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Preparation and physicochemical evaluation of chitosan/poly(vinyl alcohol)/pectin ternary film for food-packaging applications

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ABSTRACT

Chitosan/poly(vinyl alcohol)/pectin ternary film was prepared by solution casting method in this study. The prepared ternary film was characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and X-ray diffraction (XRD). The characteristic change of shapes in the IR spectra are observed in a strong peak at 1620 cm^{-1} for the interchain or intermolecular ionic salt bonds between amino groups of chitosan and carboxyl groups of pectin of the ternary film. The XRD result proves that the chitosan–poly(vinyl alcohol)–pectin ternary film is crystalline. The result of SEM indicates that the surface of chitosan–poly(vinyl alcohol)–pectin ternary film is rough, and heterogeneous. The thermogravimetric analysis (TGA) depicts the weight losses at 200–300 °C resulting from ternary film for degradation of chitosan molecule. The microbiological screening has demonstrated the antimicrobial activity of the film against pathogenic bacteria viz., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas*, and *Candida albicans* against the measurement of clear zone diameter included diameter of film strips, the values of which were always higher than the diameter of film strips. Overall, the ternary film happens to be a suitable material for food-packaging applications.

1. Introduction

Recently antimicrobial packaging has emerged as one of the most reliable and promising tool in the search for the next generation of 'active' packaging (Brody, 2001; Dutta, Tripathi, Mehrotra, & Dutta, 2009; Salleh, Muhamad, & Khairuddin, 2007; Tripathi, Mehrotra, & Dutta, 2008). Food quality and safety are major concerns in the food industry as consumers prefer fresher and minimally processed products. In particular, bacterial contamination of ready to eat products constitutes one of the most serious health hazards to human population. Antibacterial sprays or dips have been formulated to overcome many such contaminations (Ouattara, Simard, Piette, Begin, & Holley, 2000). However, direct surface application of antibacterial substances has some limitations because the active substances can be neutralized, evaporated or diffused inadequately into the bulk of food (Siragusa & Dickson, 1992; Torres, Motoki, & Karel, 1985).

Edible films or coatings have been investigated for their abilities to retard moisture, oxygen, aromas, and solute transports (Gennadios & Weller, 1990). It is one of the most effective methods of maintaining food quality. This is further improved by film carrying food additives such as antioxidants, antimicrobial, colorants, flavors, fortified nutrient, and spices (Pena & Torres, 1991). In many cases, the agents being carried are slowly released into the food surface and therefore remain at high concentrations for extended periods of time (Coma, Sebti, Pardon, Deschamps, & Pichavant, 2001).

Chitosan (poly-β-1, 4-linked glucosamine) is a cationic polysaccharide derived by alkaline deacetylation of chitin, a major structural component of the exoskeletons of crustaceans such as crab, lobster, crawfish, and shrimp (Kurita, 2006). As the second most abundant natural biopolymer, chitosan has attracted increased attention for its commercial applications in the biomedical, chemical, food, cosmetic, and many other industries due to its biocompatibility, biodegradability, and non-toxicity (Jayakumar, Prabaharan, Reis, & Mano, 2005; No, Meyers, Prinyawiwatkul, & Xu, 2007). Chitosan film may be used in wound dressing, tissue repairing, and food packaging (Jayakumar, Nwe, Tokura, & Tamura, 2007; Khan, Peh, & Ch'ng, 2000; Sezer et al., 2007; Tripathi, Mehrotra, & Dutta, 2009).

Chitosan is well known for its excellent film-forming property and broad antimicrobial activity against bacteria and fungi (Cagri, Ustunol, & Ryser, 2004; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003). The exact antimicrobial mechanism of chitosan is still unclear, but several have been proposed. The most feasible hypothesis is the leakage of cellular proteins and other intracellular constituents caused by the interaction between the positively charged chitosan and negatively charged microbial cell membranes. Other mechanisms proposed are the inhibition of microbial growth and toxin production by the chelation of essential metals and nutrients, spore components, as well as the penetration of

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the nuclei of the microorganisms, which leads to the interference of mRNA and protein synthesis (Rabea et al., 2003).

