1. Introduction

Environmental concerns have motivated the use of renewable and biodegradable resources for polymer synthesis. Major problems with petroleum-based non-biodegradable polymers are their disposal and non-renewable characters. Developments of biodegradable and edible polymers are alternative solutions to these problems (Ali, 2010). Recently, there has been great attention, towards studies and applications of edible polymers; they are renewable too. Edible polymers are alternative solutions to these problems (Ali, 2010). Most of the common edible film-forming materials can be classified as either polysaccharides (e.g., agar, alginate, carrageenan, cellulose and their derivatives, chitin, chitosan, pectin, and starch) or proteins (e.g., collagen, gelatin, keratin, whey, casein, zein, wheat gluten, and soy protein), (Valdés et al., 2015). In addition, additives such as crosslinker, plasticizer, antimicrobial agent, antioxidant, etc. are also used to enhance film properties (Zheng and Zhang, 2012). Solvents for edible films synthesis are generally ethanol, water, or a mixture of both. Proteins and polysaccharide-based edible films have good mechanical and barrier properties for gaseous exchange (Menezes and Athmaselvi, 2016). Polysaccharides such as sodium alginate and pectin also show potential in production of edible polymer film. Sodium alginate, a copolymer consisting of D-mannuronic and L-guluronic acid monomers, has been known as a pH and electric field-responsive polymer. It has non-toxic and unique colloidal properties such as suspension forming, gel producing, film forming and as an emulsion stabilizer. Alginate form transparent, uniform and good oxygen barriers but it has poor water resistivity due to hydrophilic nature. Alginates are extracted from various species of brown seaweeds, belong to Phaeophyceae class (Costa et al., 2018). On the other hand, main source of pectin are citrus fruits (Thakur et al., 1997). Pectin is mainly composed of linear homogalactururonan (-1,4-galacturonic acids) chains interspersed with branched rhamnogalacturonan (-1,4-galacturonic acid to -1,2-rhamnose) chains. In rhamnogalacturonan, neutral sugar branches are attached to rhamnose residues (Kang et al., 2007). Pectin is usually used as stabilizers and gelling agent in the food industry for making dairy products, jams, and jellies (Kang et al., 2007). However, it has been shown potentially as a serum hemostatic agent, cholesterol-lowering, a demulcent, and a compound preventing...
spontaneous cancer metastasis (Liu et al., 2010). Alginate and pectin, both belong to the polyuronates group, and are typical examples of natural ionic polysaccharides undergoing chain–chain association and forming hydrogels on addition of divalent cations e.g. Ca\(^{2+}\) (Galus and Lenart, 2013). Calcium crosslinked with pectin films or other multivalent cations exhibit fair mechanical properties. The plasticized pectin/starch films appear to be appropriate for use in industrial application. In recent years, several works show that the ionic linkage of calcium with alginate is crosslinked type and these types of bonding in alginate-based films lead to improvement in mechanical strength and increase the structure cohesion with low solubility in water (Costa et al., 2018; Russo et al., 2007).

In continuation with our previous research work we have explored the possibility of using alginate and pectin as renewable resources to prepare crosslinked film with biobased acids through covalent bond. To author’s knowledge there is no report in literature about the biobased crosslinked polymeric films derived from alginate and pectin based material with additional advantages of improved thermal and food packing material properties. Present work is focused on utilization of agricultural waste material as renewable source to obtain crosslinked polymeric films suitable for food packing and having edible properties. These films can be consumed by lower animals thereby solving the plastic disposal problem and saving the earth.

2. Experimental

2.1. Materials

All laboratory grade chemicals were procured either from SD fine chemicals, HiMedia or Spectrochem, India and used as such without further purification. Sodium alginate was purchased from Himedia labs Mumbai. Waste pineapple shell was purchased from the local market of Nanded, Maharashtra.

2.2. Methods

2.2.1. Pectin extraction

Waste pineapple shell was blanched with boiling water for 5 min and dried overnight at 60 °C. The final moisture content of the resulting mass was 10 % (dry basis) approximately. It was then kept in tightly closed plastic bag until used. Pectin was extracted from dried pineapple shell as reported in an earlier method (Ukiwe and Alinnor, 2011) using inorganic acid HNO\(_3\) (Ukiwe and Alinnor, 2011).

