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Physicochemical and bioactivity of cross-linked chitosan–PVA film for food packaging applications

S. Tripathi, G.K. Mehrotra, P.K. Dutta*

Department of Chemistry, Motilal Nehru National Institute of Technology, Allahabad 211004, India

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ABSTRACT

This work aimed to develop a novel antimicrobial coating based on chitosan and PVA to evaluate its effect on minimally processed tomato by means of microbiological analyses. In this report an antimicrobial film was prepared by blending chitosan (CS) and poly(vinyl alcohol) (PVA) with glutaraldehyde as the cross-linker. The miscibility and morphology of the film were investigated by X-ray diffraction (XRD) and scanning electron microscopy (SEM). The thermal property of the chitosan–PVA film was examined by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). The microbiological screening has demonstrated the antimicrobial activity of the film against food pathogenic bacteria viz. *Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis*. The obtained results indicate the film may be a promising material for food packaging applications.

1. Introduction

There is growing interest in recent times to develop materials with film forming capacity and having antimicrobial properties which improve food safety and shelf-life. Antimicrobial packaging is one of the most promising active packaging systems [1–3]. The use of such packaging is not meant to be a substitute for good sanitation practices, but it should enhance the safety of food as an additional hurdle for the growth of pathogenic and/or spoilage microorganisms. Antimicrobial packaging shows promise as an effective method for the inhibition of certain bacteria in foods, but barriers to their commercial implementation continue to exist.

Chitosan (Cs) is derived from chitin found in the exoskeleton of insects, shells of crustaceans or various fungi. Chitin and chitosan have tremendous applications in the biomedical field due to their good biocompatibility, biodegradability, and capacity to form membranes, beads, fibers, scaffold and gels [4–9]. Chitosan inhibits the growth of a wide variety of fungi, yeasts, and bacteria. Chitosan also represents interesting properties such as excellent film forming capacity and gas and aroma barrier properties at dry conditions, which makes it a suitable material for designing food coatings and packaging structures [10]. All the above-mentioned properties also make chitosan a very good candidate to design novel antimicrobial active packaging technologies to improve the quality and safety and to extend the shelf-life of perishable foods [11]. Chitosan is readily soluble in various acidic solvents and has high antimicrobial activity against many pathogenic and spoilage microorganism, including Gram-positive and Gram-negative bacteria. Chemically modified or not, chitosan has a wide range in packaging applications, and particularly for food preservation applications [12]. Recently, a chitosan–starch film has been prepared using microwave treatment which may find potential application in food packaging [13].

Poly(vinyl alcohol) (PVA) a biodegradable, synthetic polymer, innocuous, non-carcinogenic and have good biocompatible properties. Poly(vinyl alcohol) has excellent film forming properties. Because of its good film forming and highly hydrophilic water-soluble with outstanding chemical stability it was blended different synthetic and natural polymers. It is used as a water-soluble film useful for packaging. Poly(vinyl alcohol) is completely water-soluble synthetic polymer and non-toxic. It is useful in many applications such as controlled drug delivery systems, recycling of polymers, film formation and packaging. Because of the good biological activities of chitosan and PVA, a combination of chitosan and PVA may have beneficial effects on the biological characteristics of blend films. The objective of this study was to develop chitosan-based antimicrobial film and to evaluate its antibacterial activity on food pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis*. In this paper we have reported the FTIR, XRD, DSC, TGA, SEM, and antibacterial activity.

2. Experimental

2.1. Materials

Chitosan (79% deacetylated) was obtained from Central Institute of Fisheries Technology (CIFT, Cochin). PVA and glutaraldehyde

* Corresponding author. Tel.: +91 532 2271272; fax: +91 532 2545341.
E-mail address: pkd_437@yahoo.com (P.K. Dutta).

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(GA) were obtained from CDH. The test strains, *E. coli* MTCC 1303, *S. aureus* ATCC 6538, and *B. subtilis* ATCC 6633, were used by IMTECH, Chandigarh.

