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PVA-Gelatin Films Incorporated with Tomato Pulp: A Potential Primary Food Packaging Film

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A B S T R A C T

The bioactive properties of cooked and uncooked pulp of *Solanum lycopersicum* (tomato) were studied. Both cooked tomato pulp (CTP) and uncooked tomato pulp (UTP) had high total phenolic and flavanoid content and antioxidant activity was ascertained by various *in-vitro* assays. Gram positive organisms were sensitive to CTP and UTP but the extracts were ineffective against Gram negative bacteria. Composite food packaging films were prepared by blending Polyvinyl Alcohol (PVA) and Gelatin and functional properties of the films were improved by the addition of these pulps. The mechanical properties, radical scavenging activity and anti-microbial activity of the films were studied. Tensile strength, puncture strength and percentage elongation of the films decreased on addition of TP. PVA-Gelatin films incorporated with TP were effective against *S. aureus* and *B. cereus*. Incorporation of TP into the PVA-Gelatin films made the films bioactive as they showed both antioxidant and antimicrobial activity. The shelf life of chicken meat packed in the above films was extended during chilled storage. Chicken packed in these films had lowered oxidative rancidity and microbial counts. Hence, tomato pulp can be used effectively to prepare active films and thus improve the safety and shelf life of meat and meat products.

Introduction

India is one of the leading producers of tomato in the world and it is an important commercial vegetable crop for Indian farmers. It is a versatile vegetable with wide usage in Indian culinary tradition. Tomato is the world's largest vegetable crop after potato and is the most processed vegetable. Its worldwide consumption has increased due to the availability of number of products such as soups, juices, purees and sauces which are in great demand. Seasonal variations of the produce and its perishability results in fluctuation in its market value and therefore alternatives are required for value addition. Processing of fruits prolongs shelf-life, makes them available throughout the year, increases its convenience for use and can improve its nutritional and sensory characteristics.

Tomatoes constitute the major dietary source of lycopene which is a carotenoid associated with several health benefits (Rao and Rao, 2007). It is also a rich source of β carotene, ascorbic acid, tocopherol and several phenolic compounds which contribute to the beneficial health effect of tomato and its products.

Packaging of food is a challenging task because of the complexity and diversity of food materials. The use of renewable material for the production of packaging material will be of great advantage as conventional packaging material being used is based on plastics which is non-biodegradable and hence an environmental hazard. Natural polymers are preferred for use in preparation of packaging material due to their abundance in nature, low cost and biodegradable properties. There has been considerable research and development in identifying alternative bio-based packaging polymers. Major challenge facing the food packaging industry in producing bio-based packaging is to match the durability of the packing with product shelf-life.

Poly vinyl alcohol (PVA) is the only known carbon-carbon backbone polymer which is biodegradable and has gained immense attention due to the range of applications possible due to this property (Matsumura *et al.,* 1999). It can also be easily blended with other natural polymers and also has the advantage of being polar and soluble in water. It can improve the mechanical properties of the biopolymer as it has high tensile strength and elasticity. Blending of PVA with biopolymers such as chitosan, starch and have been investigated by researchers (Ke and Sun, 2003; Kanatt *et al.,* 2012). Gelatin is a fibrous protein which due its unique sequence of amino acids consisting of high content of proline, hydroxy proline and glycine that is available in nature and has film forming properties and can be blended with other polymers to improve its mechanical and barrier properties. Glycerol is a major by-product generated during the production of biodiesel and its use as a plasticizer in packaging films can increase the value addition. In recent years extensive studies have been carried out to improve the functional properties of food packaging material thereby improving the quality of the packed food. This has led to the development of active packaging and edible films. Bioactive compounds from natural sources have been frequently utilized in active packaging applications (Fang *et al.,* 2017; Romani *et al.,* 2017).

During preparation of products from tomato heating is one of the processing methods used to enhance its shelf-life. Hence, the objective of this study was to evaluate the effect of heating on the antioxidant and antibacterial activity of tomato pulp. The main aim of this work was to develop an active composite biodegradable food packaging film that can have practical application in the food industry.

Materials and Methods

Chemicals

PVA (average moleculer weight: 1.6 kDa, viscosity: 27-33 cP and degree of hydrolysis: 86.5-89 mol%) and Gelatin (from bovine source) was procured from HiMedia (Mumbai, India). β-Carotene, linoleic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and catechin were procured from Sigma Chemical Co. (St. Louis, MO). Glycerol and all other reagents used were of analytical grade and procured from Qualigens Fine Chemicals (Mumbai, India). All microbiological media used were from HiMedia (Mumbai, India).

Bacterial cultures

Escherichia coli JM109, *Pseudomonas fluorescens* ost5 (Accession no. DQ439976) a laboratory isolate, *Staphylococcus aureus* ATCC 6538P, *Bacillus cereus* MTCC 470, were stored in 20% glycerol (v⁄v) at -20^0 C. Before the start of experiment, the cultures were grown on nutrient agar. The isolates were subcultured twice before inoculation.