2.2.2. Preparation of crosslinked films

Crosslinking reaction between sodium alginate and citric or tartaric acid was performed as follows. A specified amount of CA/TA and the catalyst sodium hypophosphite (20% w/w, on weight of CA/TA acid used) and glycerol (40 % v/w sodium alginate) was dissolved in water. The entire reaction mixture was heated between 75 to 80 °C, held at that temperature for 40 min then cooled to 50 °C and poured onto silicone coated glass plates. The cast films were allowed to air dry for 30 h and later shelled from the plates. The free standing films were stored in desiccators. Pectin extracted from pineapple was also crosslinked with CA/TA acid in the same way. To enhance antimicrobial activities 0.1 % (w/w of polymeric solution) turmeric was added in viscous polymeric solutions of SACA/SATA and PCA/PTA (sodium alginate crosslinked with citric acid/tartaric acid and pectin crosslinked with citric acid/tartaric acid) respectively before pouring onto silicon-coated glass plates and were named SACA-T, SATA-T, PCA-T, and PTA-T.

2.3. Characterization

2.3.1. Fourier transform infrared spectroscopy (FT-IR)

The Fourier transform infrared (FT-IR) spectra of crosslinked polymer (crushed) were recorded on Shimadzu spectrophotometer (Shimadzu, Japan, Model No. 8400) from the range of 4000-500 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\).

2.3.2. Resistance to the chemical factors test

The resistance to chemical factor test of a) SACA, b) SATA, c) PCA, and d) PTA was studied using thin film in range of different proct as well as aprotic solvents such as sodium hydroxide (1 M), sulfuric acid (1 M), xylene, castor oil, and dimethyl sulphoxide respectively. Small pieces of all cured samples were kept in 50 ml amber glass bottles for 7 days at 30 °C and the resistance to chemical factor was monitored by the difference in their weights with the interval of every 24 h.

2.3.3. Thermogravimetric analysis (TGA)

The TGA measurements of crosslinked polymer were conducted using Perkin Elmer TGA 4000 under nitrogen atmosphere over a temperature range 30–800 °C, with a heating rate of 10 °C/min and, degradation temperatures such as Tonset (initial degradation temperature), T\(_{25}\) (temperature of 5 % weight loss), T\(_{30}\) (temperature of 30 % weight loss), char temperature and IPDT were determined from TGA thermograms.

2.3.4. Scanning electron microscopy (SEM)

Morphology of film surfaces was monitored using SEM (SEM Hitachi S-4200). The accelerated voltage was 15 kV. The surfaces of the films were coated with gold under vacuum before observation.

2.3.5. Antibacterial activity

Polysaccharides (alginate and pectin) are susceptible to bacterial infection hence the crosslinked film needs addition of 0.1 % turmeric powder to the film to improve its antibacterial activity. Two bacterial strains viz. Staphylococcus aureus (NCIM 2079) and Escherichia coli (NCIM 2065) were procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune. Bacterial cultures were grown at 37 °C for 24 h on sterile nutrient agar plates. 24 hold pure colonies of bacteria were used to prepare inoculums in Nutrient Broth and these were incubated at 37 °C on shaking incubator (100 rpm) for 24 h. The cell densities of each inoculum were adjusted in culture medium at 0.5 McFarland standards. The bacterial suspension (0.1 ml) was spread on Nutrient agar plate and wells were prepared with sterile cork-borer. Thin crosslinked polymers were crushed in mortar and pestle until fine powder and it was dissolved in DMSO (100 μg/ml) (Vásquez et al., 2009). The test samples (100 μl) were added into the wells and plates were kept at 4 °C for 10 min to diffuse the sample in agar medium. A standard solution of antibacterial agent (Gentamicin, 100 μg/ml) was taken as positive control and DMSO as negative control. The plates were kept for incubation at 37 °C for 24 h and zone of bacterial inhibition around the wells were measured in cm/mm.

