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Crosslinked Starch Polymer for Coating on Fruit/Vegetable for Preservation Derived from Waste De-Oiled Soybean Cake

Pratiksha Singh[1], Vipul Kusumkar[2], Ramkishan Adude[3], Omprakash S. Yemul[4,*], Sumit Kumar Hira[5], Partha Pratim Manna[6]

Abstract

Cross-linked starch films have been prepared by reacting starch extracted from de-oiled soybean cake (waste material from oil industry) with bio-based acids citric acid and tartaric acid. The obtained films were characterized by spectroscopic, thermal and surface morphological techniques. Thermal analysis showed that thin film has good thermal stability. The micrographs obtained from scanning electron microscopy showed the film surface has smooth and uniform morphology. Biological properties like cytocompatibility, antimicrobial activity showed positive results. Biocompatibility using seed germination and biodegradation in soil was satisfactory. Edibility of the cross-linked starch was verified using mice feed technique. Use of edible coating on capsicum, cherry and its stability was examined.

Keywords: Citric Acid, Tartaric acid, De-oiled Soyabean Starch, Edible Coating, Biological properties

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INTRODUCTION

Plastics (modern material) find applications in food packing, protective coating due to its easier use, durability, processing etc compared with traditional materials like wax, paper, resin, cardboard, gum and cloth [1]. Most plastics are prepared from fossil fuels which have very low rate of decomposition, limited resources and, associated pollution problems thus developing interest among researchers to look for suitable alternatives. Renewable resources are one of the alternative carbon sources for preparation of plastics. In general, polysaccharides, lipids, fatty acids and proteins are the main components of fruits and vegetables which are essential to humans and animals [2]. As a result, the market of this food is rapidly increasing, 10–20 % per year with rise of population worldwide [3]. Generally, fruits and vegetables are coated with gum, resin and wax to enhance the shelf life, to control pre-softening and water loss, improve glossiness and color. For this reason, in the 12th and 13th centuries in China, wax was coated on oranges and lemons while sucrose was used first in the 19th century for edible protective coating on fruits to avoid oxidation and

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rancidness during storage [4]. Hot-melt paraffin wax was increasingly used on fruits furthermore in the 1950s. Carnauba wax was also used for the same on fresh vegetables and fruits [5]. Starch, being derived from plant source can be a potential candidate for making edible coating. It is can be consumed by humans and animals [6]. Reacting starch with food additives citric/tartaric acid will impart antimicrobial, antioxidant properties to edible film and coatings offering food preservation property [7, 8]. Soybean plant seed oil and proteins have important nutrients for human and animals and its demand is increasing worldwide. In Marathwada (Maharashtra, India) region soybean is an extremely valuable crop. De-oiled soybean cake is available in large quantity which is either used as food for fodder or burned. In the present study we have used de-oiled soybean cake (waste material from oil industry) to extract starch. Extracted starch was cross-linked with citric acid (CA) or tartaric acid (TA) common food ingredients used in most jams, jellies, candy, canned foods, soft drinks, wines and meat foods etc. Biodegradation and cytocompatibility were tested. Suitability for consumption was evaluated using mice feeding test. Coating on fruit and vegetable to enhance the shelf life of food was evaluated.

MATERIAL AND METHODS

Materials

Laboratory grade chemicals were purchased either from SD fine chemicals, HiMedia or Spectrochem, India. De-oiled soybean cake was purchased from the local market of Nanded, Maharashtra.

Methods

Starch extraction

Starch was extracted from de-oiled soybean cake using modification in existing method [9]. The de-oiled soybean cake was ground into fine powder in pulveriser. Thereafter, it was soaked in 0.1% (w/v) sodium metabisulfite and filtered through a screen of 150 micron mesh size. The filtrate was left for 40 min to deposit starch. To separate other components (Protein and lipids) it was washed using of solution of 5 % toluene in 0.1 M aqueous sodium chloride and left standing for starch to settle out. The supernatant layer was decanted. This step was repeated 15–25 times. Then, obtained starch was washed three times with deionized water, twice with ethanol and then recovered by filtration using Whatman No. 4 filter paper. The refined starch cake was dried in a convection oven at 35 ºC for 48 h. The obtained starch was characterized to confirm the composition and structure. The comparative characterization data of starch obtained from potato, rice and de-oiled soybean cake has been tabulated in Table 1.

Preparation of cross-linked starch films

Crosslinking reaction between starch (1.0 g) and CA/TA acid was performed as follows. A specified amount of citric acid (0.2 g) and various catalysts were tried as sodium hydroxide or phosphoric acid or calcium fluoride or sodium hypophosphite (20% w/w, on weight of citric acid used) and glycerol (40 % v/w starch) was dissolved in the water solution. The entire reaction mixture was heated between 75 to 80 ºC, held at that temperature for 40 min then cooled to 50 ºC and poured onto silicone-coated glass plates. The cast films were allowed to air dry for about 30 h and later peeled from the plates. The free standing films were stored in desiccators.

Fourier transforms infrared spectroscopy (FT-IR)

The Fourier transform infrared (FT-IR) spectra of cross-linked 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}.

Proton nuclear magnetic resonance spectroscopy (^1H NMR)

Proton NMR spectra of cross-linked polymer were recorded on a Varian 400 MHz spectrometer by using DMSO as a solvent.

Resistance to the chemical factors test

The resistance to chemical factor test of a) starch citric acid cross linked film (SCA), b) starch tartaric acid cross linked film (STA), c) starch cross-linked turmeric blended polymer (SCA-T) and d) starch

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cross-linked neem oil blended polymer (SCA-N) were studied using thin film in range of different protic as well as aprotic solvents such as sodium hydroxide (1 M), sulfuric acid (1 M) and xylene, castor oil, dimethyl sulphoxide respectively. Small pieces of all cured samples were kept in the 50 m Lamber glass bottles for 7 days at 30◦C to study the chemical resistance factor of sample.