Preparation of tomato pulp (TP)

Ripe tomato (*Solanum lycopersicum* L) was purchased from local market. For preparation of uncooked tomato pulp the tomatoes were washed, cut into small pieces and blended in a home blender to obtain a smooth puree. The puree was transferred to a large petri dish and lyophylised to obtain a powder. The powder was stored in amber colored bottles at 4°C till further use. Cooked tomato pulp was prepared by microwaving the cut tomatoes for 5 minutes at 770Hz. It was then cooled and lyophilized powder obtained as described above.

Determination of total phenolics, flavonoids, lycopene and ascorbic acid content of TP

The total phenolic content of TP was determined using the Folin-Ciocalteu procedure (Singleton and Rossi, 1965) and the flavonoid content was evaluated according to procedure described by Kim *et al.,* (2003). The calibration curve for total phenolic and flavanoid content was prepared with catechin and the results were expressed as catechin equivalents. Ascorbic acid content of tomato pulp was determined by the method of Klein and Perry, (1982). Standard curve was plotted using ascorbic acid as a standard. Lycopene content of tomato was estimated by the colorimetric method of Rao *et al.,* (1998). Briefly, 0.5 g of the lyophilized powder was extracted with a mixture of hexane, methanol and acetone in the ratio 2:1:1 for 1h. The absorbance of the supernatant obtained after centrifugation was measured at 502 nm.

Determination of antioxidant activity

The DPPH radical scavenging activity of both cooked and uncooked tomato pulp was measured according to procedure described by Yamaguchi *et al.,* (1998). Percent DPPH-scavenging activity was calculated. Superoxide radical scavenging activity was estimated as per the method described by Liu *et al.,* (1997). Hydroxyl radical scavenging activity of TP was determined according to the deoxyribose method of Halliwell *et al.,* (1987). Iron chelation capacity of MPE was determined as described by Decker and Welch, (1990). Results were expressed as percentage iron chelation. The antioxidant activity coefficient was estimated by thermally induced beta-carotene bleaching assay, as described by Velioglu *et al.,* (1998). The reducing power of appropriately diluted TP was determined according to the method of Oyaizu, (1986). Results were expressed as OD at 700 nm.

Antibacterial activity of TP

The antibacterial activity of TP was measured against *S. aureus, B. cereus, P fluorescens* and *E. coli*. The test culture was grown overnight in nutrient broth. Appropriately diluted cell pellet was added to tubes containing TP.

Initially (0 h) sample was withdrawn, serial dilutions carried out, plated on plate count agar and counted after incubation at 37^0 C for 24 h. This gave the initial number of the test organism. All the tubes were then incubated for 3 h at 37^0 C, the aliquots were again taken and the surviving population was determined. The antibacterial activity of TP was assessed by decrease in log cfu/ ml of the test culture after incubation for 3 h.

Film preparation

Gelatin was dissolved in distilled water (70°C) for 30 min to obtain a concentration of 5% (w/v). PVA (5% w/v) was mixed in water and kept under magnetic stirring at 80°C for 2 h. The solutions of PVA and gelatin with mixing ratio of 9/1, containing glycerol at

0.5% were cast on Teflon plates. The film forming mixtures were blended by stirring on a magnetic stirrer (200 rpm) at room temperature for 1 hour, followed by homogenisation (using polytron) and then degassed to remove the air bubbles. The films were then cast by pouring the film forming solution (150 ml) onto teflon plates (15 cm×15 cm) and dried at 50ºC in a ventilated oven at 50% relative humidity (RH) to obtain films of uniform thickness.

Film characteristics were determined after all sample films were preconditioned in a constant temperature humidity chamber (Model FX 1077, Jeiotech Co., Ltd., Mumbai, India) set at 23ºC with 50% RH for 24 h. For films containing TP, CTP and UTP were added at a concentration of 5% respectively and films prepared as described above.

Film thickness measurements

Film thickness was measured using a hand-held micrometer (Mitotuyo No. 7327, Tokyo, Japan). Measurements were taken at ten different locations of each film sample and the average film thickness was calculated.

Optical properties

The transparency of the films was determined using the procedure of Han and Floros, (1997).

Mechanical properties

Tensile strength (TS), percentage elongation at break (%E) and puncture strength were measured using Texture Analyzer TA-HD plus (Stable Micro Systems, Surrey, UK) in accordance with ASTM D882-91 method (ASTM, 1991).

Each reported value corresponded to at least five determinations per type of film.