2.3.6. In vivo and in vitro testing of crosslink polymer

2.3.6.1. Mice selection as a mammalian model

Three weeks old female Mus musculus mice were used after a month of the quarantine period. Animals were housed in different cages as two mice per cage and cages were changed twice in a week. Water was provided continuously. All mice were fed standard rodent food. Survival, growth, and metabolism were monitored. Faecal sample was collected and faecal flora examined (Freece et al., 2005; Salezman et al., 2002).

2.3.6.2. Mice feeding and faecal sampling

Crosslinked polymer was in semisolid form, given to mice along with rodent food for two weeks continuously for one cage and another cage was kept as control. After two weeks of feeding, faecal samples were collected from both cages separately and were preceded for comparative microbial detection. Faecal sample (1g) from each cage was homogenized in sterile saline solution and serial dilutions were prepared. 0.1 ml of each dilution was spread on nutrient agar plates separately and the plates were incubated at 37 °C for 24 h. To study the effect of polymer on normal flora of mice gut, bacteria
were isolated from faecal sample of both test and control mice. Isolated bacteria were identified by biochemical characteristics and Bergeys manual of systemic bacteriology. Seven isolated bacteria were used to test activity of polymer by agar well diffusion method. Active culture of isolated bacteria was spread on media in petri plate and wells were prepared by cork borer. 50 μl of polymer solution were added in test well while streptomycin (50 μg/ml) was used as positive control and distilled water as negative control. After diffusion at 4 °C, agar plate was kept in incubator for incubation at 37 °C for 24 h. Comparative analysis was carried out in test and control for faecal samples. Observations of mice physical appearance, behavior, coat colour, hair, teeth, nails and other side effects were compared with control animal. All tests were performed in triplicates to avoid experimental error.

2.3.7. Crosslinked polymer as plant growth substrate

Crosslinked polymer as plant growth substrate was tested using Bengal gram bean (Cicer aritinum) also known as chickpea. Bengal gram bean seed germination was studied in SACA and PCA polymer film and the results were compared with soil as control (Pawar et al., 2016).

2.3.8. Biodegradation study by vermicompost

Vermicompost was prepared by a conventional layer method earlier reported by Mahalakshmi M. et al. with slight modifications (Science, 2017). Earthworms used in vermicompost belonged to species Eudrilus eugeniae.

3. Results and discussion

Sodium alginates are the natural well-known ionic polysaccharides mostly used as food thickener, food additive, gelling agent, and also in control drug delivery (Gomboz, 2002; Gómez-Díaz and Navaza, 2004). The polysaccharide pectin mostly is used for making of jam, jelly, etc. due to its gelling and thickening property (Padmaja et al., 2015). In this research both polysaccharides; sodium alginate and pectin were used for making crosslinked polymer films. Biobased acids like citric and tartaric acid are used for the crosslinking through covalent bonds. Citrus fruit is the main source of citric acid while tartaric acid was extracted from tamarind. Both, biobased acids are easily available, cost-effective, and greener crosslinking agents with antimicrobial and antioxidant properties. Thus, biobased acids used in this reaction improve thermal, mechanical and biological properties. Glycerol as a plasticizer has been used to ease film formation and to avoid stickiness.

Synthesized sodium alginate/pectin films were uniform and without pores and cracks. Although, the sodium alginate films were visually more clear as compared to the pectin film similar to Galus et al. work (Galus and Lenart, 2013). Synthesized crosslinked films are insoluble in most of the solvents because of the strong covalent bond formed between the polymer chains. Found on good product yield, and reaction conditions, sodium hypophosphite is suitable catalyst for this reaction. The general synthetic scheme of crosslinked polymer films is shown in Schemes 1 and 2.

Scheme 1. Synthesis scheme of cross-linking reaction between sodium alginate with citric acid (SACA) and tartaric acid (SATA).
3.1. Fourier transform infrared spectroscopy (FT-IR)

The FTIR spectra of crosslinked thin films show functional groups present in the polymer. The comparative FTIR spectra of crosslinked polymer films SACA and SATA are shown in (Figure 1).

The figure shows a broad band at 3460 cm\(^{-1}\) for stretching vibration of O–H bond for the samples SACA, SATA. The characteristic peaks at 1733 cm\(^{-1}\) in SACA and SATA polymer film are associated with carbonyl bands (carboxyl and ester) confirming the crosslinking reaction.