PVA is water-soluble synthetic polymer. Due to the characteristics of easy preparation, good biodegradability, excellent chemical resistance, and good mechanical properties, PVA has been used in many biomaterial applications (Park, Park, & Ruckenstein, 2001). Chitosan contains hydroxyl and amine groups, it is potentially miscible with PVA due to the formation of hydrogen bonds (Chuang, Young, Yao, & Chiu, 1999; Miya, Yoshikawa, Iwamoto, & Mima, 1983).

Pectin is a natural, non-toxic and anionic polysaccharide extracted from cell walls of most plants. Pectin is the methylated ester of polygalacturonic acid. The degree of methoxylation (DM) is used to classify the pectins as high methoxyl pectins (DM > 50) and low methoxyl pectins (DM < 50) (Sakai, Sakamoto, Hallaert, & Vandamme, 1993; Thakur, Singh, & Handa, 1997). The pectin, by itself or by its gelling properties, was employed in pharmaceutical industry, health promotion and treatment. It has been used potentially as a carrier for drug delivery to the gastrointestinal tract, such as matrix tablets, gel beads, film-coated dose form.

Combinations of pectin and chitosan form a polyelectrolyte complex (PEC) at pH values in the range of 3–6 (Macleod, Collett, & Fell, 1999; Meshali & Gabr, 1993). In addition to the formation of a PEC, pectin and chitosan also interact by hydrogen bonding at low pH values (pH < 2). At these pH values, pectin will be unionized and the importance of electrostatic interactions is suppressed, and an interaction between pectin and chitosan will probably take place via hydrogen bonding (Nordby, Kjøniksen, Nystrøm, & Roots, 2003).

The objective of this study was to develop and characterize physicochemical properties of chitosan based ternary film. The antibacterial activity was also evaluated on pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis, Pseudomonas*, and *Candida albicans*. We herein report the preparation and physicochemical evaluation of the ternary chitosan/poly(vinyl alcohol)/pectin films based on the FTIR, XRD, TGA, and SEM investigations and its antibacterial activity.

2. Materials and methods

2.1. Experimental materials

Chitosan (79% deacetylated) was obtained from Central Institute of Fisheries Technology (CIFT, Cochin). PVA and pectin was obtained from CDH. The test strains, *E. coli* MTCC 1303, *S. aureus* ATCC 6538, and *B. subtilis* ATCC 6633, used in this study were obtained from IMTECH, Chandigarh.

2.2. Preparation of chitosan/PVA/pectin ternary film

One gram chitosan powder was added into a 100 ml of 0.1 M acetic acid and the mixture was stirred to form a 1 wt% clear chitosan solution. Meanwhile, 1 g PVA powder was charged into 100 ml at 80 °C purified water while stirring to form a 1% clear PVA solution. Then, chitosan and PVA solutions were blended together to form a homogeneous chitosan/PVA binary blend solution. Pectin powder (1 g, 1.5 g, and 2 g) was charged into 100 ml purified water and stirred to form a 1 wt%, 1.5 wt%, and 2 wt% pectin solution, respectively. Different weight percentages (as indicated in Table 1) of chitosan/PVA and pectin solutions were homogenously blended to form a ternary blend solution. Then the ternary blend solutions were poured into the glass plate. After 48 h setting, these chitosan/PVA/pectin ternary blend solutions were de-bubbled and then transferred into a 60 °C oven for about 24 h drying. After that, these blend films were vacuum dried for 24 h at 80 °C in order to remove the residues of water and acetic acid.

Table 1
Compositions of chitosan/PVA/pectin ternary films.

Sample ID	Blending ratio
P0	Pectin solution 0 g + chitosan/PVA solution 2 g
P1	Pectin solution 1 g + chitosan/PVA solution 2 g
P2	Pectin solution 1.5 g + chitosan/PVA solution 2 g
P3	Pectin solution 2 g + chitosan/PVA solution 0 g

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). Thermal degradation processes were investigated using TGA (Perkin–Elmer Pyris 6). The morphology of the chitosan–poly(vinyl alcohol)–pectin ternary film was examined by a scanning electron microscopy (JEOL) Model JSM-6390LV.