Water vapor transmission rate (WVTR) and oxygen permeability (OP)

WVTR tests were carried out using an automatic water vapor permeability testing machine L80-5000 (PBI Dansensor, Denmark) at 37ºC and 10/15% RH. OP of the film was estimated with automated oxygen permeability testing machine OPT-5000 (PBI Dansensor, Denmark) at 23ºC and 0% RH. WVTR and OP of each sample were averaged from three separate tests.

Water solubility of films

Film's water solubility was measured by method of Stuchell and Krochta, (1994).

Antioxidant and antibacterial activity of films

Films (5 cm^2) were placed in conical flasks containing 10 ml of distilled water. These flasks were then continuously shaken in orbital shaker (100 rpm). Three sets of each film were taken. The antioxidant activity of the films was monitored by taking aliquots of the supernatant obtained from each flask after 3 hours and analyzed for DPPH radical scavenging activity. Antibacterial activity of film samples (diameter of 1.5 cm) was carried out by aseptically placing the films on PCA plates streaked with the test organisms. Zone of inhibition was measured after incubation of the plates at 37^0 C for 24 hours.

Packed inoculums studies

The antibacterial efficacy of the TP containing films was analyzed by packed inoculums studies. Minced chicken meat was irradiated at 10 kGy in Gamma Cell 5000 with ^{60}Co source at dose rate of 4.2 kGy/h to eliminate all microbial flora. *S. aureus* and *B. cereus* were grown in nutrient broth (37°C, 18 h), cells were collected and suspended in

phosphate buffer saline. Chicken was inoculated with cultures individually, packed in TP containing and control films and stored at chilled temperature. Total viable counts were determined at regular intervals. The results were expressed in log cfu/g of meat.

Oxidative rancidity

Minced chicken meat packed in control, CTP and UTP films was irradiated at 2.5 kGy in Gamma Cell 5000 with ^{60}Co source. The samples were stored at chilled temperature and at regular intervals lipid peroxidation was measured by Thiobarbituric Acid Reactive Substances (TBARS) assay according to Alasnier *et al.,* (2000).

Statistical analysis

All experiments were carried out in triplicate and the average values with standard errors were reported. Analysis of variance was conducted and differences between variables were tested for significance by one-way ANOVA. A statistical difference at $p < 0.05$ was considered to be significant.

Results and Discussion

Phenolic, flavanoid, lycopene and ascorbic acid content of TP

Plants are rich in phenolic compounds which are secondary metabolites that play an important role in their growth and protect them from various environmental stresses. The yield of the lyophilized uncooked tomato pulp powder (UTP) was 4.3% and that of the cooked tomato pulp (CTP) was found to be 4.8%. The total phenolic content of CTP was 12.32±0.54 mg in terms of catechin equivalent /g whereas that of UTP was 13.15±0.36 mg /g. Chlorogenic acid has been reported to be the most abundant phenolic acid present in tomato Martínez- Valverde *et al.,* (2002). The total flavonoid content in UTP was found to be 1.53±0.35 mg/g while that of CTP was 1.21±0.10 mg/g. amongst the phenolic compounds flavanoids constitute the largest group and contribute to the aroma and colour in addition to its antioxidant activity. Quercetin, kaempferol, catechins, naringerin, anthocyaninidins and stilbenes are the main flavanoids identified in tomatoes Fatima *et al.,* (2008). Lycopene is a carotenoid containing 13 double bonds that can exist in trans and cis configurations. Of all the carotenoid pigments, lycopene is the most efficient singlet oxygen quencher Shi and Maguer, (2000). In fresh tomato, lycopene is generally found in the trans configuration while factors such as light, acids, oxygen and thermal treatments can cause transformation to the more bioactive cis form. The lycopene content in CTP was 50.50 mg/kg fresh weight while that in UTP was 34.38 mg/kg.

Dewanto *et al.,* (2002) also found that there was an increase in total lycopene content between the raw tomato sample and the heat-treated tomato samples. Thermal processing disrupts the cellular matrix of the fruit and this improves the extractability and bioavailability. Colle *et al.,* (2010) demonstrated that thermal processing of tomato pulp at 130°C improved the *in vitro* bioaccessibility of lycopene. Hence, CTP had a higher phenolic, flavanoid and lycopene content. Our results are in agreement with Chang *et al.,* (2006) who found that hot air drying of tomato enhanced the nutritional value of tomato as it had higher total flavanoid, phenolic and lycopene content. Tomato is a rich source of ascorbic acid and hence the effect of heating TP was studied. In UTP the ascorbic acid content was 108.6 mg/kg which reduced to 84.4 mg/kg in CTP. Substantial loss of ascorbic acid has been reported during the production of dried tomato pulp at high temperatures (Dewanto *et al.*, 2002; Toor and Savage, 2006). A number of factors such as pH, moisture content, temperature, oxygen content and metal ion

catalysis can result in loss of ascorbic acid during processing.