Thermogravimetric analysis (TGA)

The TGA measurements of cross linked polymer were conducted using Perkin Elmer TGA 4000 thermal analyzer under nitrogen atmosphere over a temperature range 30 to 800 °C, with a heating rate of 10 °C/min and degradation temperatures such as T onset (initial degradation temperature), T_{d5} (temperature of 5% weight loss), T_{d30} (temperature of 30% weight loss), Heat resistance index (Ts) and char temperature were determined from TGA thermograms.

Surface morphology study

Surface morphology of films were examined using a scanning electron microscopy Hitachi S-4200. The surfaces of the films were coated with gold under vacuum.

Antibacterial study

Bacterial samples *Staphylococcus aureus* (NCIM 2079) and *Escherichia coli* (NCIM 2065) were obtained from National Collection of Industrial Microorganisms (NCIM). The 24 h old pure colonies of bacteria were used to prepare inoculums in Nutrient Broth (NB) and these were incubated at 37°C on shaking incubator (100 rpm) for 24 h. The cell densities of each inoculums were adjusted in culture medium at 0.5 McFarland standards. The bacterial suspension (0.1 ml) was spread on Nutrient agar plate (NA) and wells were prepared with sterile cork-borer. Thin cross-linked crushed polymers were dissolved in DMSO (100 µg/ml) [8]. The test samples (100 µl) were added into the wells and plates were kept at 4°C for 10 min in agar medium. Standard solution of antibacterial agent (Gentamicin, 100 µg/ml) was used as positive control and DMSO as negative control. The plates were incubation at 37 °C for 24 h and bacterial inhibition were measured in cm/mm.

Cytocompatibility study

Cell lines and cell culture

Human buccal epithelial cells (SCC-25) were purchased from American Type Culture Collection (ATCC), Manassas, Virginia, USA. The cells were cultured in 25 cm^2 tissue culture flask containing RPMI-1640 (Invitrogen, Carlsbad, CA) medium, with 10% fetal bovine serum (Hyclone, Logan, UT), 100 U/mL penicillin and 100 µg/mL streptomycin (Invitrogen, Carlsbad, CA). All the cells were devoid of mycoplasma contamination [10]. Lymphocytes and monocytes were isolated from peripheral blood as described earlier [10].

Anti-proliferative activity assay

The SCC-25 cells were used for in vitro anti-proliferative activity in presence of various concentrations of SCA, SCA-N or SCA-T. Proliferation of cells was performed with a concentration gradient (5-250 µg/ml) in order to assess the anti-proliferative effect of the compounds if any, against the proliferating cancerous cells. Anti-proliferation activity of SCA, SCA-N or SCA-T cells was performed by MTT assay. The SCC-25 cells (5×10^3 cells/well) were added in a 96-well tissue culture plate and were exposed to various concentrations of the above compounds [11]. Plates were incubated at 37 °C with 5% CO_2 for 48 h. After incubation the tissue culture plates were treated with MTT and incubated for 4 h as per manufacturer's protocol (CellTiter 96® Non-Radioactive Cell Proliferation Assay (MTT) kit (Promega, USA)). The absorbance was measured at 570 nm using Synergy HT Multi-Mode Micro plate Reader BioTek®, USA. Growth inhibition of the cells was determined with following formula:

$$\% \text{ Growth Inhibition} = [1 - \frac{\text{Experimental } OD_{570}}{\text{Target } OD_{570}}] \times 100$$

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The Experimental OD value is the reading of tumor cells exposed to various concentrations of the above compounds and the Target OD represents the corresponding value of tumor cells cultured in medium only.

In vitro cytotoxicity assay

The lytic activity of polymer samples SCA, SCA-N or SCA-T against the proliferating tumor cells was measured by cytotoxicity assay using the CytoTox 96 Non-Radioactive Cytotoxicity assay kit from Promega, USA [11]. The target cells (5×10^3) were added to a 96-well tissue culture plate and exposed to different concentrations of the above compounds and incubated for 18 h at 37 ºC in presence of 5% CO_2. Percent specific lysis was determined using the following formula:

$$\% \text{ Cytotoxicity} = \frac{(\text{Experimental - Effector Spontaneous - Target Spontaneous})}{(\text{Target Maximum - Target Spontaneous})} \times 100$$

In vitro cell viability assay

Effect of SCA, SCA-N or SCA-T on the viability of normal human lymphocytes and monocytes was evaluated by a colorimetric XTT (sodium 3-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate) assay (Roche Molecular Biochemicals, Indianapolis, IN) [10]. Cells were added (5×10^3 cells/well) in a 96-well plate and exposed to serial concentrations of the compounds and incubated at 37 °C, 5% CO_2 for 18 h. The absorbance was measured at 450 nm using Synergy HT Multi-Mode Micro plate Reader BioTek, USA and data was calculated as the percentage of viable cells calculated from the following formula:

$$\% \text{ Cell Viability} = \frac{\text{Experimental OD}_{450}}{\text{Control OD}_{450}} \times 100$$

Hemolysis assay

Lysis of RBC was determined according to the protocol described by Kuznetsova et al. [11, 12] with minor modifications [11].

Statistical analysis

The mean ± SD was calculated for each experimental group. "n" represents the number of times the experiment was performed. Differences between the groups were analyzed by unpaired Student's t-test and one- or two-way ANOVA analysis of variance depending on the requirement. Significant differences among the groups were calculated at $P<0.05$ or less (*=<0.05, **=<0.001 and ***=<0.0001).