3. Results and discussion

3.1. FTIR spectroscopy

The infrared spectra of chitosan as well as its films viz., P0, P2, and P3 are depicted in Fig. 1. In the IR spectra, stretching vibration of the amide group of chitosan film appears 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 is evident in P0 film. In the spectrum of P2 film two relatively broad bands appear in the region 1800–1600 cm^{-1} which can be ascribed to the overlapping of bands due to amino and carboxylic groups of the interacting molecules. A strong peak at 1620 cm^{-1} (asymmetric stretching vibration of carboxylate) also indicates the interchain or intermolecular ionic salt bonds, i.e. polyelectrolyte complex (PEC) between amino groups of chitosan and carboxyl groups of pectin (Rashidova et al., 2004). In the other words, it has been assumed that PEC formation proceeded at the expense of electrostatic interaction between the positively charged amino groups on C_2 of the chitosan pyranose ring and the negatively charged carboxyl groups on the C_5 of the pectin pyranose ring. There was no significant

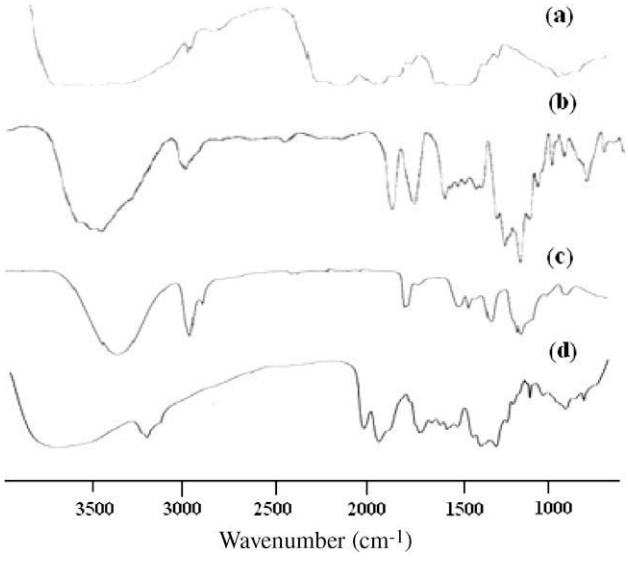

Fig. 1. FTIR spectra of (a) chitosan, (b) P3, (c) P0, and (d) P2 films.

change observed due to the presence of poly(vinyl alcohol) in the ternary film.

3.2. X-ray diffraction

The X-ray diffraction patterns of chitosan, P0, P2, and P3 films are shown in Fig. 2. Chitosan exhibits three reflection falls at $2\theta = 11°$, 20°, and 22°. Chitosan shows very broad lines especially for the smaller diffraction angles, thereby indicating that long range disorder is found in polymer samples. The P0 film shows two diffraction peaks at about 19° and 22°, which are characteristic of the crystalline peaks of PVA and the hydrated crystalline structure of chitosan (Park et al., 2001), respectively. P3 film shows diffraction peak at 21° indicating its largely amorphous nature. P2 film shows the intensity of diffraction peaks at 19° for PVA appearing as sharp while the peak due to hydrated crystalline structure of chitosan at 22° becoming flat. This result proves that the chitosan–poly(vinyl alcohol)–pectin ternary film is crystalline. This phenomenon is largely due to presence of strong intermolecular interactions such as hydrogen bonds as well as ionic interaction among chitosan, poly(vinyl alcohol), and pectin molecules.

3.3. Thermogravimetric analysis

Fig. 3 shows TGA curves of chitosan, P0, P2, and P3 films. Two weight losses are observed in the chitosan TGA curve. The weight loss at 50–150 °C is due to the moisture vaporization. The other weight loss at 200–300 °C is due to the degradation of chitosan molecule. These depict the weight losses resulting from chitosan, poly(vinyl alcohol), and pectin. The first weight loss at 50–150 °C is due to the moisture vaporization while the second weight loss at 250–350 °C is due to the thermal degradation of chitosan, poly(vinyl alcohol), and pectin. The third weight loss at 400–450 °C is due to the by-products generated by poly(vinyl alcohol) during the TGA thermal degradation process as mentioned by Hay (Holland & Hay, 2001).

3.4. Scanning electron microscopy

Fig. 4 shows SEM photographs of the top surface of the chitosan–poly(vinyl alcohol)–pectin ternary film. The result of SEM indicates that the surface of chitosan–poly(vinyl alcohol)–pectin ternary film is rough, and heterogeneous. It may have resulted from the reorientation of polar functional groups toward the top surface of chitosan–poly(vinyl alcohol)–pectin ternary film. This phenomenon implies that the top surface of the film is hydrophilic. Some immiscibility among the components has also been observed in the film.