Antioxidant activity of TP

Using various *in vitro* assays the antioxidant potential of tomato pulp was investigated. DPPH is a stable free radical commonly used for testing radical scavenging activity of natural plant extracts. Figure 1A shows the percentage DPPH free radical scavenging activity of tomato pulp. The IC_{50} value, which is the concentration at which 50% scavenging of the free radical is obtained, of CTP was found to be 2.11 mg/mL, whereas, that of the UTP was 2.23 mg/mL. Hydroxyl radical is the most reactive oxygen species that can cause damage to biomolecules. In hydroxyl radical scavenging assay the IC_{50} value of UTP, was found to be 4.50 mg/mL, whereas, that of the CTP was 2.63 mg/mL indicating that CTP was significantly ($p < 0.05$) more efficient in scavenging the hydroxyl radical as compared to UTP (Fig. 1A). Although superoxide radical is not a potent oxidant, it is the precursor for formation of reactive oxygen species such as hydroxyl radical and singlet oxygen. Superoxide radicals were generated in a PMS-NADH system and assayed by the reduction of NBT. Figure 1A illustrates the superoxide radical-scavenging ability of TP. Both the tomato extracts showed a concentration-dependent super-oxide radical scavenging activity. CTP had an IC_{50} value of 0.36 mg/mL and UTP had an IC_{50} value of 0.37mg/mL. In the β carotene bleaching assay the peroxyl free radicals generated due to oxidation of linoleic acid bleach β carotene and this can be prevented in the presence of antioxidants as they scavenge the peroxyl radical and thereby reduce the bleaching of β carotene. At a concentration of 10 mg/ml, the AAC of UTP and CTP was 564 and 765 respectively demonstrating that CTP was a better antioxidant (Fig. 1B). Iron reduction is often used as an indicator of electron donating activity which is an important mechanism of antioxidant activity of a phenolic compounds present in natural extracts. From Figure 1B it can be seen that there was concentration dependent increase in the reducing power of both CTP and UTP and the reducing power of CTP was greater than UTP. Iron chelating agents can inhibit oxidative rancidity and is estimated in the assay by the decrease in the red colour formed due to the ferrozine-Fe complex. The iron chelating ability of CTP was significantly ($p < 0.05$) greater than UTP at all the concentrations studied (Fig. 1B). Chen *et al.,* (2000) reported that a higher antioxidant activity was obtained through thermal treatments such as steaming, microwaving and frying of tomato. Most of the phenolics present in plant extracts are in their glycosidic form. On heating this bond is broken and they are present in the more reactive aglycone form. Hence, as can be seen from Figure 1B CTP had a significantly higher antioxidant potential as compared to UTP. According to Re *et al.,* (2002), the overall antioxidant activity of the hydrophilic extract of tomato paste increased after processing. Heat treatments can also inactivate endogenous oxidative enzymes and thus prevent enzymatic oxidation reactions which cause loss of antioxidant activity in the raw plant materials (Dewanto *et al.,* 2002; Choi *et al.*, 2006).

Antimicrobial activity of TP

In addition to antioxidant activity many bioactive compounds present in plant extracts also show antibacterial activity. Results of antimicrobial activity of TP indicated that Gram positive *S aureus* and *B cerues* were more susceptible to the antibacterial activity of TP as compared to Gram negative *E. coli* and *P fluroscence* (Fig. 2). TP was most effective against *B cereus* were 3 log cycle reduction was obtained while in case of *S aureus* 2 log reduction was seen. Some authors have described that hydroxyl groups of polyphenols cause inhibitory action in

microorganisms, and these groups can interact with the cell membrane of bacteria to destroy the membrane composition and cause the loss of cellular components. The position of OH group in the aromatic ring of polyphenols as well as the length of the saturated side chain can also increase the antimicrobial activity.

Films

Films were prepared with PVA and gelatin and their mechanical properties were studied. To develop functional films that could be used in food packaging for shelf life extension of the packed food, TP was added.

Thickness and appearance of films

Films could be easily removed from the plates and were not brittle. All the films were flexible and homogenous. The thickness of films prepared with PVA-gelatin with/without TP is shown in Table 1. Incorporation of CTP/UTP in the films had significant ($p < 0.05$) effect on the thickness of the films. Neat films without TP had thickness of 285 µm and there was 37% and 44% increase in thickness of films on addition of CTP and UTP respectively. Other researchers have also reported increase in thickness of films containing natural extracts (Benavides *et al.*, 2012).