In vivo testing of crosslink polymer

Mice selection as a mammalian model

Three weeks old female *Musmusculus* mice were used after a month of quarantine period. Animals were housed as two mices per cages and cages were changed twice in a weak. Water was provided continuously. All mice were fed food and their survival, growth and metabolism were monitored. Fecal sample were collected and fecal flora examined [13], [14].

Mice feeding and fecal sampling

Cross linked polymer along with rodent food was given to test mice while another cage as control for two weeks. Fecal samples were collected from test and control mice were tested for microbial detection. Fecal sample (1g) from mice was homogenized in sterile saline solution. It was spreaded on nutrient agar plates separately and the plates were incubated at 37 °C for 24 h. Comparatively analysis was carried out in test and control cage fecal samples. Observations of mice physical appearance, behaviour, coat colour, hairs, teeth and nails, any side effects with reference to control animal were also examined. All tests were performed in triplicates.

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Biodegradation and seed germination studies

For the Biodegradation studies, soil burial method was used earlier reported [15] with some modifications. Garden soil (pH 6.3) was mixed with cow dung and sewage water to maintain the humidity of the soil. The seed germination was tested using black eyed peas (*Vigna unguiculata*). Black eyed peas were sown in starch crosslinked polymer and the results were compared with control (in soil) [16].

Application as coating on fruits/vegetables to preserve the natural quality

Viscous cross-linked starch solution prepared as per the procedure described earlier in experimental section. It was coated on fruits and vegetables using brush or dip coating method. The coated fruits/vegetables were cured at room temperature for 6 h.

RESULTS AND DISCUSSIONS

Starch (natural polymer) has physical properties such as odorless, transparent, tasteless and it forms semi-permeable membranes [7]. Native starch lacks the desired properties suitable for coating and thin film formation on food and vegetables. To obtain the desired properties it is cross-linked using citric/tartaric acid. In the present study we have extracted starch from de-oiled soya bean cake. This starch has been analyzed and compared with potato and rice starch [9] as presented in Table 1.

Table 1. Comparative data of iodine affinities, amylose contents of starches from potato rice and de-oiled soybean cake.

Variety[a]	Iodine affinity[b]		Amylose content (%)[c]		A-B
	Amylose	*Amylopectin*	*Apparent (A)[d]*	*Absolute (B)[e]*	
Extracted starch	3.84±0.01	1.14±0.02	19.3	13.9	5.4
Potato starch	7.20±0.01	4.6±0.1	36.0	16.9	19.1
Rice starch	5.00±0.02	1.11±0.00	25.0	20.5	4.5

[a]Determined by X-ray diffraction[17] , [b]Averaged from at least three replicates ± standard deviation of each sample, except for defatted green leaf canna starch, of which the iodine affinity was averaged from two replicates, [c]Iodine affinity for pure amylose was assigned as 20% (Takeda and Hizukuri 1987), [d]Calculated as: C = 100 × IAS/0.20 where C is the percentage of apparent amylose content and IAS is the iodine affinity of the whole defatted starch, [e]Calculated as: C = (IAS – IAAP+IC)/{0.20 – (IAAP+IC/100)} where C is the percentage of absolute amylose content, IAS is the iodine affinity of whole defatted starch, and IAAP+IC is the iodine affinity of the amylopectin and the intermediate component mixture.

Native starch properties vary with its source and composition. Starch extracted from de-oiled soyabean cake has less amylose and amylopectin content. In order to improve its mechanical properties it needs to be modified by reacting with biobased cross linking agent as citric or tartaric acid. Citric and tartaric acid are common food ingredients used in jam, jelly etc. Biobased acids such as citric acid extracted from citrus food and tartaric acid from tamarind used as greener cross-linking agents are easily available and cost effective, and they bear antimicrobial, antioxidant properties too. Hence, the use of these acids in this reaction improves thermal, mechanical and biological properties. The multi-carboxyl structure in CA and TA triggers interaction between starch and CA/TA which assists in improving properties such as solubility of films, moisture absorption etc. Based on product yield, reaction conditions sodium hypophosphite catalyst found to be suitable for this reaction. The plasticizer glycerol has been used to ease film formation. Plasticizers have hydroxyl groups allowing compatibility with starch granules and they plasticize starch by breaking the internal hydrogen bonding between the glucose rings in starch. An efficient plasticizer needs to be polar, hydrophilic and small enough to fit between the starch chains. Additionally, the boiling point of the plasticizer must be higher than the manufacturing conditions so that it should not evaporate during processing. The general synthetic scheme of cross-linked edible film is shown in scheme 1.

Fourier transforms infrared spectroscopy

FT-IR spectrum of crosslinked polymer film SCA supplementary file 1. As seen in the spectrum, the broad band at 3398 cm^{-1} is assigned for -OH stretching. The main peak of interest occurs at 1733 cm^{-1} which is associated with (carboxyl and ester) carbonyl bands confirming the cross linking reaction between citric acid and starch [17].