2.2. Preparation of antimicrobial film

2.2.1. Preparation of chitosan homogeneous film

Chitosan was dissolved into 2 wt% acetic acid (HOAc) to prepare a 1 wt% chitosan solution. The mixture was stirred continuously at room temperature for 24 h to obtain a homogeneous solution. After that the homogeneous solution was filtered through a synthetic cloth to remove undissolved material and degassed by keeping the solution into vacuum oven for 3 h to remove the trapped air bubbles. The air bubble free chitosan solution was clear and transparent. The resulting solution was cast on a ceramic plate in a dust free environment and dried in air at room temperature for 48 h. The resulting film was washed with 2 wt% NaOH solution to neutralize HOAc, and then with distilled water and kept in a 2 wt% H_2SO_4 cross-linking solution for 1 h. After fully cross-linking, the film was washed with distilled water.

2.2.2. Preparation of chitosan/poly(vinyl alcohol) films

PVA was dissolved in hot water at 100 °C and stirred for 6 h to prepare a 10 wt% solution and cast on a chitosan film. The resulting film was kept in desiccators at room temperature for 24 h, and then cross-linked using 0.01 wt% GA and 0.5 wt% sulfuric acid (H_2SO_4)

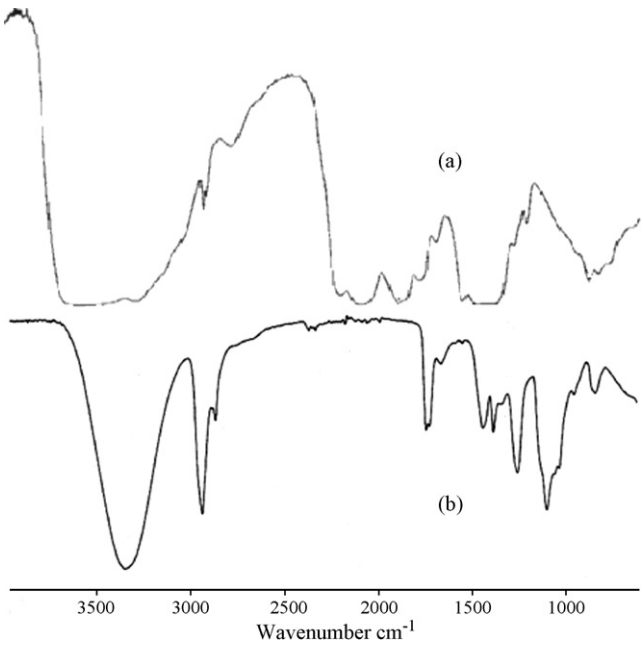

Fig. 1. FTIR spectra of (a) chitosan and (b) chitosan–PVA film.