3.5. Antimicrobial activity

Inhibitory effect of chitosan/poly(vinyl alcohol)/pectin ternary film against *E. coli, S. aureus*, *B. subtilis, Pseudomonas*, and *C. albicans* are shown in Fig. 5(a)–(e). The inhibitory effect was measured based on clear zone surrounding circular film strips. Measurement of clear zone diameter included diameter of film strips, therefore, the values were always higher than the diameter of film strips 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/poly(vinyl alcohol)/pectin ternary film did not show inhibitory effect against all tested microorganisms. The antimicrobial effect of chitosan occurred without migration of active agents (Brody, Strupinsky,

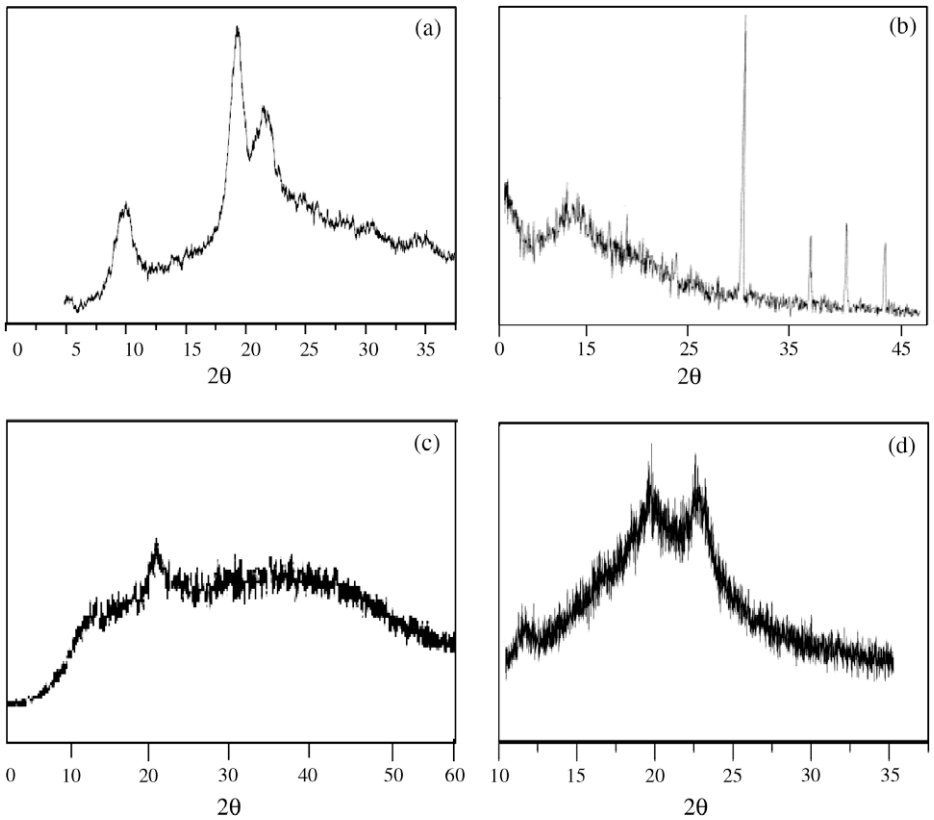

Fig. 2. XRD analysis of (a) chitosan, (b) P0, (c) P3, and (d) P2 film.