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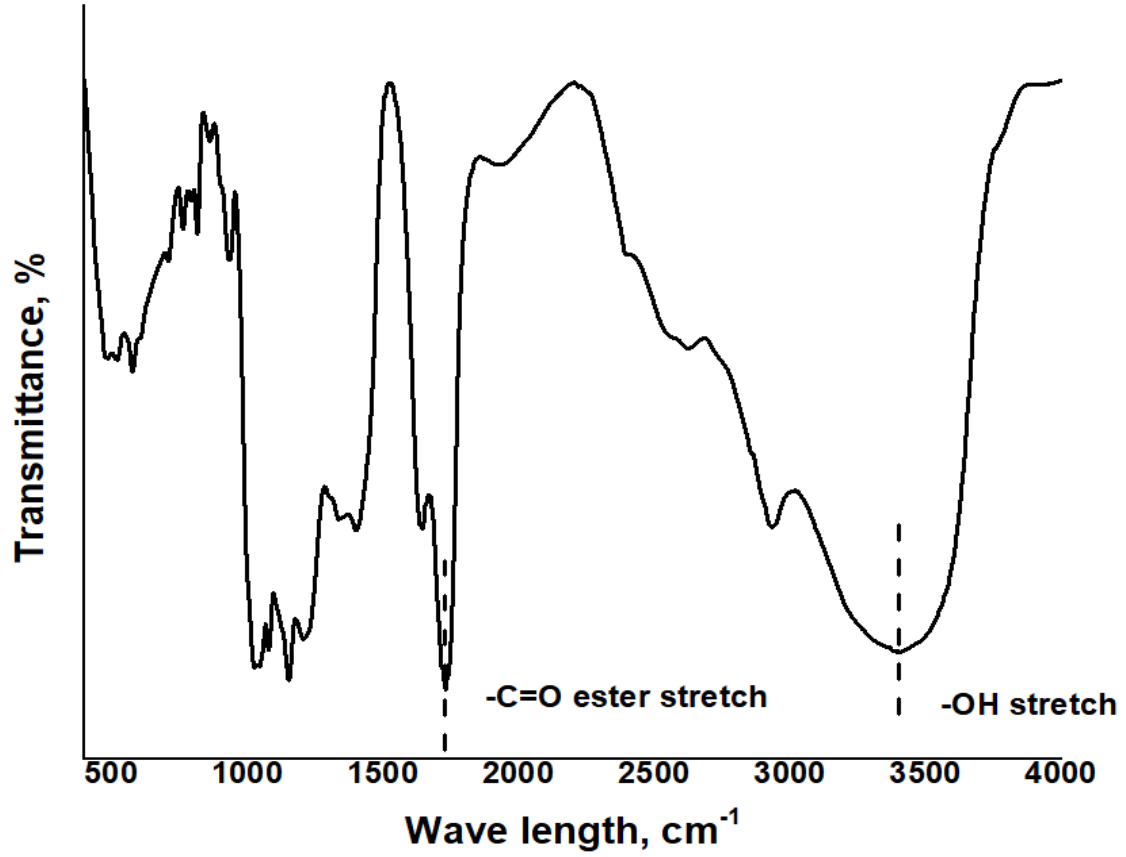
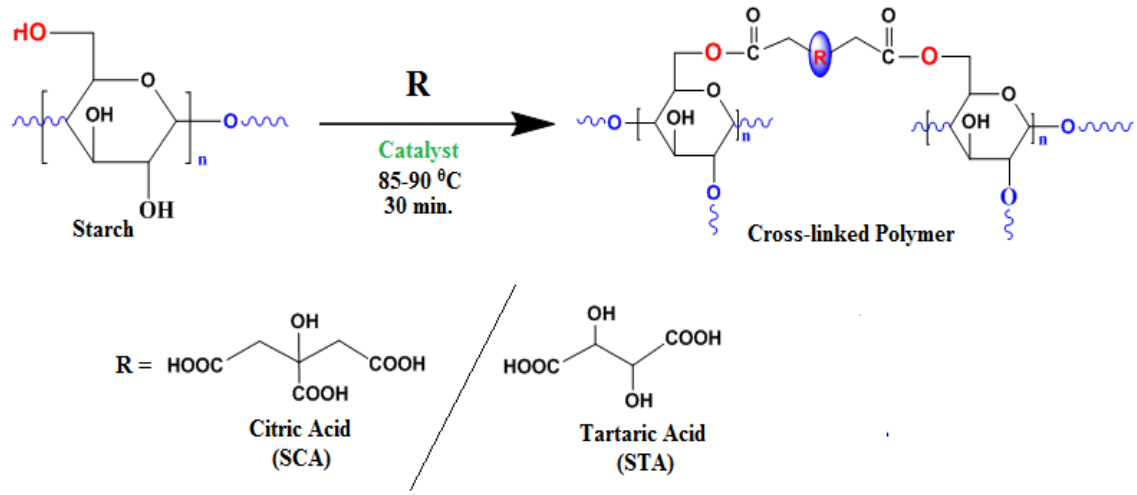


Supplementary File 1. FT-IR spectrum of crosslinked polymer film SCA.



Scheme 1. Synthesis scheme of cross-linking reaction between starch with citric acid (SCA) and tartaric acid (STA).

Proton nuclear magnetic resonance spectroscopy

Assignment of peaks in proton NMR spectrum of crosslink polymer film is shown as follows, 2.54 ppm for -CH_2 protons of citric acid, -CH_2 and -CH groups of starch-furanose ring proton appeared at 3.7 ppm (in this region water peak also appears if proton NMR recorded in DMSO-D_6) [18]. The peak at 6.46 ppm was for the anomeric hydrogen of furanose ring. The peak at 7.44 ppm is the hydroxyl proton of citric acid shown in Figure 1.

