscosity even at small concentrations. Natural gums are chemically inert, biocompatible, odorless, inexpensive, non-toxic and water soluble. Natural gums serve as

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sources of reserve nutrients, structural entities, dietary fiber and water-binding moieties, therefore gums have many industrial applications such as gelling agents, stabilizers, thickening agents and emulsifying agents (Saha, 2017). Film-forming properties of natural gums involve intermolecular forces such as crosslinking, electrostatic, hydrophobic, ionic interactions or intermolecular/intramolecular hydrogen bonding. Natural gum includes guar gum, xanthan gum, gellan, gum Arabic, almond gum used in preparing edible films and coating (Alizadeh-Sani, 2019).

Almond gum is a copious gum exuded by the trunk, branches and fruits of *Prunus dulcis* trees. Almond gum is mainly composed of 92.36% of polysaccharides with the major constituent of arabinose (46.83%), galactose (35.49%), and uronic acid (5.97%) along with less amount of protein (2.45%) (Mahfoudhi, 2015). Almond gum is a natural biopolymer produced by almond trees and found worldwide. It possesses numerous properties such as preservative, emulsifier, stabilizer, food strengthener and increases the dietary fiber content in the food. It could also serve as functional ingredient to improve the physical and structural properties of hydration, oil-holding capacity, viscosity, texture, sensory characteristics and shelf-life (Rezaei, 2016). Alginate is a naturally occurring polysaccharide derived from brown algae species (*Laminaria hyperborean*). Sodium alginate, potassium alginate, ammonium alginate, and calcium alginate are monovalent salts of alginic acid. Sodium alginate is the most commonly used salt of alginate. The U.S. Food and Drug Administration (FDA) classifies food grade sodium alginate as GRAS (generally regarded as safe) substance in Title 21 of the Code for Federal Regulations (CFR) and lists its usage as an emulsifier, stabilizer, thickener, and gelling agent (Müller, 2018).

From the previous study, various types of almond gum or alginate or combination with other biopolymer edible film have already been prepared. For example, Alginate based edible film enriched with citric acid and ascorbic acid shows significant difference in anti-browning of fresh-cut apples (Ma, 2018). A capsaicin incorporated alginate/pullulan edible film shows great anti-microbial activity against *E. coli*, *S. aureus* with improved tensile strength, water contact angle and water vapor permeability (Najafi Marghmaleki, 2021). Kumar et al 2021 has reported that the increase in shelf-life of postharvested tomatoes by 9 days using chitosan-pullulan with pomegranate seed oil composition at 23 °C and 4 °C storage time. However, based on the existing literature knowledge, the combination of almond gum/alginate has not been explored considering the uniqueness of the both materials. Almond gum has high medicinal values such as reducing body heat, chloestrol control, weight gain, natural dietary fibre and delay premature aging. Like wise, alginate also have excellent film forming ability, biodegradability, mechanical, barrier and antimicrobial activity with low cost and relatively abundant in india. Therefore, the aim of this work was to develop a novel edible coating from almond gum/alginate composite to enhance the shelf-life of post-harvest *Solanum Lycopersicum*. L (tomatoes).

2. Materials and Methods

2.1. Chemicals

Almond gum was purchased in amazon (ASIN B07JFCH58J) (India). Sodium alginate was purchased in Sigma Aldrich (CAS 9005-38-3) (India). Glycerol (G89129) and sorbitol (S23429) were obtained from Nice Chemical, India.

2.1.2. Plant source

Tomatoes were collected from the local market around sathyamangalam (11.5167°N 77.2500°E) and gopichettipalayam (11°27'13"N 77°26'18"E), coimbatore, India. Tomatoes (*Solanum lycopersicum* L.) were harvested at the mature-green stage of ripening. Tomatoes with similar size, shape, color and free from any defects were selected. Initially all the treated tomatoes were washed with a solution of sodium hypochlorite of 0.05% (w/w) for 3 mins and air-dried at ambient temperature (23±2°C). All the glasswares are sterilized previously.

2.2. Preparation of film forming solution

AG/AL composite was prepared with three different ratios of plasticizer. Initially, almond gum exudate was grinded as a fine powder using mixer grinder. It was dried at 60°C to remove moisture. To prepare coating formulation 0.3 % of almond gum and 0.67 % of sodium alginate were dissolved in 150 ml of water. Each composite solution was stirred using magnetic stirrer for 30-45 min at 75°C on 500 RPM to get homogenized. After the addition of plasticizer (glycerol and sorbitol), each solution was stirred for 1 hr for the interaction between the biopolymer (almond gum and alginate) and plasticizer. The composite solution was kept in vacuum oven to remove the air bubbles during film casting. Then, it was casted in glass petri dish (90 × 15 cm) using solution casting technique and the volume of the solution used in the petri dish was 80 ml. The solution was air-dried at room temperature for 24 hrs to remove volatile substance. The air-dried solutions were dried at hot air oven for 3 to 4 hrs at 85°C to prepare edible film. The prepared films were peeled off and stored in vacuum desiccators at 50 ± 2% Relative humidity (RH) and 23±2°C temperature for further analysis.

2.2.1. Characterization of edible film Film thickness

Film thickness: Thickness was measured using electronic screw gauge at 5 different positions of the film and mean thickness was calculated. Samples with air bubbles and mean thickness variation greater than 5% were excluded from the analysis. Moisture content (MC): MC was calculated using hot air oven method in triplicates. The samples were cut into 2 cm × 2 cm, dried at 105°C and weighed at each one-hour interval until constant weight is obtained. Film solubility: The prepared films were cut into 2 cm × 2 cm and immersed in 50 ml of distilled water with constant agitation at 23±2°C for 24 hrs using orbital shaker in triplicates. After agitation, the solution was filtered with filter paper. The filter paper contains undissolved residues which were weighed and dried at 103°C for 1 hr by using hot air oven and weighed. The total soluble matter was calculated from initial and final dry weight of films and measured on dry weight basis as reported by (Zhang, 2018). Water contact angle: Contact angle measurement of film was measured using OCA20 video based contact angle measurement unit (Dataphysics, GmbH, Germany) at ambient conditions (23±2°C) by recording the side profiles of deionized water drops (5 µL) deposited on the film surface. The measurement was taken for 5s after the drop deposition to ensure its stabilization, yet to minimize water absorption and evaporation. At least three drops were observed on different areas for each film (both on the top and on the bottom side), and contact angles were reported as the average value ± standard deviation (Siracusa, 2018).