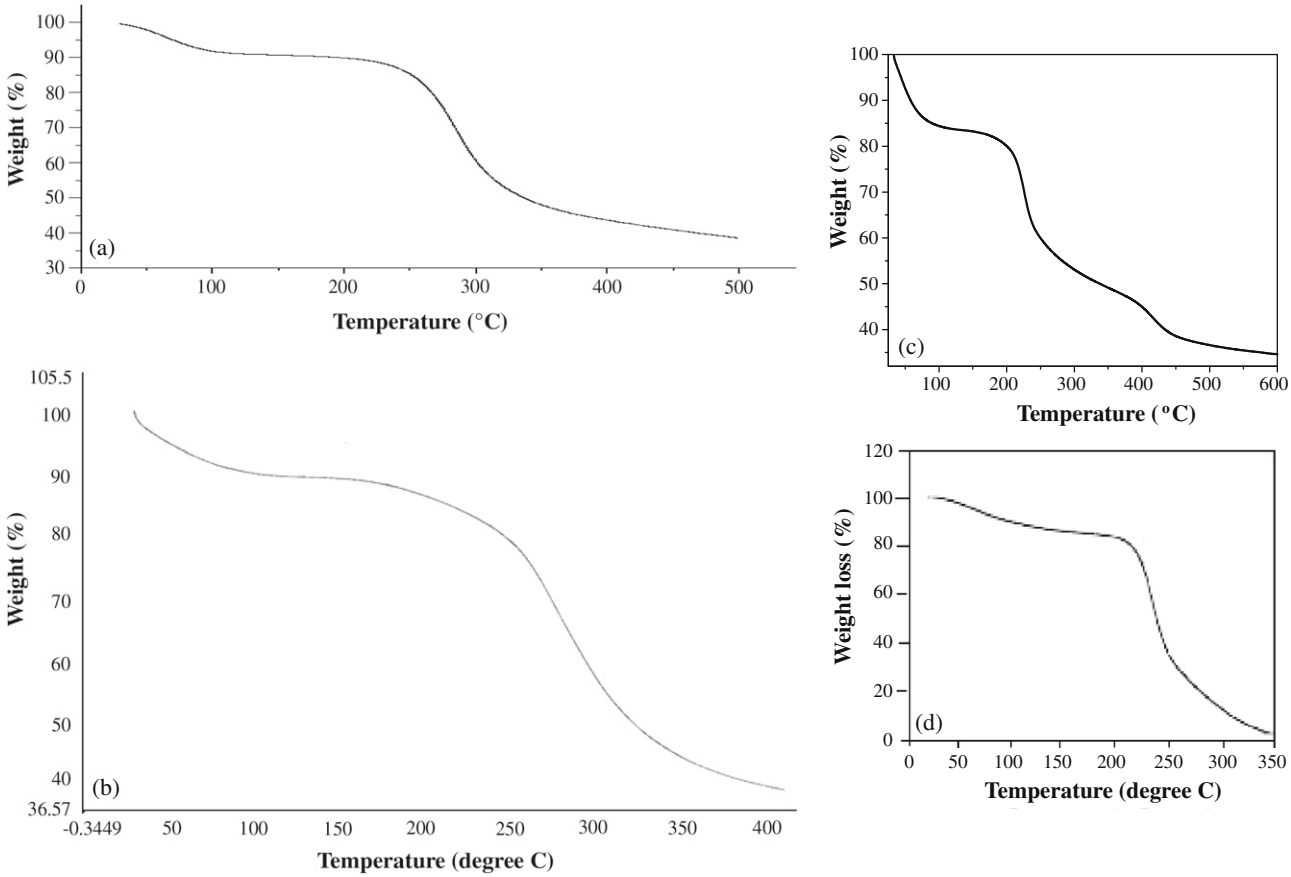

Fig. 3. TGA curves of chitosan (a), P0 (b), P2 (c), and P3 (d) films.

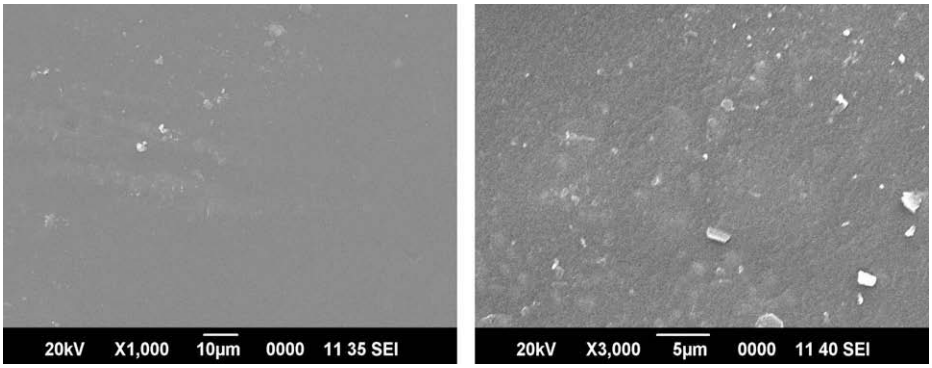

Fig. 4. (a and b) SEM images of chitosan–poly(vinyl alcohol)–pectin ternary film.