![Scheme 2. Synthesis scheme of cross-linking reaction between pectin with citric acid (PCA) and tartaric acid (PTA).](image)

![Figure 1. Comparative FT-IR spectra of SACA and SATA polymer film.](image)
3.2. Resistance to the chemical factors test

The chemical resistivity of crosslinked films a) SACA, b) SATA, c) PCA, d) PTA were tested under various protic and aprotic solvents like sodium hydroxide (1M), sulfuric acid (1M), xylene, castor oil, and dimethyl sulphoxide for 10 days at room temperature. The results have been tabulated in Table 1.

Crosslinked films are soluble in acidic and basic solutions which is suitable for animal and human digestion. Also, they are soluble in organic solvent DMSO and insoluble in rest solvents. This property will help to act as barrier for organic solvents so that food contamination can be avoided. After use of this film, we can dissolve them in acidic or alkaline solution and dispose them in liquid form.

3.3. Mechanical analysis

Mechanical properties of crosslinked films are directly associated with their composition. Earlier research reports explained the reason of high tensile strength and young's modulus values obtained for ionic crosslinked film of Ca²⁺ and alginate by formation of “egg-box” structure since it reduces free volume in structure (Costa et al., 2018). In current study, tensile strength and young's modulus of acid crosslinked (covalent bond) films show low mechanical strength compared to Ca²⁺ ion containing crosslinked alginate. Mechanical strength in CA crosslinked film is higher as compared to TA crosslinked film (Table 2). Mechanical properties of the CA and TA crosslinked films differ due to polymerization behavior. Reactivity of CA is more as compared to TA i.e. better crosslinking occurs with CA rather than TA.

3.4. Thermogravimetric analysis (TGA)

The thermal stability of polymer is greatly induced by structure, chemical composition diverse interaction parameters, and other chemical factors. Thermal stability of the crosslinked polymer was studied by using TGA instrument. Thermogravimetric analysis for crosslinked films is extremely important since the thermal properties may limit their processing temperature and application area where the atmosphere temperature might be the reason of thermal degradation. The comparative thermogravimetric curves have been presented in (Figure 2). The characteristic values calculated from thermogravimetric analysis curves for crosslinked films are given in Table 3. Crosslinking through covalent bonding enhances the thermal stability of films as compared to non-crosslinked or ionic crosslinked films. The crosslinked polymer undergoes single step degradation in nitrogen atmosphere with an onset thermal degradation temperature of approximately 99.8 °C and 99.9 °C in SACA and PCA respectively, a characteristic of the thermal stability of cured films. Temperatures at 5% weight loss (Td5) and 30% weight loss (Td30) of the sample is obtained by TGA. Integral procedure composition temperatures (IPDTs) proposed by Doyle were calculated (Doyle, 1961). PCA crosslinked film has more ipdt value than SACA film probably due to the pectin molecules present in the polymer. Both these polymer films have moderate thermal stability.

3.5. Scanning electron microscopy (SEM)

The surface morphology of crosslinked films was examined using SEM micrograph. Surface morphology of the film depends on the reactivity of dicarboxylic to form crosslinked film through ester linkages. Although both crosslinked films have shown a uniform phase. i.e. there is no phase separation found thereby indicating the polymer is in homogenous phase. The crosslinked SACA film has smooth surface while PCA film has little roughness. A surface micrograph of SACA and PCA film has been presented in (Supplementary Figure 1).

3.6. Antibacterial activity

One of the important applications of plastic film is wrapping of food to enhance the shelf life, control infection and avoid contamination. Antibacterial activity of polysaccharide films can be improved by adding 0.1% of turmeric powder in crosslinked film (SACA-T). SACA-T has been tested for antibacterial activity against common infectious bacterial Escherichia coli (NCIM 2501) and Staphylococcus aureus (ATCC 6538). SACA-T has 12 mm and 13 mm inhibition zone against Escherichia coli and Staphylococcus aureus respectively, shown in Figure 3. These results show that SACA-T crosslinked film has antibacterial properties. A crosslinked film with antibacterial properties has advantages in food wrapping to avoid the growth of bacteria over the food surface.