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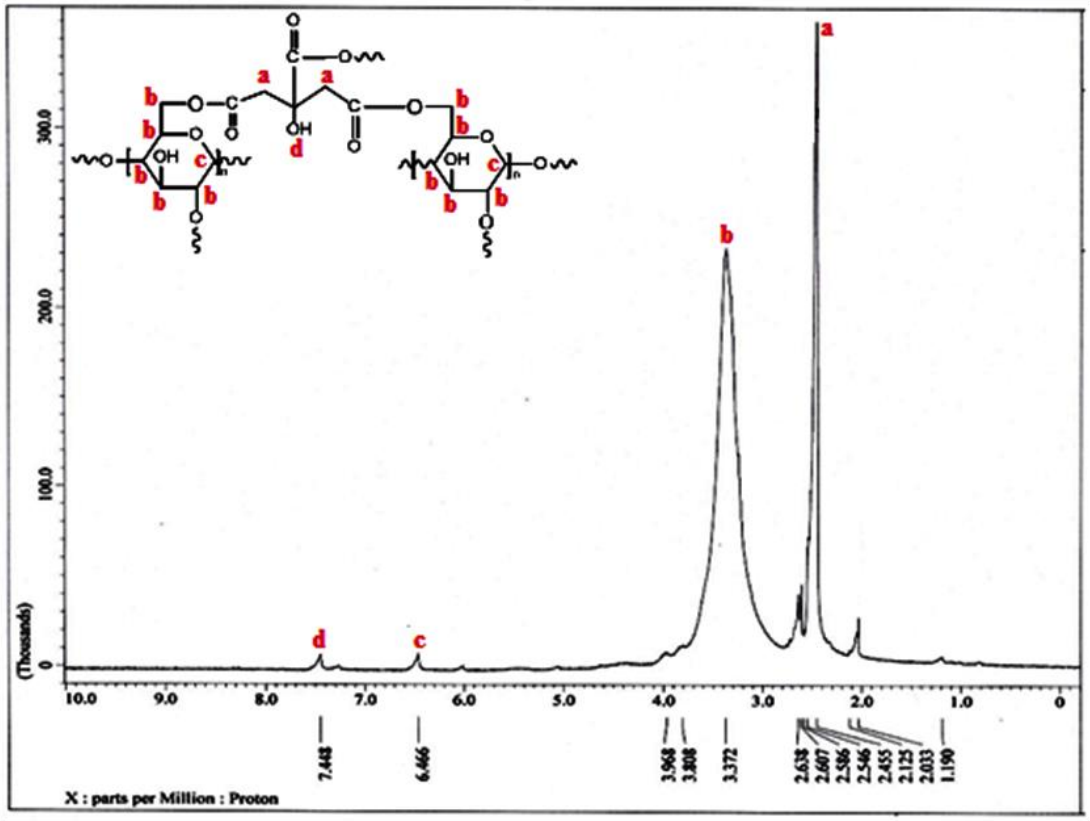

Figure 1. Proton NMR spectrum of crosslink polymer film SCA.

Resistance to the chemical factors test

The resistance to chemical factor test of a) SCA, b) STA c) SCA-T, and d) SCA-N were studied using thin film in a range of different protic as well as aprotic solvents such as sodium hydroxide (1M), sulfuric acid (1M) and xylene, castor oil, dimethyl sulphoxide respectively. The result data has been tabulated in Table 2. It was found that cross-linked films of a) SCA, b) STA, c) SCA-T, and d) SCA-N all films are solubilised in both acidic H_2SO_4 and basic NaOH solution as well as in DMSO and xylene solvent, while showing resistance to ethyl methyl ketone, acetone and chloroform.

Table 2. Chemical resistivity test of cross-linked polymer films.

Solvents	SCA	STA	SCA-T	STA-N
Acetone	-	-	-	-
1 M NaOH	+	+	+	+
1 M H_2SO_4	+	+	+	+
Xylene	+	+	+	+
Castor oil	-	-	-	-
Methanol	#	#	#	#
Chloroform	-	-	-	-
DMSO	+	+	+	+
Distilled water	#	#	#	#

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

Thermogravimetric analysis

The thermal degradation properties of the cross-linked film were measured by TGA analysis. The characteristic values calculated from these curves are given in Table 3. The edible film presents single step degradation under nitrogen atmosphere, with an onset thermal degradation temperature at 99 °C, a characteristic of the thermal stability of a cured film. The values are estimated from the temperatures at 5% weight loss (T_{d5}) and 30% weight loss (T_{d30}) of the sample obtained by TGA. The statistic heat

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resistant index temperature (Ts) and integral procedure composition temperatures (IPDTs) proposed by Doyle were calculated [19].

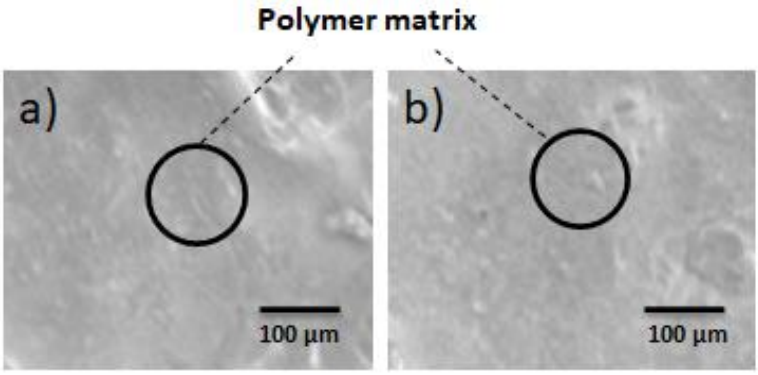
Table 3. Parameters of thermal stability.