& Kline, 2001). As chitosan is in a solid form, therefore, only organisms in direct contact with the active sites of chitosan are inhibited. Chitosan is incapable of diffusing through the adjacent agar media (Coma et al., 2002). 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 crosslinking level of the films also affect this phenomenon (Cagri, Ustunol, & Ryser, 2001). Furthermore, it has been found that the chitosan based ternary film can be used to extend food shelf-life.

4. Conclusions

Chitosan/poly(vinyl alcohol)/pectin ternary film was prepared successfully by solution casting method. IR spectra and SEM analyses of ternary film have indicated the PEC (polyelectrolyte complex) formation between pectin and chitosan. The XRD study shows that the chitosan–poly(vinyl alcohol)–pectin ternary film is crystalline. The result of SEM indicates that the surface of the ternary film is rough, and heterogeneous. The thermal study is also quite reasonable for its food-packaging applications which depicts the weight losses at 200–300 °C resulting from ternary film for degradation of chitosan molecule. The microbiological screening

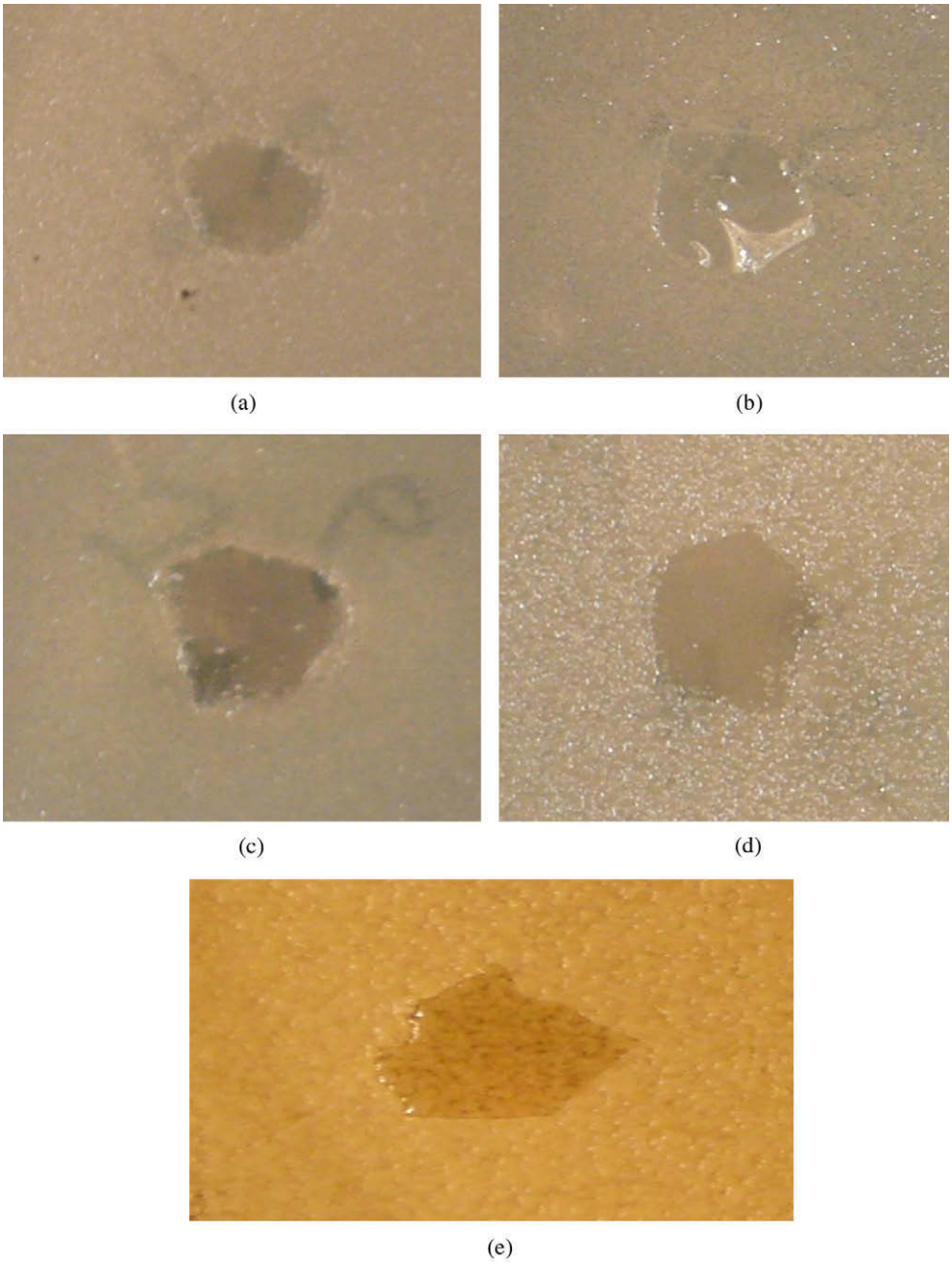

Fig. 5. Inhibitory effect of chitosan/poly(vinyl alcohol)/pectin ternary film against (a) *E. coli*, (b) *S. aureus*, (c) *B. subtilis*, (d) *Pseudomonas*, and (e) *Candida albicans*.

has demonstrated the positive antimicrobial activity of the film against pathogenic bacteria. The study has affirmed the potential of chitosan/poly(vinyl alcohol)/pectin ternary film as a universal antimicrobial food-packaging material.

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References

Brody, A. L. (2001). What's the hottest food packaging technology today? *Food Technology, 55*, 82–84.

Brody, A. L., Strupinsky, E. R., & Kline, L. R. (2001). *Active packaging for food applications*. Lancaster: Technomic Publishing Inc. p. 218.