3.7. Mice feeding and faecal sampling

Edibility of food/material can be tested using the mice feed technique and analysis of faecal matter (Sung et al., 2017; Rodriguez et al., 2019). Type of food consumption of mice determines the microflora of gut, accordingly to their physiology. To obtain the best results one must isolate and characterize the gut microbiomes which is difficult due to lack of facilities and Government regulations to perform the test. In the

Table 1. Chemical resistivity data of crosslinked polymer films.

| Solvents      | SACA | SATA | PCA | PTA |
|---------------|------|------|-----|-----|
| Acetone       |      |      |     |     |
| 1 M NaOH      | +    | +    | +   | +   |
| 1 M H₂SO₄     | +    | +    | +   | +   |
| Xylene        | +    | +    | -   | -   |
| Castor oil    |      |      |     |     |
| Methanol      |      |      |     |     |
| Chloroform    |      |      |     |     |
| DMSO          | +    | +    | +   | +   |
| Distilled water | * # | *   |     |     |

Note: ‘+’ is Soluble, ‘-‘ is Insoluble and ‘#’ is Swelling.

Table 2. Mechanical analysis data of crosslinked films.

| Sample | Tensile strength (MPa) | Young's modulus (MPa) |
|--------|------------------------|-----------------------|
| SACA   | 18.38                  | 0.25                  |
| SATA   | 17.03                  | 0.21                  |
| PCA    | 17.20                  | 0.21                  |
| PTA    | 16.43                  | 0.19                  |

Figure 2. Comparative thermogravimetric curves of cured SACA and PCA polymer film.
present investigation, we have tested the edibility of sodium alginate and pectin crosslinked films by feeding to mice. We have limited our experiments to faecal analysis. The test sample, SACA-T shows antibacterial activity in the faecal microflora by decreasing the number of coliforms by ~ 1 log c.f.u. shown in Figure 4. There was negligible zone of inhibition seen in normal flora of mouse gut which is not affected by SACA polymer but some amount of bacteriostatic effect is seen in SACA-T (Supplementary Table 1). Physical fitness was confirmed by coat colour and physical appearance of the test animal was normal and similar to control animal.

Mice feed study results suggest that these polymeric films are safe for animal consumption. These crosslinked films after use can be fed to lower animals to avoid waste plastic problem.

### 3.8. Crosslinked polymer as plant growth substrate

Prepared films are substitutes for soil as plant growth substrate and have potential in greenhouse application. In addition, biobased material from renewable resources could be best environment-friendly i.e. nontoxic plant growth substrate. Yoshioka et al. reported the use of biodegradable polymer, water-absorbent polymers, polyvinyl alcohol-based films as plant growth substrate. They have cultivated and grown various vegetables in polyvinyl alcohol films as soilless cultivation method (Yoshioka et al., 2000). Similarly, in order to test the feasibility and suitability of crosslinked polymers as plant growth substrate we have tested them by growing the seed (Bengal gram bean) in crosslinked films (SATA and PCA). Seed germination was examined up to 16 days and healthy plant growth is observed in both (control and test samples). Thus, seed germination study reveals that crosslinked films can be alternative source as a plant growth substrate. Seed germination study is shown in Figure 5.

### 3.9. Biodegradation study by vermicompost

Biodegradation study of crosslinked film was done by conventional layer method. Biodegradation is revealed through changes and deterioration of key material properties (e.g. cracking, breakage and fragmentation). These primarily changes will express the polymer properties. Biodegradation by vermicompost method results revealed that there was cracking, breaking and holes on edible polymer and complete

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**Table 3.** Thermal stability data of SACA and PCA polymer film.

| Sample Code | \(^a\)Tonset (°C) | \(^b\)T10 (°C) | \(^c\)T30 (°C) | \(^d\)Char temp (°C) | \(^e\)IPDT (°C) |
|-------------|------------------|----------------|----------------|---------------------|----------------|
| SACA        | 99.8             | 100            | 270            | 600                 | 344.96         |
| PCA         | 99.9             | 150            | 312            | 685                 | 398.82         |

\(^a\) Tonset Temperature of as given by TGA.