Sample code	Tonset (° C)[a]	T_{d5} (° C)[b]	T_{d30} (° C)[c]	Heat-resistant index (Ts)[d]	Char temp (° C)[e]	IPDT (° C)[f]
SCA	99	100	250	93.10	445	223

[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]Statistic heat resistant index temperature, [e]Char temperature at 800 ˚C, [f]Integral procedure decomposition temperature

Scanning electron microscopy

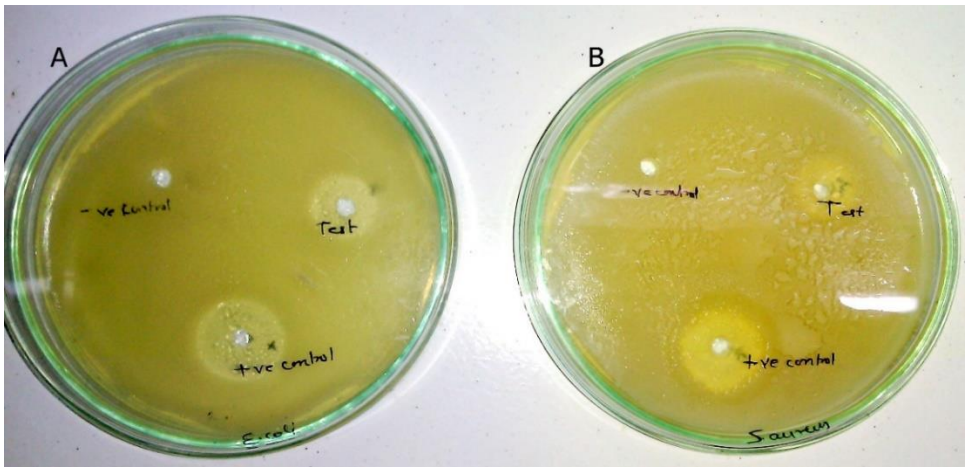
The surface morphology of crosslink film was studied by FE-SEM. The FE-SEM image shown in supplementary file 2 reveals that the SCA film has smooth surfaces and compact structure, indicating their uniformity and structural reliability. Appearance of the cross linked film surface depends on the reactivity of dicarboxylic acids to form ester linkages.



Supplementary File 2. The SEM images of the edible films a) SCA and b) STA

Antibacterial activity

The antibacterial activity is helpful in protection of food material viz. fruits and vegetables from bacterial infection. Crosslinked polymer film SCA-T showed 14 mm zone of inhibition against *Escherichia coli* (MTCC 227) and 16 mm zone of inhibition against *Staphylococcus aureus* (MTCC 3160) indicating that SCA-T is possessing good antibacterial activity the comparative images of antibacterial activity has been presented in supplementary file 3.



Supplementary File 3. Petri plate showing antibacterial activity (Zone of inhibition) Sample A test, +

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ve control (Gentamycein) and E Coli, Sample B test, + ve control (Gentamycein), - ve control against S. aureus.

Cyto-compatibility study

Effect of SCA, SCA-N or SCA-T on cell proliferation and cytotoxicity

The effect of developed polymeric films on the proliferating cells was studied to demonstrate possible toxicity of the films following its ingestion along with the food. Buccal epithelial cells were used in this study since the lining of the gut including the buccal cavity and the stomach is constituted by epithelial cells. Our data suggests that all the three compounds moderately prevent the proliferative potential by inhibiting the cellular growth. Normal human epithelial cells were not proliferating, while SCC-25 cells are transformed epithelial lines that are capable to grow in culture media. The polymers SCA, SCA-N or SCA-T at a concentration of 250 µg/ml prevents the cell proliferation up to 50% while lower concentrations are relatively tolerant to the proliferating cells (Figure 2A). The DMSO was used as diluents in order to dissolve the compounds for this study. Free DMSO had no effect on cell proliferation while the compounds dissolved in same amount of DMSO had moderate effect on cell proliferation, specifically at highest concentration tested (Figure 2A). We also studied the direct cell-lysis in presence of the above thin films. Our results reveal that the thin films show base line cytotoxicity against proliferating SCC-25 cells with SCA likely become more cytolytic against the proliferating cells compared to other 2 films (Figure 2B).

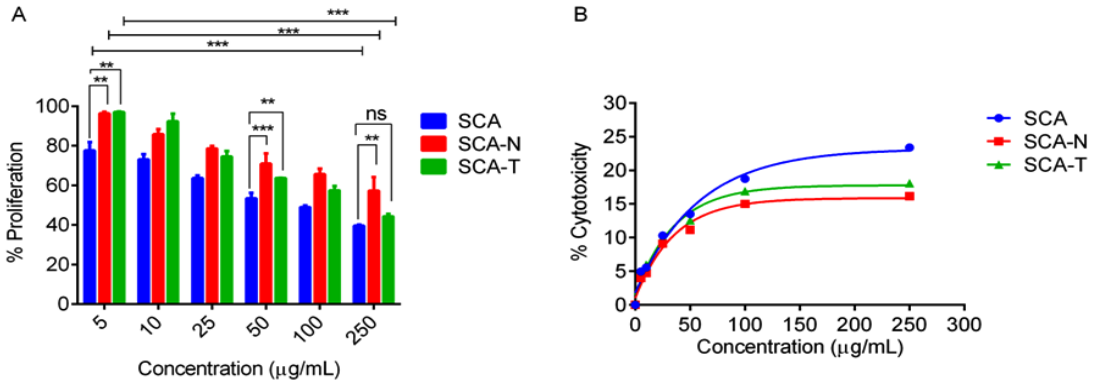

Figure 2. Concentration dependent anti-proliferative effect of SCA, SCA-N or SCA-T against SCC-25 cells (A). Cytotoxicity of the SCA, SCA-N or SCA-T against SCC-25 cells (B). Data presented as mean±SD, n=3.

Normal human cells are tolerant to SCA, SCA-N or SCA-T

Unlike SCC-25, normal human monocytes and lymphocytes are largely unaffected by the compounds with respect to cell viability, studied in presence of various concentrations of SCA, SCA-N or SCA-T. Both monocytes and lymphocytes remain unaffected to moderately high concentrations (50-100 µg/ml) of SCA, SCA-N or SCA-T (Figure 3A & B). Higher concentration (200 µg/ml) however, caused a moderate loss in viability in both the cell types, suggesting the compounds could be toxic if ingested in higher amount (Figure 3A & B). The compound SCA showed moderate hemolysis compared to other 2 thin films. At lower concentrations, all three compounds were found to be tolerant to human RBC. As the concentrations increased above 100 µg/ml, the thin films showed increasing haemolytic activity (Figure 3C).