Cagri, A., Ustunol, Z., & Ryser, E. T. (2001). Antimicrobial, mechanical, and moisture barrier properties of low pH whey protein-based edible films containing p-aminobenzoic or sorbic acids. *Journal of Food Science, 66*, 865–870.

Cagri, A., Ustunol, Z., & Ryser, E. T. (2004). Antimicrobial edible films and coatings. *Journal of Food Protection, 67*, 833–848.

Chuang, W. Y., Young, T. H., Yao, C. H., & Chiu, W. Y. (1999). Properties of the poly(vinyl alcohol)/chitosan blend and its effect on the culture of fibroblast in vitro. *Biomaterials, 20*, 1479–1487.

Coma, V., Marital-Gross, A., Garreau, S., Copinet, A., Salin, F., & Deschamps, A. (2002). Edible antimicrobial films based on chitosan matrix. *Journal of Food Science, 67*, 1162–1169.

Coma, V., Sebti, I., Pardon, P., Deschamps, A., & Pichavant, F. H. (2001). Antimicrobial edible packaging based on cellulosic ethers, fatty acids, and nisin incorporation to inhibit *Listeria innocua* and *Staphylococcus aureus*. *Journal of Food Protection, 64*(4), 470–475.

Dutta, P. K., Tripathi, S., Mehrotra, G. K., & Dutta, J. (2009). Perspectives for chitosan based antimicrobial films in food applications. *Food Chemistry, 114*, 1173–1182.

Gennadios, A., & Weller, C. L. (1990). Edible films and coatings from wheat and corn proteins. *Food Technology, 44*, 63–69.

Holland, B. J., & Hay, J. N. (2001). The thermal degradation of poly(vinyl alcohol). *Polymers, 42*, 6775–6783.

Jayakumar, R., Nwe, N., Tokura, S., & Tamura, H. (2007). Sulfated chitin and chitosan as novel biomaterials. *International Journal of Biological Macromolecules, 40*, 175–181.

Jayakumar, R., Prabaharan, M., Reis, R. L., & Mano, J. F. (2005). Graft copolymerized chitosan – present status and applications'. *Carbohydrate Polymers, 62*, 142–158.

Khan, T., Peh, K., & Ch'ng, H. (2000). Mechanical, bioadhesive strength and biological evaluations of chitosan films for wound dressing. *Journal of Pharmacy & Pharmaceutical Sciences, 3*, 303–311.

Kurita, K. (2006). Chitin and chitosan: Functional biopolymers from marine crustaceans. *Marine Biotechnology, 8*, 203–226.