2.2.2. Barrier Properties

Water vapor transmission rate (WVTR) was measured using water vapor transmission rate analyzer (Make-SDL Atlas, Model: M261) with ASTM E 96/E96M-16-Method-B. Relative humidity is maintained at 50±2% and temperature was maintained at 23±2°C.

2.2.3. Mechanical properties

The tensile strength and elongation at break of the film was determined by using INSTRON 3366 according to ASTM D882 Standard. Each Films were cut in the form of strips with 2 cm × 2 cm in triplicates. The strips were clamped between two tensile grips and the initial gauge length was set at 50 mm. Films were pulled using a crosshead speed of 25 mm/s. During the stretching, force (N) and deformation (mm) were recorded. Measurements were carried out on 3 different specimens. The tensile strength was calculated by using a formula, $TS = L / A$, where TS corresponds to tensile strength (MPa), L is the load at tensile strength, A is the cross sectional area of the film. The elongation at break was calculated by the equation, $\text{Elongation at break (\%)} = \text{change in length} \times 100 / \text{Original length}$. The tensile strength and elongation at break were calculated as average value from the obtained results (Sanyang, 2015).

Table 1
Different composition of almond gum, alginate and glycerol, sorbitol as plasticizer for the preparation of edible film

S.No	Composite Film	Almond Gum (W/W %)	Alginate (W/W %)	Glycerol (V/V %)	Sorbitol (V/V %)
1	A1	0.5	0.67	0.2	0.2
2	A2	0.4	0.67	0.26	0.26
3	A3	0.3	0.67	0.33	0.33

2.2.4. FT-IR Analysis

The Fourier transformed infrared (FT-IR) spectra were recorded on a Bruker-IFS 48 FT-IR spectrometer (Ettlingen, Germany) to analyze the chemical structure of AG/AL-plasticized films. Films were placed in the support and pressed by the measuring sensor. Ten scans were done for each samples, from a spectral range of 400–4000 cm^{-1} and a resolution of 4 cm^{-1} (Król, 2016). The AG/AL composite solution without plasticizer was used as control.

2.2.5. Thermo gravimetric analysis (TGA)

Thermo-gravimetric analysis (TGA) was carried out under nitrogen atmosphere by means of Toshvin TG-50 instrument (USA). Gas flow of 50 mL/min and a heating scan of 15°C/min, over a temperature range 15–600°C, were used for the analyses. Sample mass of 10 mg was used for the experiments (Chakravartula, 2019).

2.2.6. Morphological Analysis of Edible film using SEM

Morphology of the edible film was studied using SEM (Scanning Electron Microscopy) (Zeiss Model Evo18, USA). Edible films were cut into 1 $\text{cm} \times 1$ cm . The films containing various plasticizers were mounted on aluminum stubs using double-sided adhesive tape and sputtered with a thin layer of gold using a BAL-TEC SCD 005 sputter coater (BAL-TEC AG, Blazers, Liechtenstein). All samples were examined using an accelerating voltage of 20.0 kV (Aadil, 2016).

2.3. Preparation of coating solution

To prepare coating solution, A-1 composite film (Table 1) were chosen due to the presence of low plasticizer content compared to A-2 and A-3 as described in the 2.2 method section. 0.3 ml of sorbitol and 0.3 ml of glycerol was added to the solution. It was stirred for 2 hrs at 50°C to get the homogenous solution. The said polymer solution was used to coat the tomatoes by dipping method. The dipping was done directly by immersing the tomatoes for 1 minute and rotated 360-degree angle to ensure the uniformity of the coating and were exposed to the atmospheric condition. The sample without coating was taken as control. Control tomatoes were dipped in double distilled water. The coated/uncoated samples were exposed to atmospheric condition and analysis was done for alternative days (1,3,5,7..) in the storage period of 40 days. WL, TA, TSS, AAC were analyzed up to day 40.

Analysis of Coated tomato for optimization of coating formulation

2.3.1. Weight loss (WL)

For each treatment, the measurement of WL was carried out on ten tomatoes in each replication. The weight loss was determined using electronic weighing balance. The weight differences after storage compared to initial weight were expressed in percentage (Lin, 2017).

2.3.2. Titratable Acidity (TA)

TA were recorded using potentiometric titration of N/10 of NaOH an Phenolphthalein as an indicator using 1 mL of diluted juice in 25 mL distilled H₂O and results were the mean \pm SE expressed as g citric acid equivalent per 100 g^{-1} fresh weight (Zapata, 2008).

2.3.3. Total Soluble Solids (TSS)

The TSS of the tomatoes were determined with the help of hand refractometer calibrated in °Brix at 40°C with necessary correction factor. TSS was individually measured for each fruit on an alternative day basis.

2.3.4. Ascorbic acid content (AAC)

Titration methods were used to determine AAC using 2,6-dichlorophenol indophenol dye, as described by Liguori (2021). In this redox reaction, the indophenol dye is oxidised to DHAA and then reduced to a colourless compound with a pale pink end point. When an excess of unreduced dye is present, a pale pink colour is obtained.

2.3.5. Anti-microbial activity test

Anti-microbial activity was performed by agar diffusion method based on Van Der Watt and Pretorius, 2001. Agar well diffusion method has been used to determine the antimicrobial activities and minimum inhibitory concentrations of plant extracts against Gram positive, Gram negative bacteria (Gonelimali, 2018). Chloramphenicol is used as an control which is a semisynthetic antibiotic originate from *Streptomyces venuelae* with primarily bacteriostatic activity. The stock culture of bacteria (*Staphylococcus aureus*, *E.coli*, *Bacillus subtilis*, *Enterococcus faecalis*) were received by inoculating in nutrient broth media and grown at 37°C for 18 hrs. The agar plates of the above media were prepared. Each plates were inoculated with 18 hrs old cultures of bacteria in the sterile plates using swab method. 5 wells were cut and the sample was poured in ratio of 25 μl , 50 μl , 75 μl and 100 μl . All the plates were incubated at 37°C for 24 hrs and the diameter of inhibition zone was noted in cm.

2.3.6. Statistical Analysis

All the experiments were repeated three times in parallel and the results were expressed as mean value \pm standard deviation. Significant values of edible coating was evaluated using graph pad prism software by means of two-way analysis of variance (ANOVA). The significant values (P value) were included.

3. Result and discussion

3.1. Characterization of edible film

The dried films were peeled off and the visual appearance of film was transparent, flexible and odorless. Film thickness, MC, solubility, Mechanical and barrier properties were employed to the check the suitability of the film for food applications.