Table.1 Characterization of composite films with/without TP

	90 : 10 (CTP)	90: 10 (UTP)	90:10 (without TP)
Thickness (µm)	390±5^{b}	410±4^{c}	285±5^{a}
Transparency	3.16±0.09^{c}	3.66±0.02^{b}	1.02±0.1^{a}
Puncture (N)	7.33±0.20^{a}	8.73±0.42^{b}	7.12±0.19^{a}
Moisture (%)	14.82±0.30^{a}	13.74±0.74^{a}	14.56±0.29^{a}
Tensile strength (MPa)	6.86± 0.65^{b}	4.45±0.52^{a}	10.98±0.48^{c}
Elongation (%)	75.38±0.21^{a}	74.10±0.14^{a}	110±0.38^{b}
WVTR (g/m^2/day)	4008±67^{a}	4091±12^{a}	4082±41^{a}
Solubility (%)	37.78±0.10^{b}	31.51±0.18^{a}	71.21±0.21^{c}
Color			
L*	37.82±0.88^{a}	39.75±0.93^{a}	97.22±0.28^{b}
a*	0.98± 0.07^{b}	1.00±0.06^{b}	0.15±0.05^{a}
b*	9.59±0.73^{c}	5.11±0.83^{b}	0.85±0.11^{a}
Antibacterial activity (zone of inhibition in cm)			
S aureus	2.5±0.1^{c}	2.1±0.1^{b}	0±0^{a}
B cereus	2.3±0.1^{c}	1.9±0.15^{b}	0±0^{a}

D Data are expressed as mean ± standard deviation of three independent experiments. Different super script letters within the same row indicate significant ($p< 0.05$) differences of means.

Table.2 Antibacterial activity of PVA-Gelatin films with/without CTP/UTP in minced chicken meat inoculated with Gram positive organisms

Organism	Film	Storage (days)			
		0	3	7	12
***B cereus* (CFU/g)**	Control film	2.29±0.05^{a}	2.35±0.06^{a}	2.39±0.01^{ab}	2.40±0.02^{ab}
	CTP film	2.20±0.02^{b}	0±0^{a}	0±0^{a}	0±0^{a}
	UTP film	2.25±0.04^{b}	0±0^{a}	0±0^{a}	0±0^{a}
***S aureus* (CFU/g)**	Control film	2.44±0.02^{a}	2.89±0.06^{b}	3.52±0.04^{c}	3.68±0.01^{d}
	CTP film	2.34±0.01^{c}	1.03±0.01^{b}	0±0^{a}	0±0^{a}
	UTP film	2.38±0.03^{c}	1.29±0.01^{b}	0±0^{a}	0±0^{a}

Data are expressed as mean ± standard deviation of three independent experiments. Different superscript letters within the same row indicate significant ($p< 0.05$) differences of means.

Fig.1 [A] DPPH, hydroxyl and superoxide radical scavenging activity of cooked and uncooked tomato pulp. [B] Beta carotene bleaching assay, reducing power and iron chelation capacity of cooked and uncooked tomato pulp. Values are mean ± SD of three independent experiments