\(^b\) Temperature of 5% weight loss as given by TGA.

\(^c\) Temperature of 30% weight loss as given by TGA.

\(^d\) Char temperature at 800 °C.

\(^e\) Integral procedure decomposition temperature.

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Figure 3. Antibacterial activity A: Test, +ve control (Streptomycin), -Ve control against E. coli. B: Test, +ve control (Streptomycin), -ve control against S. aureus.

Figure 4. Comparative study of intestinal micro-flora of test organisms. Cage I: intestinal flora isolated from control mice and Cage II: intestinal flora isolated test mice.
degradations of the edible polymer were observed within 4 days and 22 days. Biodegradable polymer usually consists of ester, amide, or ether bonds due to which degradation proceeds. After degradation of crosslinked film in vermicompost, compost can be used as nutrient supplement material for healthy plant growth. The compost was characterized as pH (6.3), soluble salt or dissolved salt (82 ppm), black colour, and muddy odour. The compost was further tested by germinating cottonseed and seed growth was monitored (Supplementary Figure 2). Healthier growth of cotton plant indicates that composting of crosslinked polymer has improved. Chemical resistivity and thermal properties, were found to be comparable. Edibility test by mice feeding method shows that these crosslinked polymers are edible. Crosslinked polymers are very good plant growth substrate. When used food wrapping.

3.10. Application of SACA and PCA film

The newly prepared crosslinked polymer films exhibit good thermal, antibacterial activity, biodegradability, flexible, and transparency. Because of these properties these polymer films can be green alternatives for edible food packaging. Chocolate has been wrapped for 1 month and Indian vegetable puff for 1 week using these crosslinked films and their shelf life was examined. The comparative images of unwrapped and wrapped food material have been presented in supplementary (Supplementary Figure 3).

| Days | Sample | Colour | Flavour | Smell | Overall acceptability |
|------|--------|--------|---------|-------|-----------------------|
| 0-7  | PCA 1  | No change | No change | Good | Accepted               |
| 8-14 | PCA 2  | Little change | Little change | Bad | not accepted          |
| 15-21| PCA 3  | Completely change | Completely change | Very bad | not accepted          |
| 22-28| PCA 4  | Completely change | Completely change | Very bad | not accepted          |

Figure 5. Crosslinked polymer as plant growth substrate photographs of comparative images of seed germination in a) control (in soil), and b) SACA, c) PCA polymer films from 0 day to 16 days.

Table 4. Comparative Organoleptic test results before and after packaging for Indian veg. puff.

3.11. Organoleptic test

Organoleptic evaluation of food, or drugs, is that which stimulates the sense organs taste, colour, odor, and feel. Organoleptic tests is a very important quality control tool in food industry (Yi et al., 2015). In present work, the crosslinked films were used as wrapping film on chocolate and Indian vegetable puff. Effect of this green wrapping film on the quality, shelf life of food have been evaluated using organoleptic tests. The results have been tabulated in Tables 4 and 5.

Organoleptic tests results (Tables 4 and 5) reveal that the unwrapped chocolate and Indian vegetable puff have less shelf life as compared to wrapped (with SACA/PCA) chocolate and Indian vegetable puff. Thus, crosslinked films could be a green substitute for food packaging.

4. Conclusions

Alginate from sea weed, pectin from pineapple rind was extracted. The extracted polysaccharides were modified by covalent bond formation using biobased acids as citric acid and tartaric acid. Tensile strength of crosslinked polymer has improved. Chemical resistivity and thermal properties, were found to be comparable. Edibility test by mice feeding method shows that these crosslinked polymers are edible. Crosslinked polymers are very good plant growth substrate. When used films were thrown in soil it doesn’t harm plant growth. Crosslinked film has been successfully composted using earthworms. The converted compost is very good soil enriching medium. Finally crosslinked film has been used as green food wrapping material to enhance shelf life of chocolate and Indian vegetable puff. Waste (waste pineapple shell) has been successfully converted to edible packing film which has value added application in food wrapping.

Declarations

Author contribution statement

Omparaksh Shrinivas Yemul, Pratiksha Singh, Pankaj Baithakur: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.
Additional information

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