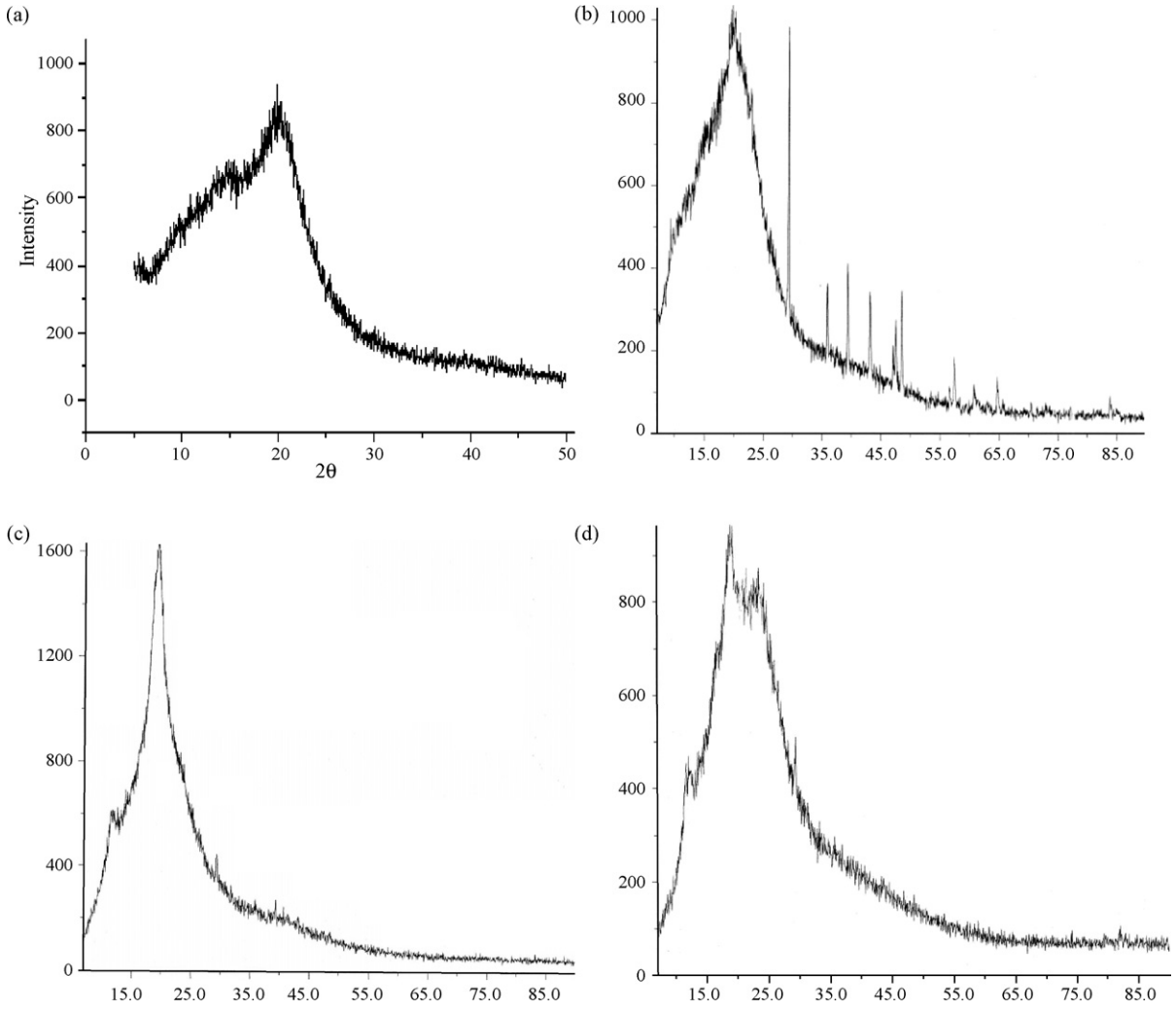

Fig. 2. X-ray diffraction patterns of chitosan (a) and chitosan–PVA films (b) 1:1, (c) 1:3, and (d) 3:1.

as a catalyst for 1 h. The final film was obtained after washing with distilled water.

2.3. Characterizations

The infrared spectra were recorded on Perkin Elmer RX1 FTIR spectrophotometer model. X-ray diffraction patterns were analyzed for the films dried naturally in room temperature by Rigaku X-ray diffractometer. The X-ray source was Ni-filtered Cu Kα radiation (40 kV, 30 mA). The film samples were scanned from 10° to 60° 2θ at a scanning rate of 3° min^{-1}. Thermal degradation processes were investigated using TGA (Perkin Elmer Pyris 6) at a heating rate of 5 °C under Ar atmosphere. Differential scanning calorimetry (DSC) with STA 600 thermal analyzer was used to examine the thermal property of chitosan–PVA film. The morphology of the bioactive films was examined by a scanning electron microscopy (JEOL) Model JSM-6390LV.

3. Results and discussion

3.1. FTIR spectroscopy

FTIR spectra of chitosan and chitosan–PVA antimicrobial film are shown in Fig. 1. Stretching vibration spectra of the amide group of chitosan films appear at 1560 cm^{-1}. The change in the characteristic shape of the chitosan spectrum as well as shifting of peak to a lower frequency range due to hydrogen bonding between –OH of PVA and –OH or $–NH_2$ of chitosan in the blended films. Kim et al. [14] reported that the crystallization-sensitive band of PVA at 1140 cm^{-1} is observed with a similar intensity without a significant change in frequency with PVA and blended films.