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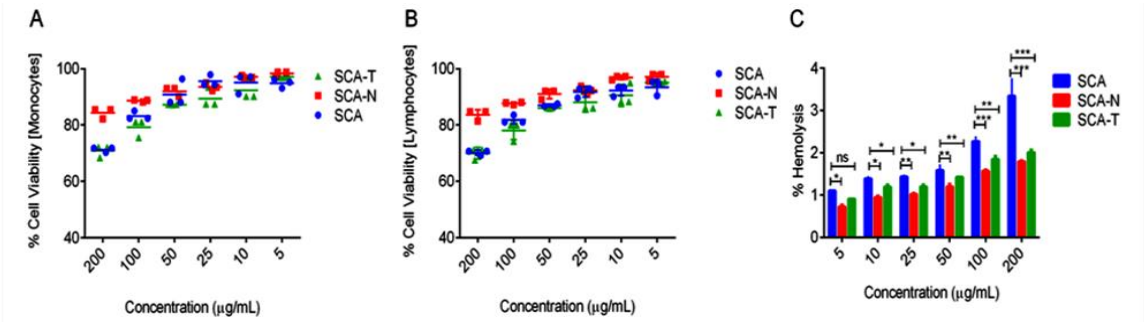
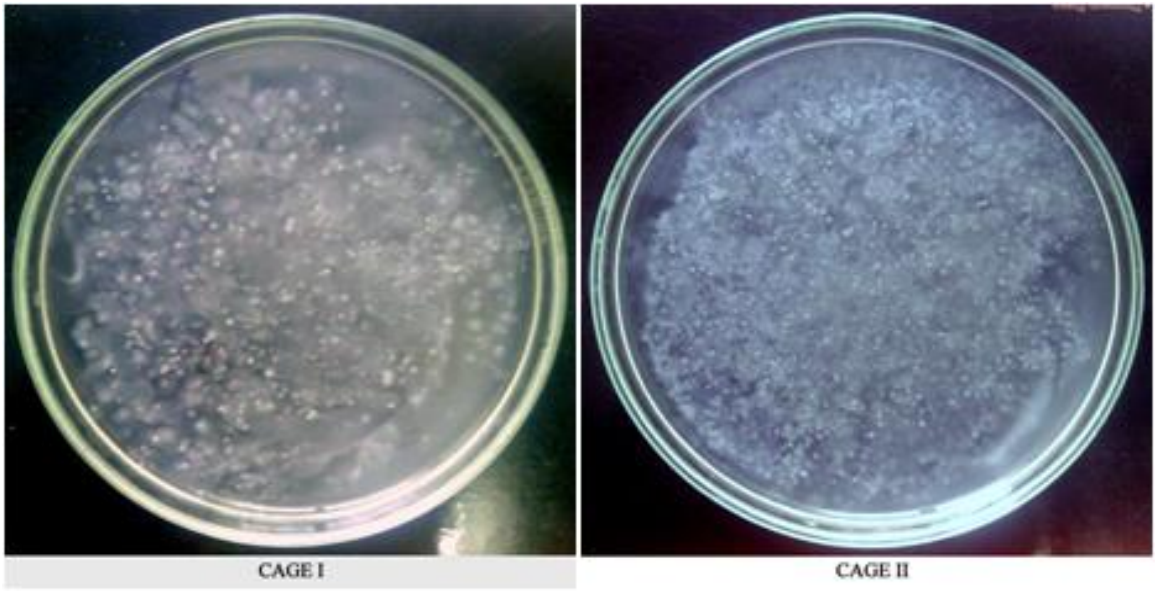


Figure 3. Viability of normal human peripheral blood monocytes and lymphocytes (A & B) and percent hemolysis of human RBC in presence of SCA, SCA-N or SCA-T (C), mean±SD, n=3.

In vivo testing of cross-linked polymer

Mice feeding and fecal sampling

Mice feeding and fecal sample testing was carried out to evaluate suitability of crosslinked product for human consumption. Since in the present study we have used the starch extracted from de-oiled soybean cake. Crosslink polymer film SCA-T shows positive influenced the fecal microflora by decreasing the number of coliforms by ~ 1 log c.f.u. shown in supplementary file 4. Coat colour and physical appearance of test animal was normal and similar with control animal. The results validated that crosslinked starch prepared in the present study is suitable for human consumption though its source is waste material.



Supplementary File 5. Cage I: intestinal flora isolated from test mice and Cage II: intestinal flora isolated control mice.

Biodegradation and seed germination studies

Nature of the polymer and the neighboring environment affects the biodegradation of the polymers. Changing and deformation of properties viz fragmentation, breaking or cracking is considered as degradation. Biodegradation results revealed that cracking, breaking and holes on crosslink polymer was appeared within 10 days and in 50 days 86% degradation of the crosslinked polymer was observed as shown in supplementary file 5. Complete degradation of the crosslinked polymer takes approximately 60 days. Biodegradable polymer usually consists of amide, ester, or ether bonds due to which degradation proceeds. In our synthesized polymer degradation could be due to the presence of ester linkages [20].