3.1.1. Film thickness

Film thickness is one of the key tool to determine the mechanical ability of the edible film. The obtained film thickness for A1, A2 and A3 films were 0.17 ± 0.01 μm , 0.18 ± 0.02 and 0.19 ± 0.01 μm respectively as shown in Figure 1a. The homogeneity of the films increased with the solvent evaporation time. Small difference in film thickness was due to solvent evaporation time, density of both polymer networks and plasticizer. The addition of plasticizer to an edible film formulation can result in thickening of the film because the plasticizer molecule can occupy a cavity in the polymer matrix and interact with the film-forming polymer, causing more space between the polymers (Ekrami, 2014). The higher the plasticizer, greater the swelling as a result, the films were thicker. Compared to A1 and A2, A3 has high film thickness. The acceptable thickness of edible film from the literature is ~ 0.1 to 0.25 mm; hence the prepared films were within the range.

3.1.2. Moisture content (MC)

The moisture content of the film was measured to estimate the water binding capacity of the film. MC is significant in edible film since it

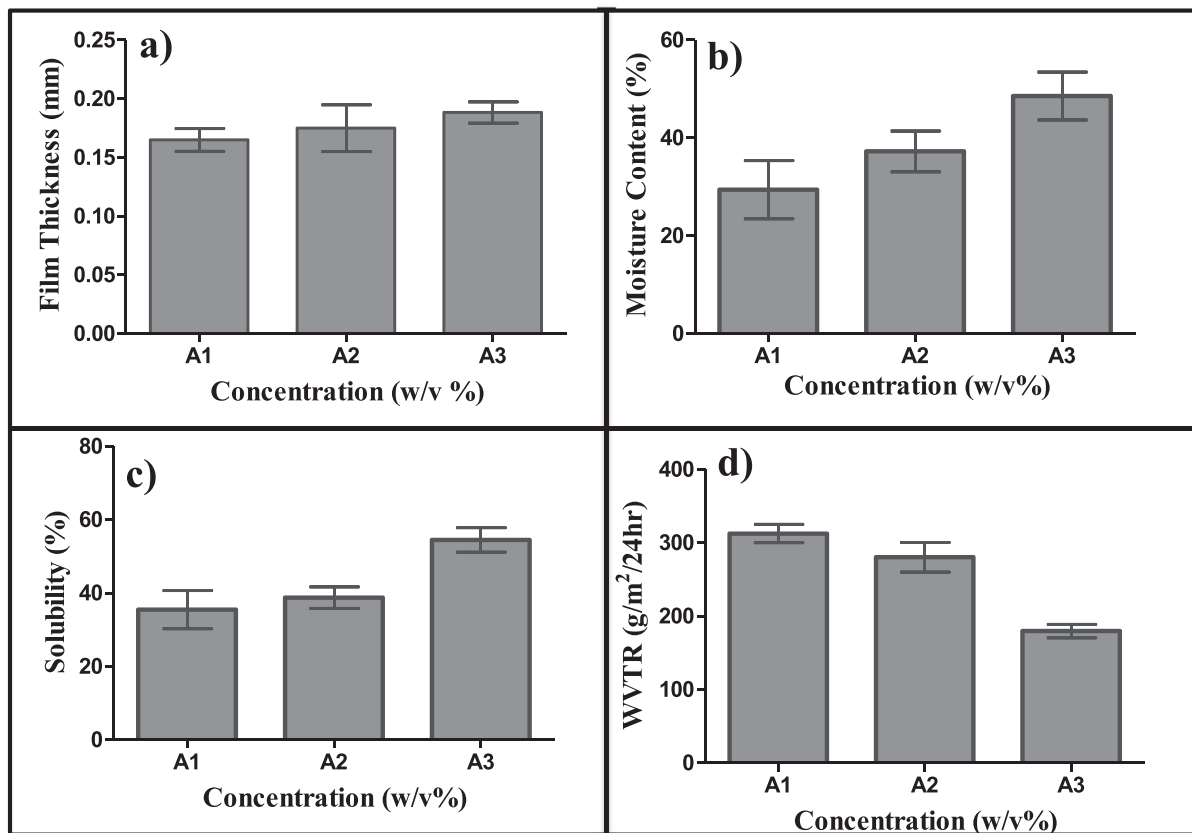


Figure 1. a) Film thickness, b) Moisture Content, c) Solubility and d) WVTR analysis of AG/AL edible film

affects the film's stability. MC of A1, A2 and A3 were 29.41 ± 4.16 %, 37.25 ± 4.16 % and 48.58 ± 4.90 % respectively as shown in Figure 1b. A3 film shows high moisture content due to hygroscopic nature of biopolymer and hydrophilic nature of plasticizer. The addition of plasticizer affects the moisture sorption. The reduced similarity of glucose units in the molecular structure of the plasticizers caused a weak molecular connection between the plasticizer and the intermolecular of biopolymer chains, which was linked to the difference in water retention of plasticized films. As a result, the plasticizer's chances of interacting with water molecules increased (Adhikari, 2010). Thus, the film having higher plasticizers show high moisture content due to the presence of almond gum and swelling ability of plasticizer as they absorb moisture. In addition, plasticizer might interact with water and disturb the polymer network because glycerol and sorbitol are the most basic glyceride chemicals, having a hydrophilic and hygroscopic hydroxyl group that makes it easy to attach to water. Thus, moisture content was increased with increasing plasticizer.

3.1.3. Film Solubility

Solubility of the edible film depends on agitation, temperature and solute/solvent ratios. The solubility of the prepared film was 35.50 ± 5.17 %, 38.75 ± 2.94 % and 54.50 ± 3.35 % for A1, A2 and A3 film respectively. There were significant ($P < 0.05$) increase from 35% to 54% with increasing plasticizer concentration as shown in Figure 1c. The film (A1) with 0.2% of 1:1 ratio of glycerol and sorbitol as plasticizer has low solubility than other two films. This indicates that increase in solubility might be due to the increase in plasticizer concentration from A1 to A3. It is possible that the higher the plasticizer content, the more plasticizer molecules that might be not accommodated in the cross-linked network and can escape into solution, and vice versa, hence, solubility increased with plasticizer content (Nogueira, 2021). In addition, gly-

cerol is present in all films, and its plasticizing impact may interact with water, disrupting the film network with hydrogen bonds, lowering the cohesiveness of the polymer matrix and increasing water solubility. Plasticizers diminish interactions between polymer molecules, resulting in more free space in the polymer chain. This, in turn, allows water fragments to penetrate the film matrix, increasing the plasticized film's solubility. (Razavi, 2015), also discovered that increasing the volume percentage of plasticizers (glycerol or sorbitol) in sage seed gum films from 40% to 100% resulted in increase in the water solubility.