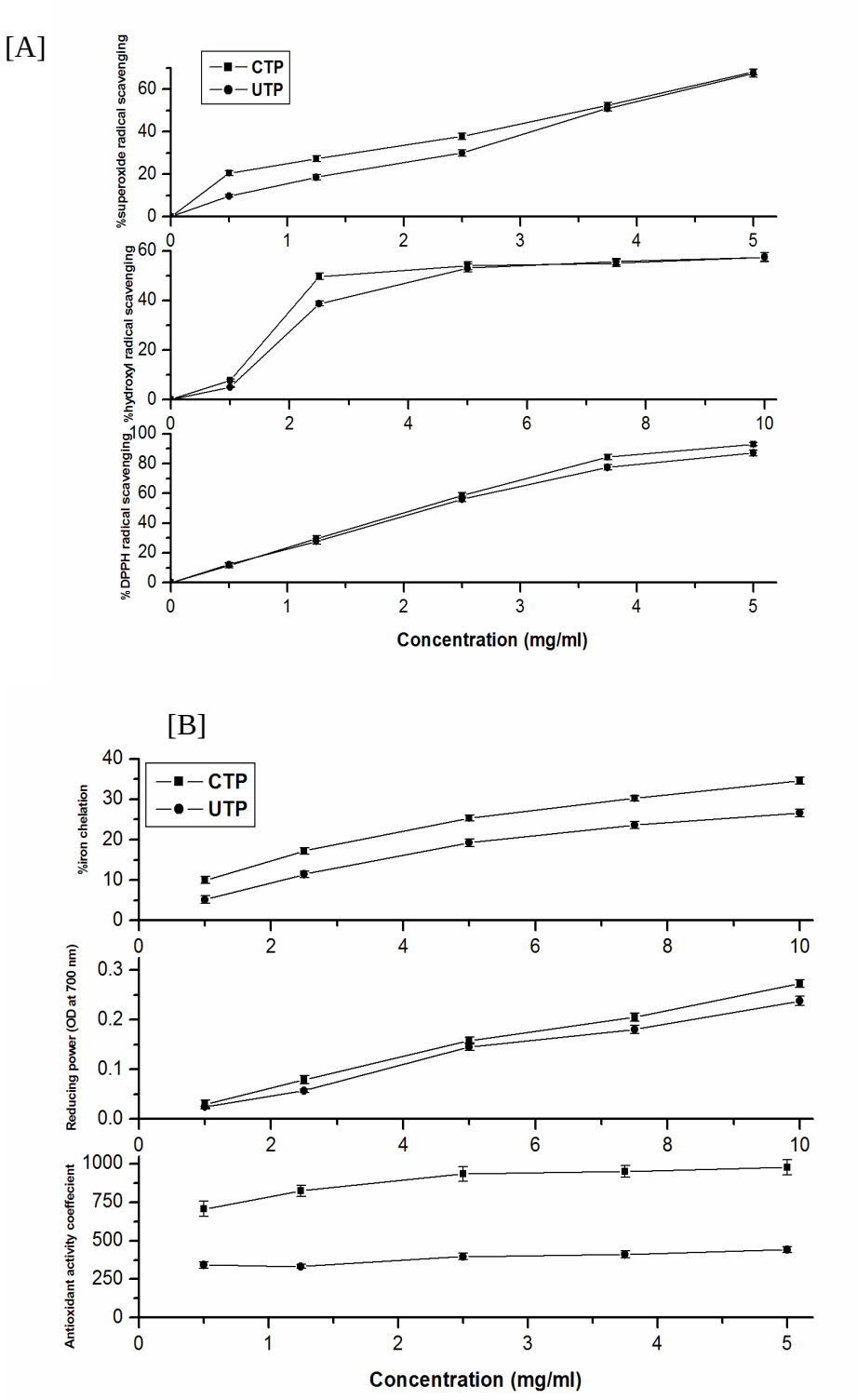


Fig.2 Antibacterial activity of CTP and UTP against common food spoilage and pathogenic organisms

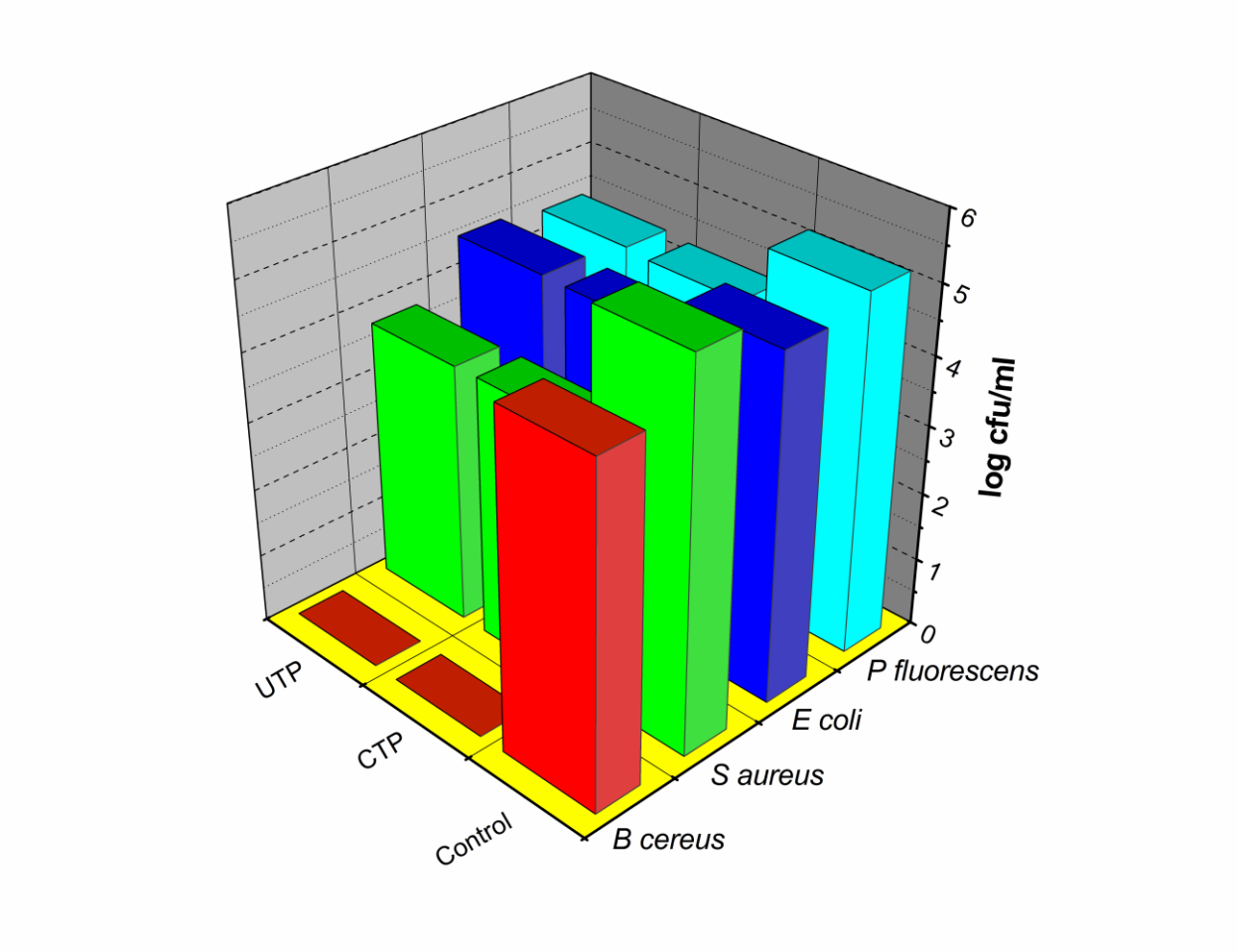
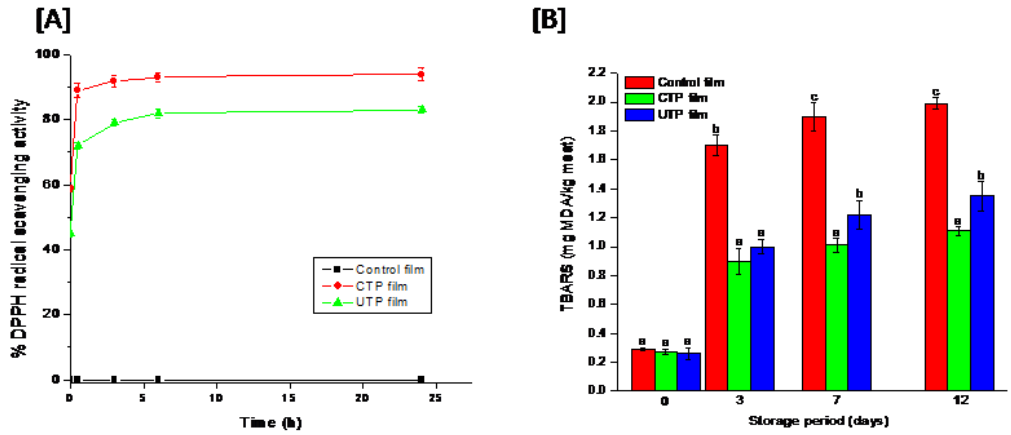