3.2. X-ray diffraction

X-ray diffraction is a proven tool to study crystal lattice arrangements and yields very useful information on degree of sample crystallinity. X-ray patterns of chitosan and chitosan–PVA blends are shown in Fig. 2. The diffraction peak of chitosan is at around 10° and 20° of 2θ. X-ray diffraction patterns of chitosan:PVA (1:3) show diffractive peak in the region from $2\theta = 11°$ to 48° with the highest intensity of about 1452 counts at $2\theta = 19°$. Chitosan:PVA (1:1) shows highest peak intensity of about 1085 counts at $2\theta = 19.91°$ and chitosan:PVA (3:1) shows intensity peak of about 863 counts at $2\theta = 18.96°$. From these diffractograms, it is obvious that chitosan:PVA (1:3) is more crystalline than other two samples.

3.3. Differential scanning calorimetry and thermogravimetric analysis

DSC thermograms of chitosan and chitosan–PVA film are presented in Fig. 3. DSC thermogram of chitosan film (Fig. 3a) exhibited sharp exothermic peaks at 290 °C, indicating decomposition of chitosan. The endothermic peak at 100 °C is attributed to water loss, represent the energy required to vaporize water present in the film. All chitosan films were degraded at <280 °C to 300 °C, which agrees well with the result of our study [15]. The broad exothermic peak of chitosan–PVA film (Fig. 3b) at 310 °C corresponds to thermal degradation of chitosan film. The endothermic peak at 60 °C is associated with loss of water from the chitosan–PVA film. The results indicated that the structure of chitosan–PVA film is approximately similar to chitosan film.

Thermographs of chitosan and chitosan–PVA film are shown in Fig. 4. The weight loss at 40–150 °C is due to the moisture vaporization. The other weight loss at 200–300 °C is due to the degradation of chitosan film. There are also two significant weight losses are observed in the TGA curve of chitosan–PVA film. One weight loss at 40–120 °C is due to the moisture vaporization. The second weight loss at 170–300 °C is due to the thermal degradation of chitosan–PVA film.

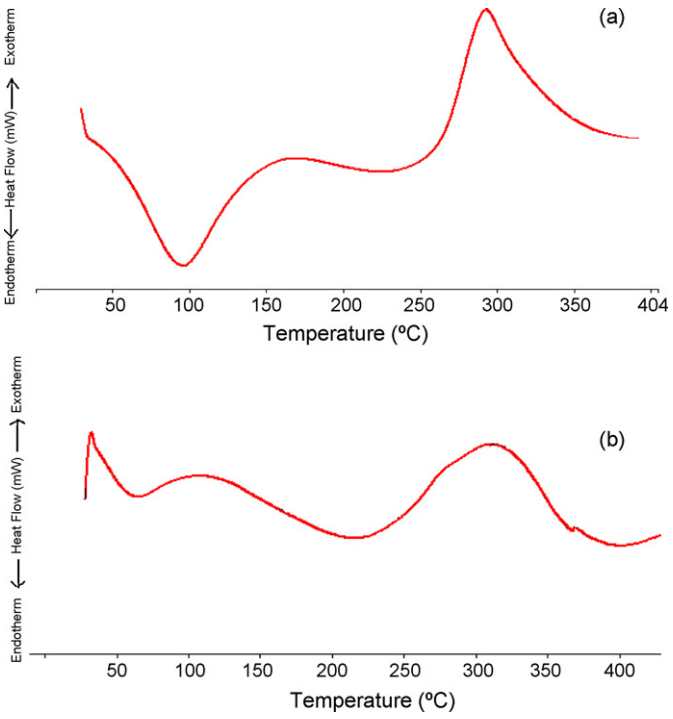

Fig. 3. DSC thermogram of (a) chitosan and (b) chitosan–PVA film.