3.1.4. Barrier Properties -Water vapor transmission rate (WVTR)

The result of WVTR significantly ($P < 0.05$) affect the AG/AL edible film. The obtained WVTR results for A1, A2 and A3 films were 212.66 ± 12.54 g/m²/24 hr, 179.33 ± 20.14 g/m²/24 hr and 160.66 ± 9.08 g/m²/24 hr as shown in Figure 1d. All three films have high WVTR due to the influence of almond gum concentration. Though, the highest value of WVTR was observed in A1 film (212 ± 12.5) due to higher concentration of almond gum since it has high moisture retaining ability, hydrophilicity and cooling property. When plasticizer is increased. WVTR of the sample was decreased as shown by (Urbizo-Reyes, 2020) who reported his finding on chia seed mucilage edible film with polyol mixture.

3.1.5. Mechanical properties

Tensile strength and elongation at break: Mechanical property is one of the essential parameter for edible film to improve the quality and structure. These measures demonstrate the film's ability to withstand a variety of forces encountered during the preparation, handling and storage of packaged food while retaining its integrity. The result of tensile strength and elongation at break was 3.16 ± 0.10 Mpa, 2.16 ± 0.10 Mpa and 1.90 ± 0.18 Mpa for A1, A2 and A3 films. Tensile strength was low

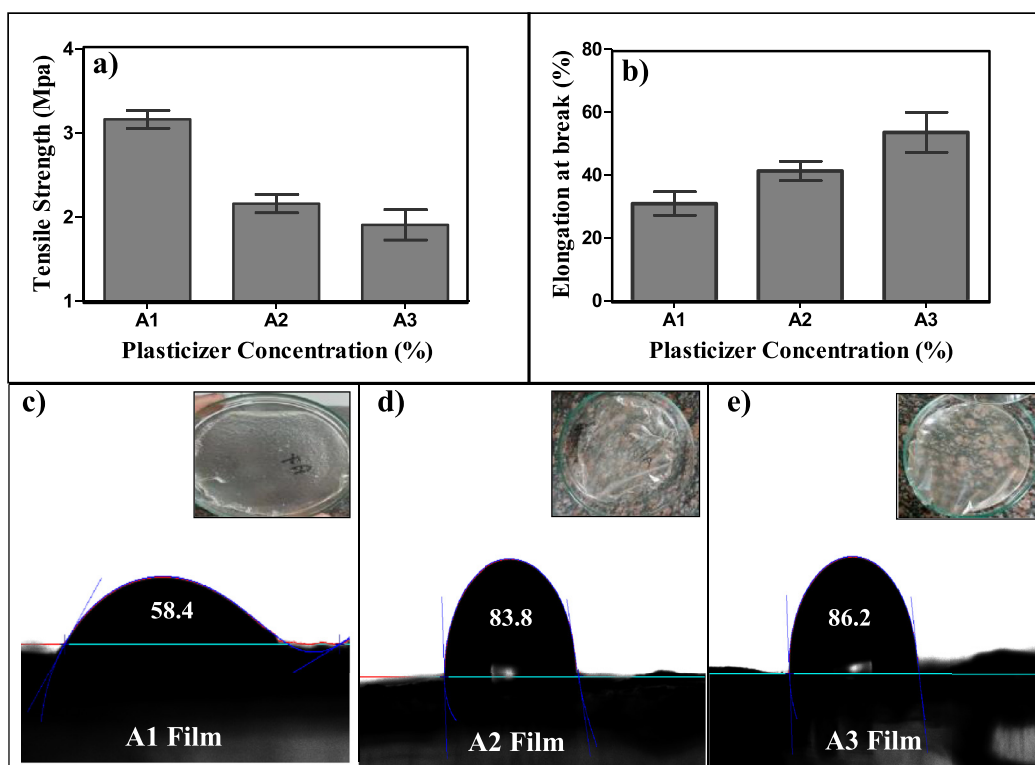


Figure 2. a) Tensile strength, b) elongation at break and c), d), e) were water contact angle of A1, A2 and A3 film.

in A3 film due to high content of plasticizer. This high plasticizer content makes the film brittle and stiff than A1 and A2 film (shown in figure 2a). This behavior can be explained by the fact of anti-plasticizing effect. The elongation at break results shows that the film having higher plasticizer has high elongation value which leads to increase in the flexibility to the film. The obtained elongation for A1, A2 and A3 was $30.93\% \pm 3.79$, $41.30\% \pm 3.04$ and $53.60\% \pm 6.35$ (shown in figure 2b). It suggests that edible film with a higher plasticizer concentration may be more stretchable. Similarly, (Dick, 2016) found that adding glycerol to CMS (Chia flour: Maize Starch) edible film, increased the elongation and water permeability while lowering tensile strength.

3.1.6. Water contact angle

The water contact angle was measured to predict the hydrophobicity of the film surface by measuring the wetting of the solid by liquid quantitatively. The measured water contact angles were $58.4^\circ \pm 3.2$, $83.8^\circ \pm 1.7$ and $86.2^\circ \pm 3.2$ for A1, A2 and A3 films (Figure 2c, 2d and 2e). Absorption, swelling, spreading and evaporation are known to be the key driving forces influencing drop evolution on biopolymer surfaces. It is well known that $\theta < 60$ is hydrophilic and $\theta > 60$ is hydrophobic. Therefore, this result indicates that A2 and A3 film have good hydrophobicity than A1 film. In contrast to moisture and solubility, contact angle shows higher hydrophobicity in A2 and A3 films which is due to crosslinking formed during the heat treatment that played a substantial role in lowering the hydrophilic properties of the polymeric surface. Similar results found in pectin and brea gum film showed contrast result to moisture and solubility because the hydrophilic groups present in pectin are orientated towards the inside of the film, thus it shows slight hydrophobic surface (Slavutsky, 2018).