Fig.3 [A] Antioxidant release from films as assayed by DPPH radical scavenging assay [B] Oxidative rancidity of chicken meat packed in films with/without CTP/UTP



Thickness of films is important as it could affect the mechanical properties and permeability of the films. The films containing CTP/UTP were visually red in color. Siripatrawan and Vitchayakitti, (2016) also reported that incorporation of propolis to chitosan films resulted in deep orange color films.

Transparency

Neat films were transparent (1.02) however addition of CTP/UTP significantly reduced the transparency of films (Table 1). Clarke *et al.,* (2016) also reported that addition of antimicrobials to gelatin based films reduced the transparency of the films. Transparency of a film to some extent is determined by the miscibility of the various components in the film forming solution and hence transparency values can provide information about the regularity of the microstructure of the blends.

Moisture content and solubility

The moisture content and solubility of the different films is shown in Table 1. Incorporation of CTP/UTP did not have any significant affect on the moisture content. Lower water solubility of film is mostly desired for application as a packaging material. There was very significant reduction in the solubility of films containing TP. In films prepared with CTP the solubility was reduced by 47% while in UTP containing films the decrease was by 56%. As TP is rich in lycopene, a carotenoid which is water insoluble its addition to the film must have increased the hydophobicity of the films.

Mechanical property

Mechanical properties of a film play a major role in food packaging as it affects the handling and the storage of the packed food. The mechanical properties of the films were investigated with respect to tensile strength (TS), puncture strength and percentage elongation (Table 1). Neat PVA-Gelatin films had TS of 10.98 MPs. The hyrodxyl groups present in the backbone of PVA is responsible for the strong intra and inter molecular hydrogen bonds, and thus endowing it with high tensile strength (Dorigato and Pegoretti, 2012). To improve the functional properties of this film CTP and UTP was added and its and mechanical properties were studied. Reduction in TS of PVA: gelatin films incorporated with CTP/UTP was observed. The tensile strength reduced from 10.98 MPa to 6.86 MPa and 4.45 MPa for CTP and UTP films respectively. The extent to which a film stretches from its original length to the point where it breaks is defined as the elongation of the film. The percentage elongation of neat films was 110. Addition of CTP/UTP reduced % elongation by around 32%. Significant increase in puncture strength was seen on addition of UTP in films while addition of CTP to films did not have any effect on the puncture strength. Reduction in the mechanical properties of biodegradable films containing natural extracts has been reported by other researchers. Kowalczyk and Biendl, (2016) found that the incorporation of ethanolic hop extract into gelatin and CMC film formulation caused a significant reduction ($p < 0.05$) in tensile strength and puncture strength. With addition of tea extracts into chitosan films significant decrease in tensile strength and elongation at break was reported by Peng *et al.*, (2013).