3.4. Scanning electron microscopy

Scanning electron micrographs (SEMs) of chitosan–PVA film are shown in Fig. 5. The pure PVA film exhibit characteristic patterns on the film surface. These patterns represent the withered ghost granules of starch. The surface of chi-

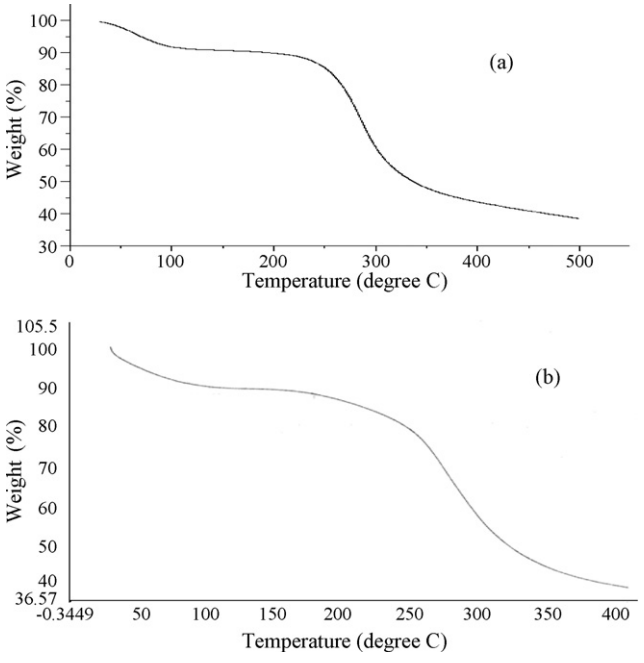

Fig. 4. TGA curve of (a) chitosan and (b) chitosan–PVA film.

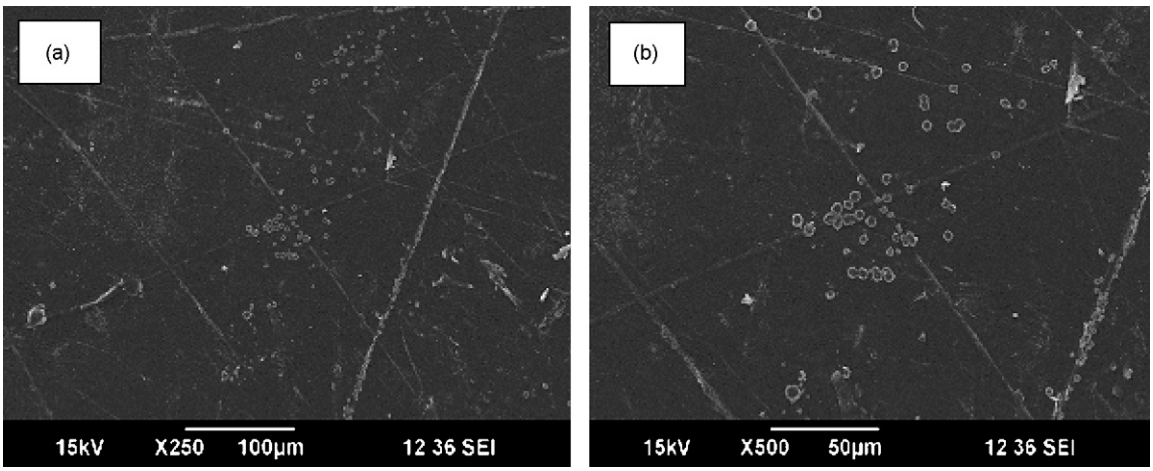

Fig. 5. SEM images of chitosan–PVA film.

tosan films to be relatively smooth, to be homogeneous and to be a continuous matrix without any pores or cracks with good structural integrity. It is flat and compact with very sparsely distributed small particles without any phase separation. The chitosan–PVA film also exhibits such patterns. Chitosan microdomains are dispersed within PVA matrix in the blend film with relatively good interfacial adhesion between the two components.