3.1.7. FT-IR Analysis of edible film

FT-IR analysis was carried out to determine the structural modification and functional group present in 3 different ratios of prepared edible film with a control. In all 4 peaks, the intense band at 3230 cm^{-1} to 3350 cm^{-1} is responsible for abundant intermolecular hydrogen bonding due

to stretching vibration of -OH in the polysaccharides and plasticizer (glycerol and sorbitol). The Peak observed at 1635.64 cm^{-1} is due to the asymmetric vibration of -COO group in the sodium alginate. In control (AG/AL composite solution without plasticizer) the -OH peaks is observed at 3340 cm^{-1} whereas in A1, A2 and A3 films the peaks were shifted to lower wavelength (3294 cm^{-1} , 3271 cm^{-1} , 3348 cm^{-1} for A1, A2 and A3 films respectively). This is due to the polymer-plasticizer interaction. The Peak observed at 1635.64 is due to the asymmetric vibration of -COO group in the sodium alginate. Peaks at 1265 cm^{-1} indicating the presence of -CO group which is shifted to higher wavelength 1280 cm^{-1} , 1273 cm^{-1} , 1269 cm^{-1} . Peaks under 700 cm^{-1} to 400 cm^{-1} was not significant. Therefore, from the peak at 3230 cm^{-1} to 3350 cm^{-1} it proves the presence of polymer-plasticizer interaction (Shown in Figure 3). Expected spectra for alcohols were obtained for plasticizers alone, with major signals corresponding to the O-H bond at $3000\text{-}3500\text{ cm}^{-1}$ (Murrieta-Martínez, 2019). In general, most of the edible films have certain functional group which are -OH , -CH , -CO and -COO which reflected in the present work as well (Darni, 2017). Therefore, according to the results of the FTIR study, there do not appear to be alterations in a functional group as a result of changes in plasticizer concentration which is essential for keeping the properties of biopolymer in the edible film.

3.1.8. Thermo gravimetric analysis (TGA)

Thermal stability is an important criterion for edible film in packaging application. The result of TGA demonstrate that all the film exhibit similar WL with three major WL stage (as shown in Figure 4). WL around 100°C might be due to the loss of excess absorption of water present in all the film. The amount of weight loss at 100°C for A1, A2 and A3 films were 1.473%, 0.975% and 1.478% respectively. Major WL was observed in second stage for all films. In that, A1 film has WL of 3.767% (195°C), A2 film has WL of 2.715% (217°C) and A3 has 2.025% (192°C). This is due to the dehydration of carbon containing compounds present in almond gum. The second stage of WL is also due to the dehydration of glycerol in the range of 190°C to 250°C . Third stage of WL for all films

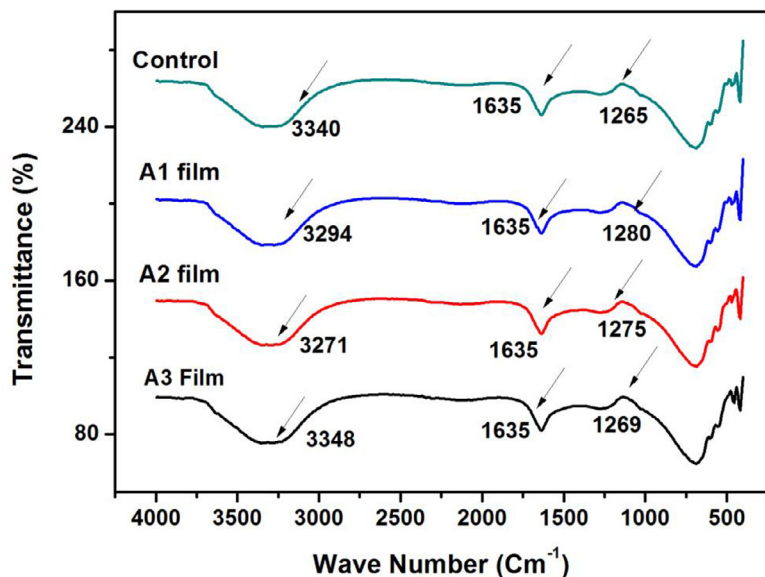


Figure 3. FT-IR analysis of AG/AL edible film with different glycerol:sorbitol ratio.

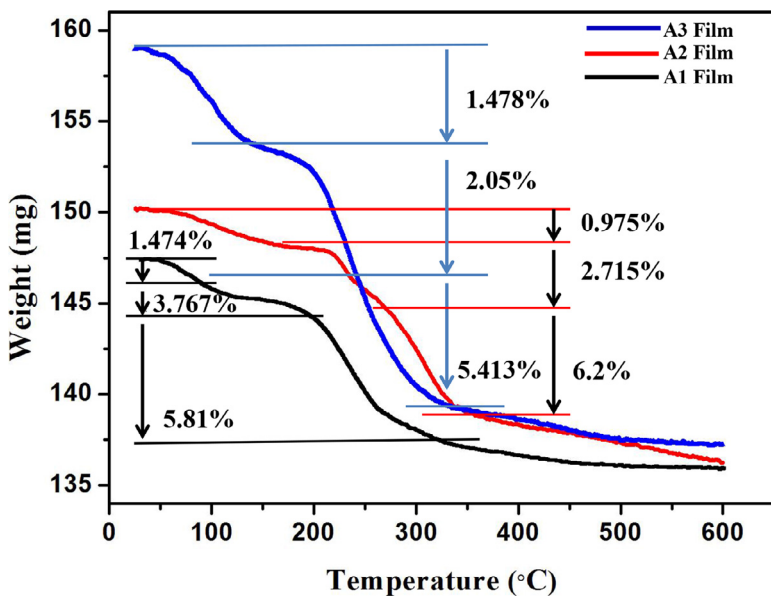


Figure 4. TGA analysis of AG/AL edible film with 3 different ratios of polyol Concentration

were 5.81%, 5.413% and 6.2% around 350°C to 400°C. This is due to the decomposition of crosslinked bonds present in both alginate and almond gum. Therefore, the TGA results demonstrate that all AG/AL films exhibit same thermal stability about 100°C and this is more enough for an edible film to be wrapped on food surface (Salama, 2018).

3.1.9. Morphological Analysis using SEM

Scanning electron microscopy is used to identify the structural morphology of edible film. Control (Figure 5a) shows that the AG/AL composite forms aggregates due to the immisible nature of almond gum and alginate. This will be homogenized by the addition of plasticizer. Compare to control A1, A2 and A3 films shows smooth and even surface indicating that the plasticizer can interact with polymer matrix and produce homegenous surface. Yet, A3 film alone shows highly even and smooth surface. In that, internal hydrogen bonding was reduced while intermolecular space was increased, reducing brittleness, enhancing permeability and flexibility of the material. This is because glycerol and sorbitol are mixed together evenly. In addition, connecting the glycerol:sorbitol mixture with almond gum and alginate composite in a network of hydrogen bonds molecular chains, make the film more flexible,

soft, supple and transparent. Similar research finding was observed by (Pak, 2020) with increasing the concentration of glycerol to more than 35% (w/w) caused softening and stickiness in SPG films by providing sufficient molecular ability and intermolecular spacing between polymer chains gives more flexibility to the film.