Macleod, G. S., Collett, J. H., & Fell, J. T. (1999). The potential use of mixed films of pectin, chitosan and HPMC for bimodal drug release. *Journal of Controlled Release, 58*, 303–310.

Meshali, M. M., & Gabr, K. E. (1993). Effect of interpolymer complex formation of chitosan with pectin or acacia on the release behaviour of chlorpromazine HCl. *International Journal of Pharmaceutics, 89*, 177–181.

Miya, M., Yoshikawa, S., Iwamoto, R., & Mima, S. (1983). Mechanical properties of poly (vinyl alcohol)–chitosan blend films. *Kobunshi Ronbunshu, 40*, 645–651.

No, H. K., Meyers, S. P., Prinyawiwatkul, W., & Xu, Z. (2007). Applications of chitosan for improvement of quality and shelf life of foods: A review. *Journal of Food Science, 72*, R87–R100.

Nordby, M. H., Kjøniksen, A. L., Nystrøm, B., & Roots, J. (2003). Thermoreversible gelation of aqueous mixtures of pectin and chitosan. Rheology. *Biomacromolecules, 4*, 337–343.

Ouattara, B., Simard, R. E., Piette, G., Begin, A., & Holley, R. A. (2000). Diffusion of acetic and propionic acids from chitosan-based antimicrobial packaging films. *Journal of Food Science, 65*(5), 768–773.

Park, J. S., Park, J. W., & Ruckenstein, E. (2001). Thermal and dynamic mechanical analysis of PVA/MC blend hydrogels. *Polymers, 42*, 4271–4280.

Pena, D. C., & Torres, J. A. (1991). Sorbic acid and potassium sorbate permeability of an edible methylcellulose–palmitic acid films: Water activity and pH effects. *Journal of Food Science, 56*(2), 497–499.

Rabea, E. I., Badawy, M. E., Stevens, C. V., Smagghe, G., & Steurbaut, W. (2003). Chitosan as antimicrobial agent: Applications and mode of action. *Biomacromolecules, 4*, 1457–1465.

Rashidova, S. S., Milusheva, R. Y., Semenova, L. N., Mukhamedjanova, M. Y., Voropaeva, N. L., Vasilyeva, S., et al. (2004). Characteristics of interactions in the pectin–chitosan system. *Chromatographia, 59*, 779–782.

Sakai, T., Sakamoto, T., Hallaert, J., & Vandamme, E. J. (1993). Pectin, pectinase, and protopectinase-production, properties, and applications. *Advances in Applied Microbiology, 39*, 213–294.

Salleh, E., Muhamad, I., & Khairuddin, N. (2007). Preparation, characterization and antimicrobial analysis of antimicrobial starch-based film incorporated with chitosan and lauric acid. *Asian Chitin Journal, 3*, 55–68.

Sezer, Ali Demir, Hatipoglu, Fatih, Cevher, Erdal, Oğurtan, Zeki, Bas, Ahmet Levent, & Akbuğa, Jülide (2007). Chitosan film containing fucoidan as a wound dressing for dermal burn healing: Preparation and in vitro/in vivo evaluation. *AAPS PharmSciTech, 8*, E94–E101.

Siragusa, G. R., & Dickson, J. S. (1992). Inhibition of *Listeria monocytogenes* on beef tissue by application of organic acids immobilized in a calcium alginate gel. *Journal of Food Science, 57*(2), 293–296.

Thakur, B. R., Singh, R. K., & Handa, A. K. (1997). Chemistry and uses of pectin review. *Critical Reviews in Food Science and Nutrition, 37*, 47–73.

Torres, J. A., Motoki, M., & Karel, M. (1985). Microbial stabilization of intermediate moisture food surfaces: I. Control of surface preservative concentration. *Journal of Food Processing and Preservation, 9*, 75–92.

Tripathi, S., Mehrotra, G. K., & Dutta, P. K. (2009). Physicochemical and bioactivity of crosslinked chitosan–PVA film for food packaging applications. *International Journal of Biological Macromolecule, 45*, 372–376.

Tripathi, S., Mehrotra, G. K., & Dutta, P. K. (2008). Chitosan based antimicrobial films for food packaging applications. *E-Polymers, 093*, 1–7.