WVTR and oxygen gas permeability

The water vapour transmission rate of films plays an important role in deteriorative reactions of food; therefore, it is the most extensively studied property of films. The WVTR of control films was 4082 g/m^2/day and incorporation of TP didn't make significant difference in WVTR of the films

(Table 1). Oxygen permeability of packaging materials is of great importance for food preservation. Barrier to oxygen in a packaging system can increase food product shelf-life and also improve food quality. All the films were impermeable to oxygen. This result indicates that films prepared with PVA-gelatin can be used as a natural packaging to protect food from oxidative rancidity. PVA has been reported to be an excellent gas barrier due to its small, dense and closely packed monoclinic crystal structure (Jang and Lee, 2003).

Antibacterial activity of films

Antibacterial activity of the films was tested only for Gram-positive organisms as tomato pulp was found to be ineffective against Gram negative organisms (Fig. 2). Films prepared with only PVA and Gelatin did not have any antibacterial activity. PVA –gelatin films incorporated with TP were effective against both the Gram positive organisms i.e. *S aureus* and *B cereus*. Zone of inhibition was larger in case of *S aureus* as compared to *B. cereus* (Table 1).

Released Antioxidant activity of films

Active packaging is a promising technology that can limit the use of synthetic additives in food industry. The active compounds present in the film when released into the food enhance its shelf life due to its antimicrobial and antioxidant activities. The release of antioxidant compounds from PVA-gelatin film incorporated with CTP/UTP was monitored at regular intervals by estimating its DPPH radical scavenging activity (Fig. 3A). The control films (not containing CTP/UTP) did not show any radical scavenging activity. Films containing CTP had significantly higher ($p < 0.05$) antioxidant activity as compared to those containing UTP. In the first 3 h itself 80-90% of the antioxidant compounds are released and further that activity was maintained over a period of time. Hence these films containing CTP/UTP have quick release from the film matrix. The release rate of active compounds from the films to the food surface would depend on the physical, chemical and biological properties of the food. When a film is placed over the food surface, its solubility also largely determines the release of bioactive compounds (Peng *et al.,* 2013). Others have also shown that the inclusion of plant extracts in food packaging films improved the oxidative stability of the packed food (López-de-Dicastillo *et al.*, 2012).

Prevention of oxidative rancidity of radiation processed meat by active packaging

Lipid peroxidation is a major problem that affects the quality of food. Oxidative rancidity in meat and meat products is minimized generally by addition of synthetic antioxidants like BHT, BHA and others. The application of active packaging in minimizing oxidative rancidity is a new concept that not only prevents the use of synthetic antioxidants but also minimizes the use of plastics in packaging. Radiation processing is known to accelerate lipid peroxidation. Hence the effect of active packaging was studied in irradiated meat. In food systems extend of rancidity is generally measured in terms of TBARS. Chicken packed in film not containing TP had higher TBARS values throughout the storage period (Fig. 3B).

Compared to UTP containing films CTP containing films were more efficient in minimizing rancidity in meat. After 12 days of chilled storage chicken stored in control films had TBARS value of more than 2 mg MDA/kg meat while in CTP packed films it was only 1.1 mg MDA/kg and was acceptable.

Packed inoculums studies

At present, meat industry uses various chemical additives in meat products to prevent growth of food spoilage bacteria, pathogens and extend the shelf life during refrigerated storage. Concern over the safety of chemical additives has arisen in recent years and consumers are demanding use of natural products as preservatives in foods. Active packaging is a viable option that can minimize the use of synthetic additives. Efficacy of films containing CTP/UTP in reducing the numbers of Gram positive micro flora was ascertained using minced chicken meat as a model food system. In case of *B. cereus*, active packaging led to complete elimination of *B. cereus* (Table 2). Films incorporated with both UTP and CTP were equally efficient in completely eliminating *B. cereus*. For *S. aureus* meat packed in neat films had a count of 2.44 log cfu/g which was maintained during chilled storage. After 7 days of chilled storage chicken packed in CTP/UTP incorporated films did not show the presence of any *S. aureus*. Compatibility of antimicrobial compounds released from the film with the food components have to be considered as residual antimicrobial activity could change due to complexes formed between them.

This study demonstrated that active PVA-Gelatin films can be made by incorporation of TP. CTP is a better additive as compared to UTP in development of functional active films. The films showed antibacterial activity against Gram-positive food pathogens. Active packaging is a promising system for the improvement of quality and shelf-life extension of food. Thus, this study demonstrates that the addition of natural extracts to bioactive biopolymers has great potential for being developed into functional packaging material for food and is a potential substitute for synthetic materials.

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