3.2. Analysis of coated tomato for optimization of coating formulation

Based on the AG/AL edible film results, A1 film has less moisture, less solubility and high water vapor permeability with lower amount of plasticizer. Hence, in this research work A1 film forming solution was used as edible coating solution to enhance the shelf-life of tomatoes. The reason for choosing tomatoes is: tomatoes are perishable in nature, with thin skin, and is a mandatory element for preparation of indianized recipes and easily ripen at room temperature. The overall visual appearance of tomatoes for both coated and control tomatoes is illustrated in Figure 6. In control tomatoes, bacterial infection was noted in the top of the tomatoes due to the surrounding environmental condition and contamination. Furthermore, control tomatoes started to ripen in day 5 itself and 80% of tomatoes decayed in day 25 to 27. Nevertheless, in

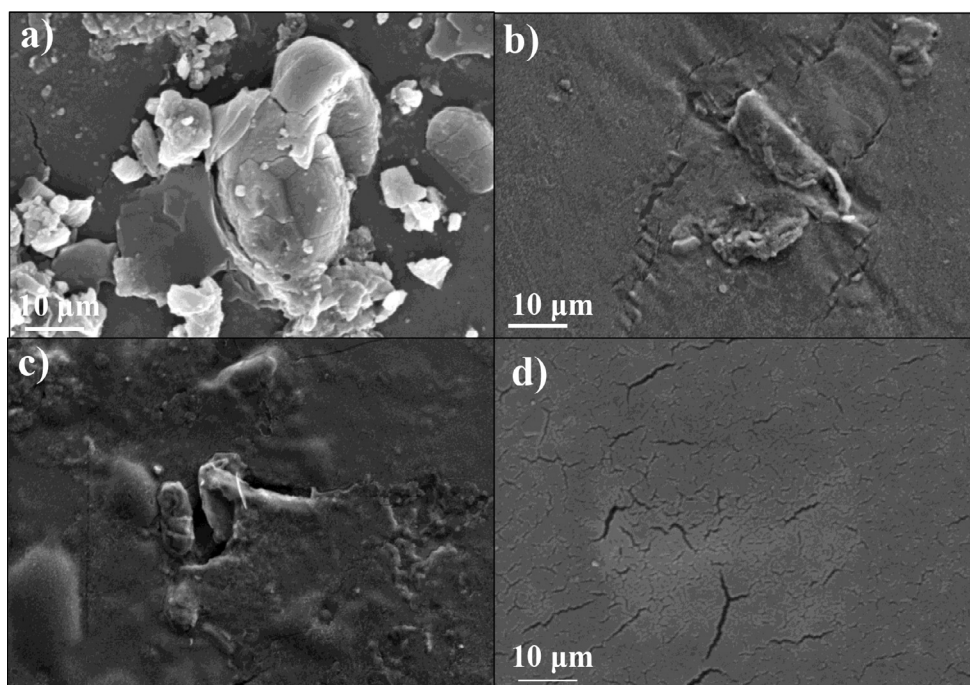


Figure 5. SEM image of a) AG/AL composite without plasticizer, b) A1 film, c) A2 film and d) A3 film

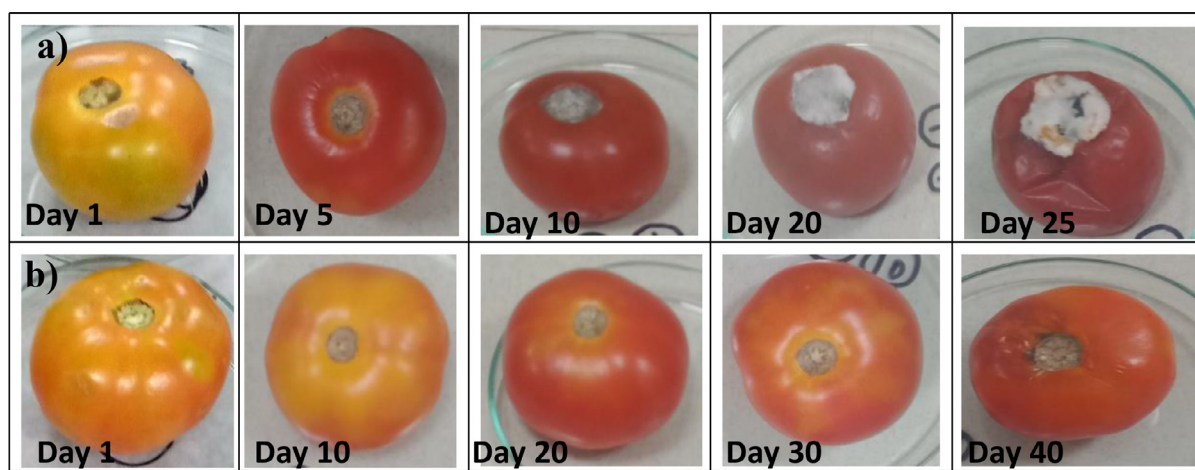


Figure 6. Visual appearance of a) control tomatoes at day 1,5,10,20, 25 and b) coated tomatoes at day 1,10,20,30 and 40.

case of coated tomatoes no such bacterial infection was viewed by naked eye and also 80% of tomatoes did not decay until day 38 to 40.

3.2.1. Weight Loss (WL)

The WL of coated and uncoated tomatoes as function of storage time and treatments are presented in Figure 7a. WL during respiration is primarily due to transpiration and the loss of carbon atom from the fruit in each cycle of respiration. In coated vegetable, it can be noted the weight decreased gradually for all tomatoes during the storage time of 40 days while control samples unevenly lost the weight day by day. The control tomatoes lose 20% of its weight in the day 12 itself whereas coated withstand its 20% WL up to day 35. The control vegetables started to decay from the day 25 itself while coated tomatoes maintain its rigidity, hardness and more than 70% of the tomatoes did not decay. This could be attributed to the hygroscopic nature of alginate and presence of plasticizer as combination of glycerol and sorbitol. It produces barrier to the vegetable surface and prevent the tomatoes from water loss. The alginate based coating with essential oil on fresh cut papaya study findings revealed that substantial ($P < 0.05$) impact on weight loss was

observed in different atmospheric condition (Tabassum, 2020). Similar results were found in (Ruelas-Chacon, 2017) who reported the effect of guar gum edible coating on postharvested tomatoes and shows the delay in weight loss over 15 days. Similarly, the AG/AL coating would reduce the WL during storage period of 40 days and also the addition of small quantity of glycerol and sorbitol as a plasticizer helps to reduce WL. All coated samples lost their weights almost uniformly throughout the storage period.