3.5. Antimicrobial activity

The chitosan–PVA solution for preservation of fruit (tomato): (a) as a whole and (b) into 2 pieces is shown in Fig. 6. Inhibitory effect of chitosan–PVA solution and inhibitory effect of chitosan–PVA film against *E. coli, S. aureus* and *B. subtilis* are shown in Figs. 7(a)–(c) and 8(a)–(c). The inhibitory effect was measured based on clear zone surrounding circular film strips/solution. Measurement of clear zone diameter included diameter of film strips/solutions, therefore, the values were always higher than the diameter of film strips/solutions whenever clearing zone was present. If there is no clear zone surrounding, we assumed that there is no inhibitory zone, and furthermore, the diameter was valued as zero.

In terms of surrounding clearing zone, chitosan–PVA film did not show inhibitory effect against all tested microorganisms. The chitosan–PVA film forming solution showed a clear inhibitory zone of 1.5, 1.2, and 1.4 cm against *E. coli, S. aureus* and *B. subtilis* respectively. However, increasing level of starch at higher concentration did not reveal significant an increased inhibitory effect. It was generally caused by the maximum capability of chitosan polymer to carry active agents beside the occurrence of functional groups interaction phenomenon. The antimicrobial effect of chitosan occurred without migration of active agents [16]. As chitosan is in a solid form, therefore, only organisms in direct contact with the active sites of chitosan is inhibited. Chitosan is incapable to diffuse through the adjacent agar media [17]. The agar diffusion test is a method commonly used to examine antimicrobial activity regarding the diffusion of the compound tested through water-containing agar plate. The diffusion itself is dependent on the size, shape and polarity of the diffusion material. The chemical structure and the cross-linking level of the films also affect this phenomenon [18]. The chitosan–PVA solution shows stronger inhibitory effect against *E. coli* and *B. subtilis* than *S. aureus*. Furthermore, it was found that the bioactive chitosan–PVA film can be used to extend food shelf-life.

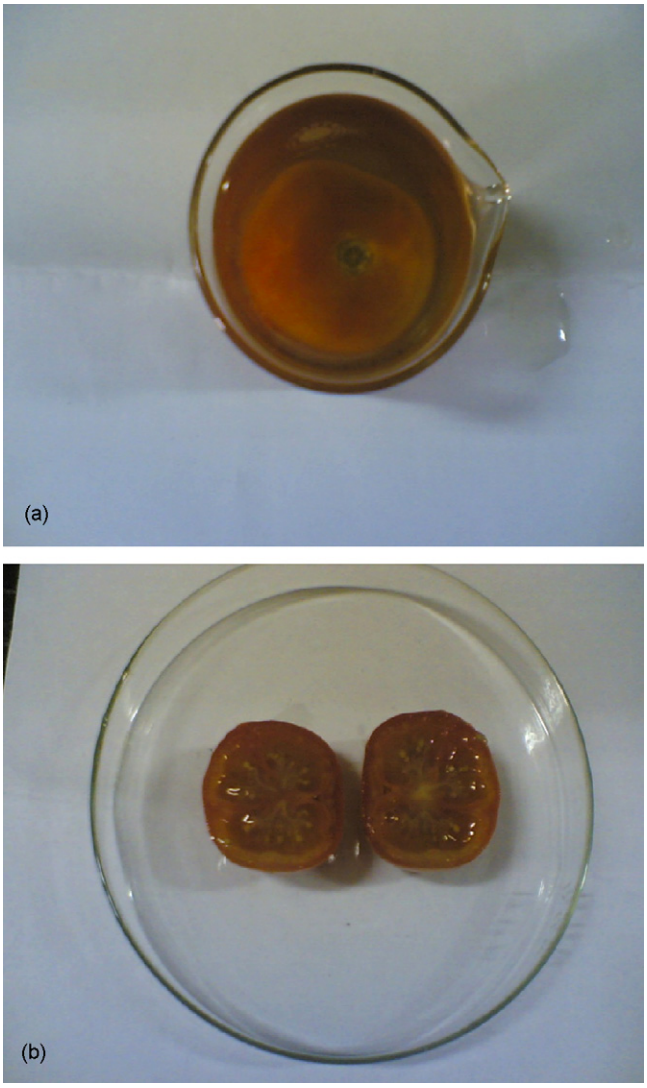

Fig. 6. Chitosan–PVA solution for preservation of fruit (tomato): (a) as a whole and (b) into 2 pieces.