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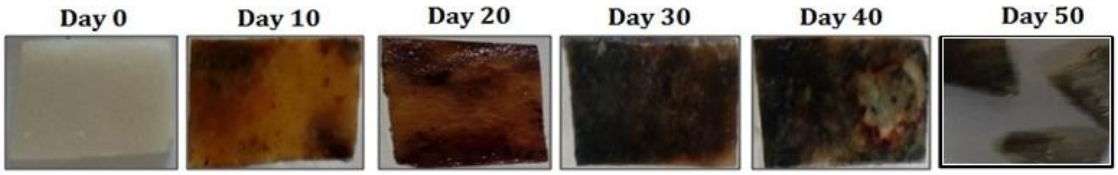

Supplementary File 4. Progressive images of cross-linked polymer film SCA biodegradation.

In seed germination study, germination of black eyed peas (*Vigna unguiculata*) seed was initiated in the control within 3 days while in the polymer sample germination was started after 3 days. Seed germination study results indicate that healthy plant growth occurs in crosslink polymer in a similar manner to that in the control. Test results signified that, crosslink polymer can also be used as a suitable plant growth substrate. The seed germination data is as shown in Figure 4.

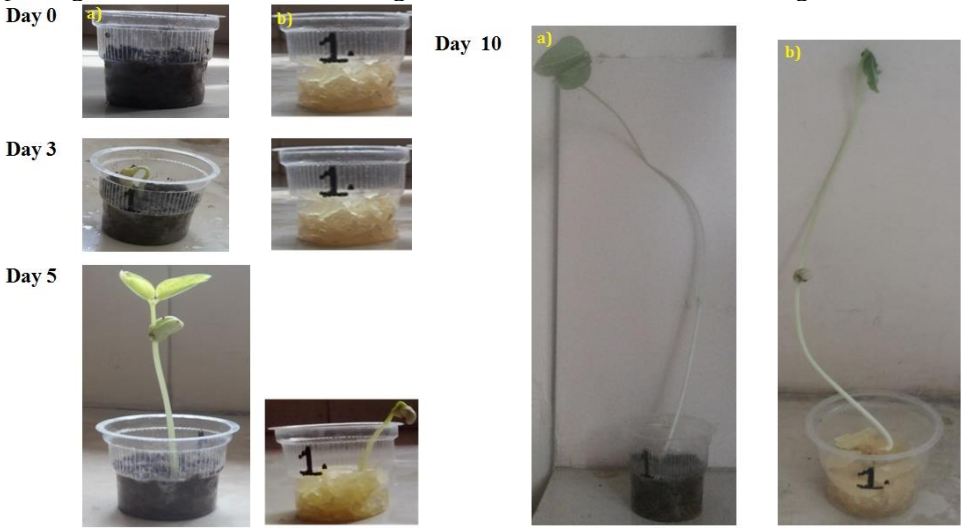

Figure 4. Comparative images of seed germination a) control (in soil), b) crosslink polymer film SCA from 0 day to 10 days.

Application as coating on fruits/vegetables to preserve the natural quality

The coating of starch crossed linked polymer on fruits and vegetables was carried out using brush or dip coat method. The comparative images of uncoated and coated material have been presented in Figure 5. Coating is carried out to check the shelf life of fruits and vegetables by weight loss method. The weight loss and shelf life of cherry fruit and vegetable capsicum data as per supplementary file 6, shows that coating of starch cross-linked turmeric blended polymer has a better shelf life than starch cross-linked polymer. Turmeric is used to enhance antimicrobial properties of the polymer which will decrease the degradation rate of coated sample.

Figure 5. a) Uncoated, b) coated with starch cross-linked polymer and, c) coated 1 with starch cross-linked turmeric blended polymer.

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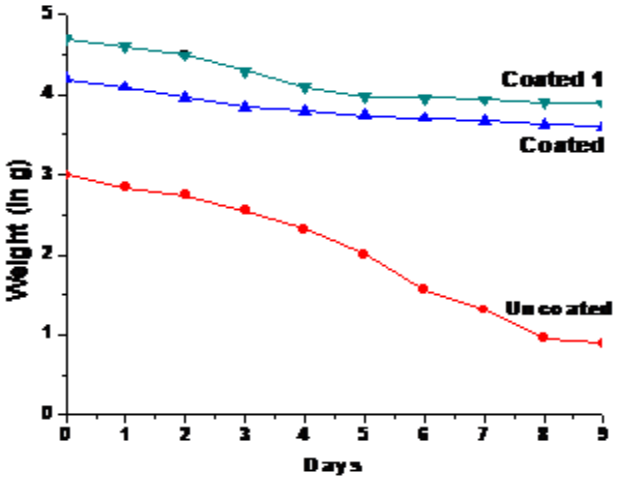

Supplementary File 6. Comparative plot of weight loss against number of days for coated vs uncoated fruit/vegetable

CONCLUSION

Starch from de-oiled soybean cake has been extracted and cross-linked using citric/tartaric acid. Thin films of cross-linked starch showed improved properties as mechanical strength, glossiness, besides low weight loss compared with neat starch. Cytocompatibility study revealed that the films show base line cytotoxicity against proliferating SCC-25 cells with SCA likely become more cytolytic against the proliferating cells. Our findings indicate that SCA-T is found as immune-modulators because it stimulated the immune humoral response and have the ability to survive and adhere in the intestinal tract of mouse and function as valuable probiotics that positively influence the intestinal micro-flora of the host. Starch cross-linked turmeric blended polymer against *Escherichia coli* (wild strain) *and Staphylococcus aureus* (ATCC 6538) shows antibacterial activity. Biocompatibility and biodegradation study of cross-linked starch was tested using seed germination and biodegradation in soil. Shelf life of capsicum and cherry fruit coated with cross-linked starch has stability up to 9 days. De-oiled soybean cake (waste material from oil industry) has been successfully used as carbon source for preparation of value added product as cross linked starch (edible polymer) which has applications as coating on fruits, vegetables.

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