3.2.2. Titratable Acidity (TA)

In TA, there was gradual decrease in titratable acidity for coated and control tomatoes. Compared to control, coated tomatoes have less decreased TA during storage time. This indicates that coating would protect the rapid decrease in TA during storage hence it evident that coating can retard the respiration rate. For coated tomatoes TA decreased from 0.469% to 0.17% during storage period of 40 days and for uncoated tomatoes, TA decreased from 0.448% to 0.129% (in 25 days) which is attributed to the increase in ethylene production and decrease in respiration rate during the ripening stage of tomatoes (shown in Figure 7b).

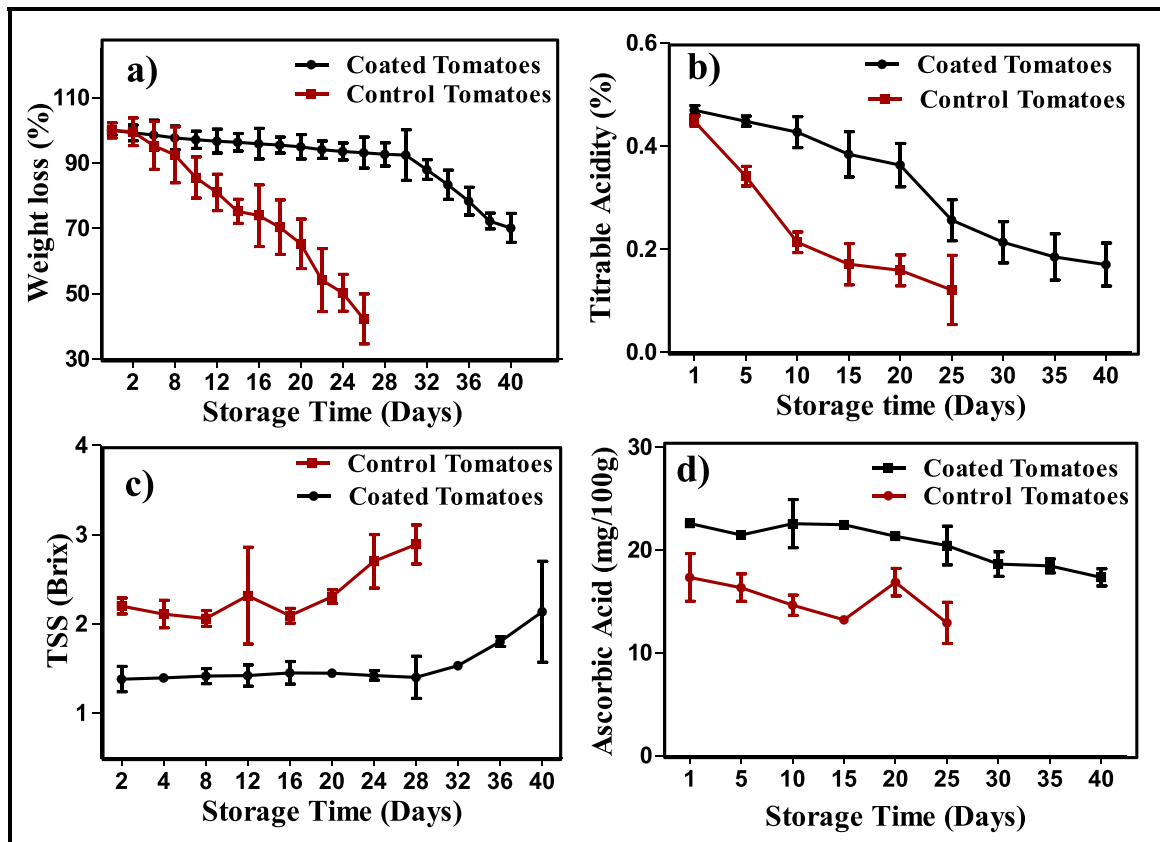


Figure 7. Variation in a) Weight loss, b) titratable acidity, c) TSS and d) Ascorbic acid content of coated and control tomatoes

The coated tomatoes show overall decrease of 63% up to 40 days while control tomatoes show 71% of drop in TA in the day 25 itself. Therefore, it can be clearly declared that compared to coated sample, loss in acidity was significantly high in control due to the early stage ripening. Therefore, it is evident that effect of AG/AL coating prevent the tomatoes from respiration and delay the ripening process which is accordance with the result of (Sree, 2020; Zhu, 2019) who reported decrease in the titratable acidity of tomatoes about 12 days with maximum of TA of 26.3% and 28.1% compare to control (30.3% at day 9).

3.2.3. Total Soluble Solids (TSS)

In general, during storage time and ripening process, the soluble solids concentration increases, mainly due to the breakdown of starch molecule into simple sugars (fructose and glucose) during ripening along with a proportional increase in TSS. In addition, sugars are the primary constituents of TSS of a product which are consumed during respiration. Since coatings formed a semi-permeable membrane around tomato, which decreases the ripening and the respiration rate by limiting the gas exchange (decrease of O₂ and increase of CO₂ levels in the tomatoes), thus the consumption of sugars will be delayed which delays the increase of soluble solids. Higher TSS may also be due to the water loss in tomatoes during storage which lead to higher concentration of sugars in tomatoes during storage. From the result of TSS (Figure 7c), it is clear that the control tomatoes (ranged from 2.2 to 2.89) have higher TSS value than the coated (ranged from 1.38 to 2.1) tomatoes. It reveals that the controlled tomatoes ripened early than coated tomatoes. Hence, this result demonstrate that coating can reduce the ripening of tomatoes by creating a semi-permeable membrane which serve as a barrier against external factors such as moisture, O₂ and CO₂ etc. Furthermore, according to (Khalil, 2020), the TSS % varied linearly with storage time, and untreated tomatoes had greater TSS values at each stage than tomatoes coated with glycerol, starch, and gelatin.