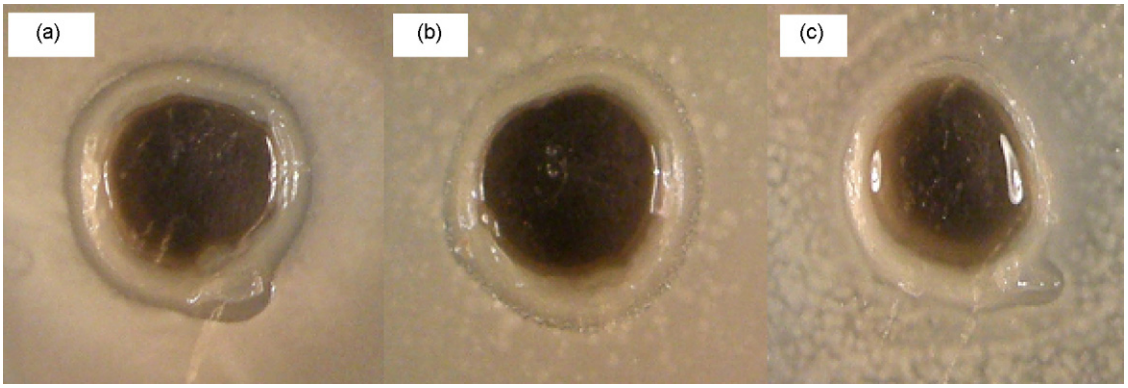

Fig. 7. Inhibitory effect of chitosan–PVA solution against (a) *E. coli,* (b) *S. aureus* and (c) *B. subtilis.*

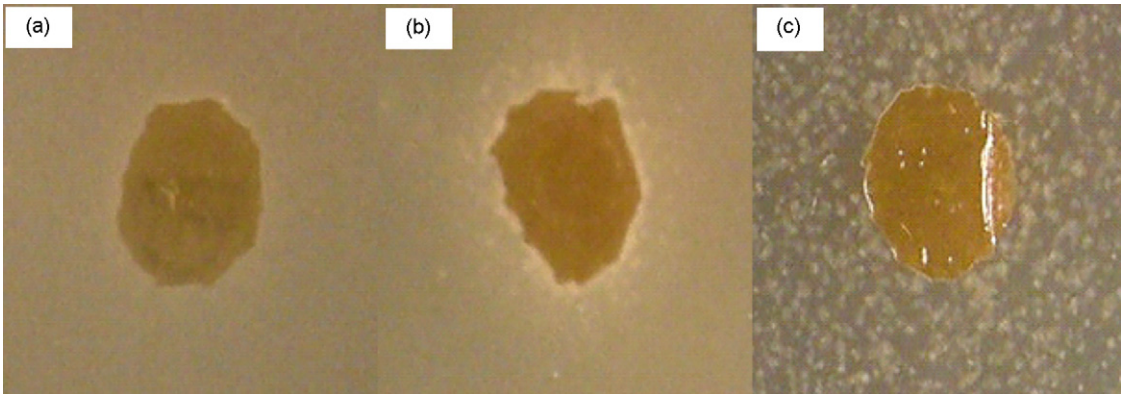

Fig. 8. Inhibitory effect of chitosan–PVA film against (a) *E. coli,* (b) *S. aureus* and (c) *B. subtilis*.

4. Conclusions

Chitosan-based antimicrobial films consisting of chitosan and PVA were prepared by solution casting method. The FTIR results pointed out that there is a molecular miscibility between PVA and chitosan. The results of this experiment showed that the use of an antimicrobial coating consisting of chitosan–added PVA is a viable alternative in shelf-life extension of minimally processed tomato combined to other types of controls, such as quality raw material, hygienic processing conditions and storage temperatures. Chitosan-based antimicrobial film may be a promising material as a packaging film.

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