3.2.4. Ascorbic acid content (AAC)

The result of AAC shows that there was no notable difference in ascorbic acid up to day 3 but from day 5 there is a difference between coated and uncoated tomatoes. It is well known that the ascorbic acid or Vitamin C content will decrease with increasing storage time. The amount of ascorbic acid in tomatoes increases with maturity and ripening; however, once the fruit is fully ripe, the amount of ascorbic acid begins to diminish. The slower ascorbic acid content increase in coated vegetables show that the coating delay the decrease in AAC but does not preclude ascorbic acid synthesis during ripening. Figure 7d shows that the control tomatoes decrease its AAC from day 5 to day 25 and a sudden increase is noted on day 15 due to leakage of juice, cell decay and water loss in selected tomato which could increase the sugar content in tomato and make it more ripen, leading to ups and downs in ascorbic acid content from the tomatoes, while in coated tomatoes, same ascorbic acid content was averagely maintained up to day 20 then it was diminished gradually up to day 40. Hence it proves that compared to control, coating delay the decrease in ascorbic content with increase in storage time even at ambient temperature (23±2°C) but it did not prevent the synthesis of ascorbic acid during storage time. In controlled tomatoes the ascorbic acid value would decrease from day 4 itself and it was decreased randomly. Similar results were reported by Tigist *et al.* (2013) who found a general trend of increase in ascorbic acid content followed by decreased during full ripening stage (Tigist, 2013).

3.1.9. Anti-microbial activity test

Antibacterial activity was performed for selected test organism (*E.coli*, *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*). The results show a clear zone around the well which indicate that the AG/AL coating has enhanced antimicrobial activity against all the test organism. This could be due to the presence of sodium alginate since it has anti-microbial property (Figure 8). In this study, it was noted that

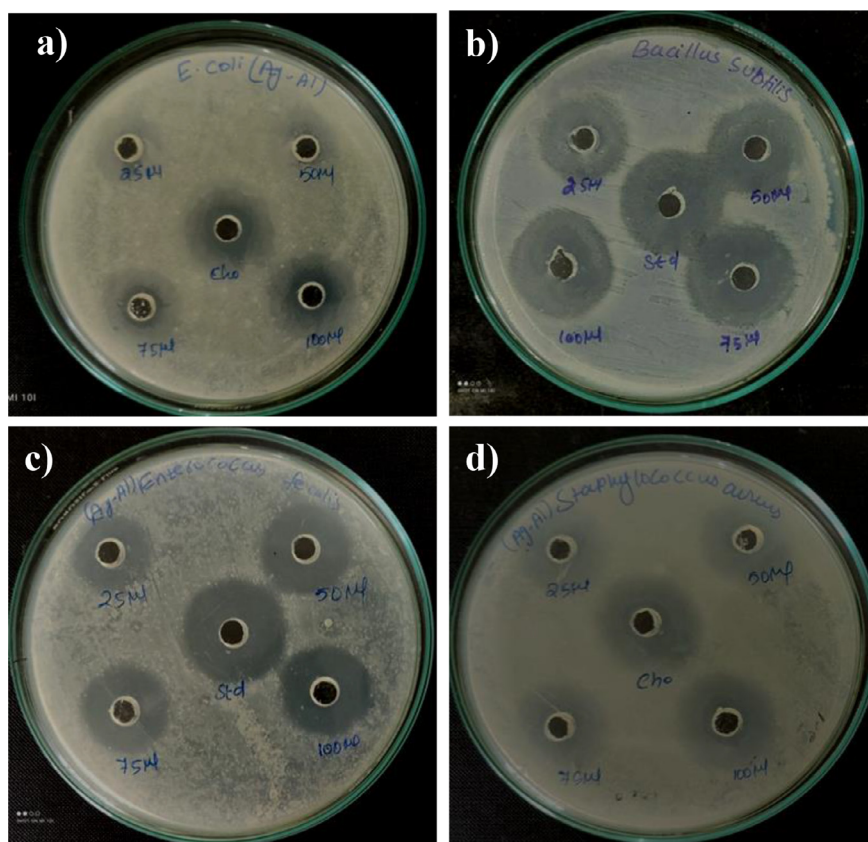


Figure 8. Antimicrobial activity of AG/AL edible film against a) *E.coli*, b) *Bacillus subtilis*, c) *Enterococcus faecalis* and d) *Staphylococcus aureus*

Table 2
Diameter zone of inhibition by edible coating for food pathogens.

Organisms	E.Coli(-) (cm)	Bacillus subtilis(+)(cm)	Staphylococcus aureus(+)(cm)	Enterococcus faecalis(+)(cm)
25 µl	0.32± 0.02	0.5± 0.04	0.2± 0.06	0.3± 0.04
50 µl	0.5± 0.01	0.7± 0.03	0.4± 0.04	0.5± 0.05
75 µl	0.7 ± 0.02	1.0± 0.02	0.7± 0.05	0.8± 0.04
100 µl	0.9± 0.02	1.2 ± 0.05	1.1± 0.03	1.0± 0.02
Standard (Chloramphenicol)	0.9± 0.03	1.2 ± 0.02	1.3± 0.02	1.2± 0.03

the inhibition zone was increased depending upon the concentration (Table 2). Hence, it proved that, due to the high antimicrobial activity, the coating solution can withstand for 40 days even at ambient temperature ($23\pm 2^{\circ}\text{C}$). Hence, this result confirmed that the prepared coating solution has microbial stability and can be used to improve the shelf life of fruits and vegetables.

4. Conclusion

Almond gum is an attractive natural gum having lots of medicinal values. The fabricated almond gum and alginate biopolymer based film has good film thickness, solubility, flexibility and is odorless. A3 films shows better flexibility than other two films due to presence of higher plasticizer and lower amount of almond gum. The WVTR results shows that all the film has higher moisture absorbing property though A3 film has less WVTR than A1 and A2 films. The effect of AG/AL coating on post-harvest tomatoes has remarkable delay in weight loss over 40 days. TA, TSS and AAC results prove that the coating significantly ($P < 0.05$) delay the ripening of tomatoes compared to control tomatoes. The antimicrobial activity test shows that the AG/AL effectively prevent the tomatoes from microbial contamination by *E.coli*, *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*. The prepared AG/AL composite film were good in flexibility, solubility and biodegradability, however barrier properties need to be improved. Thus, future research needs to

be focused on enhancing the barrier properties which helps to make this coating formulation for commercial purpose.

5. Author Contributions

Data collection, interpretation, drafting the article was done by SN. CP co-wrote and helped for the data analysis. RS designed the study, co-wrote, supervised and approved for the submission.

Declaration of Competing Interest

There are no conflicts to declare.

Data availability

Data will be made available on request.

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