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RESEARCH PAPER

Antimicrobial activity of nanocomposite films for storage of foods

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SUMMARY : Food packaging plays a major role in the food supply chain and performs tasks of fulfilling demands of manufacturers and consumers. Polymers are widely used in the food packaging industry because of its flexibility, mechanical strength and low cost. In order to prevent the contamination by undesirable microbes, novel packaging technologies like antimicrobial packaging are developed which prolongs the shelf-life of fresh foods. Nanotechnology is expected to play the greatest and most immediate role in the development of new food packaging materials. A key purpose of antimicrobial nano packaging is to control growth of microorganisms unlike conventional food packaging systems which are used for shelf life extension, quality maintenance, and safety assurance. Antimicrobial compounds are coated, blended or immobilized on the surface of polymer films. The present study was undertaken for developing and evaluating nanocomposite films incorporated with titanium oxide (TiO_2) as antimicrobial agent by melt compounding. Different polymer based nanocomposite films of (1-2 per cent) concentration and thicknesses were fabricated and fresh cut carrots were stored in the developed films without spoilage.

KEY **W**ORDS : Food packaging, Antimicrobial nano packaging, Nanocomposite films

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Antimicrobial packaging is an emerging technology that has significant impact on shelf-life extension and food safety. It is a type of active packaging that reduces, inhibits or retards the growth of microorganism which contaminates the packaged food. Antimicrobial agents in particular can be incorporated into a packaging system which can be achieved by simple blending with the packaging materials, as well as immobilisation or coating depending on the characteristics of packaging systems, antimicrobial agents and the food (Yang *et al*., 2010). The antimicrobial film when placed on a solid agar medium containing the test microorganism, a clear zone surrounding the film indicated the antimicrobial diffusion from the film and lack of growth under the film (Appendini and Hotchkins, 2002).

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Nanocomposites are a new class of mineral-field plastics that contain relatively small amounts (<10%) of nanometer-sized particles. The particles, due to their extremely high aspect ratios (about 100-15000) and high surface area (750-800 m^2/g) promise to improve structural, mechanical, flame retardant, thermal and barrier properties (Arora and Padua, 2010). Nano packaging can also be designed to release antimicrobials, antioxidants, enzymes, flavours and neutraceuticals to extend the shelf life of food products. Wang *et al.* (2010) reported that the cells of *E. coli* attached by the TiO_2 nanoparticles had severe damage. The present study was undertaken with the objectives of developing high density polyethylene and polypropylene based polymer films of 60 and 80µ thicknesses with (1-2%) titanium oxide as antimicrobial agent by melt compounding and evaluating the antimicrobial and mechanical properties of nanocomposite films for storage of foods.

EXPERIMENTAL METHODS

Fabrication of nanocomposite films:

High density polyethylene (HDPE) and polypropylene (PP) polymer pellets procured from M/s. Reliance Industries Limited, Mumbai and titanium oxide powder from M/s Sigma

Aldrich Pvt, USA was melt compounded in twin screw extruder. The dried granulated compounds were fed into multi layer blown film (Capacity 10kg/h, 120 rpm) and the film thickness was made by adjusting the air pressure, screw speed and nip roller spacing.

Scanning electron microscope (SEM):

Surface morphology of the films was evaluated using JEOL JSM-6390 SEM with an accelerating voltage between 0.5 and 20 kV. Coated samples were viewed in a scanning electron microscope and photographed.

Permeability of the films :

The oxygen permeability of the film was carried out using gas permeability apparatus (Gas and steam permeability, AtsFaar, SocietaPerAzioni, Milano, Italia). The OTR was expressed as cc m^{-2} 24 h^{-1} at 1 atm pressure. The water vapour transmission rate of nanocomposite films was measured using water vapour permeability tester (Model: L80-5000, Make: PBI Dansensor, Denmark).

Antimicrobial activity :

The inoculated *Pseudomonas* ssp. in the broth medium was allowed to grow for antimicrobial studies. The film of 2.5 cm diameter size was placed on the Petri plates with Mueller Hinton agar medium with the culture and incubated to investigate the antimicrobial activity of the films.

Beta carotene :

Acetone-petroleum ether extract method was used to determine the beta carotene in the sample (Sadashivam and Manickam, 1992).

Quantity of beta carotene (mg) present in 100 g of the sample was calculated as given below:

$$\text{Beta carotene}\left(\frac{\text{mg}}{100\,\text{mg}}\right) = \frac{\text{Absorbance at 453 nm}}{0.2592} \times \frac{\text{Tatal valume}}{\text{weight of the sample}} \times 100$$

Bacterial load :

The shelf-life and microbial quality is based on number and kind of microorganism present in it, which was assessed by serial dilution and plating method for enumeration of bacteria.

Statistical analysis :

Statistical analysis was carried out to study the effect of different parameters on all the dependent variables. Analysis of variance (ANOVA) was conducted with Completely Randomized Block Design (CRBD) using the AGRES software.

EXPERIMENTAL FINDINGS AND ANALYSIS

The present study was undertaken with the objective of developing HDPE, PP based polymer films of two thicknesses (60 and 80 µ) with different concentrations (1, 1.5 and 2 %) of titanium oxide nanopowder as antimicrobial agent. The study included the evaluation of morphological and barrier properties of nanocomposite films. The investigation also included the storage studies of fresh cut carrots in the developed antimicrobial nanocomposite films.

Surface morphology of films :

In SEM TiO_2 looked like white dots dispersed in grey patches of polymer matrix. The nanocomposites of 1.5 per cent showed better uniform distribution. For the treatment 2 per cent an aggregrated structure was observed due to the non uniform distribution of titanium oxide nanopowder in the polymer films and similar results were also reported by Wacharawich *et al.* (2009).

Permeability of films :

The reduction in OTR with increased thickness was due to the creation of more tortuous path in films which retarded the progress of gas molecules through the polymer matrix. As the concentration was increased beyond 1.5 per cent, OTR decreased in all the films due to the clustering of nanopowder in the films. The permeability was minimum in 1.5 per cent, of 80 µ thickness HDPE based nanocomposite film and maximum in 1 per cent, 60µ thickness PP based film. The maximum WVTR of 4.2 cc m^{-2} day^{-1} atm^{-1} was found in control films having 1 per cent, 60µ thickness and minimum of 2.04 cc m^{-2} day^{-1} atm^{-1} in 1.5

Table 1: OTR of nanocomposite films in cc $m^{-2}day^{-1}atm^{-1}$

HDPE				PP			
Control		1.5%		Control		1.5%	
60µ	80µ	60µ	80µ	60µ	80µ	60µ	80µ
3850	3600	2330	2250	4700	3980	2420	2410

Table 2: Antimicrobial activity of films

	Diameter of inhibition zone (cm) of films of different concentration(%) and thicknesses(µ)					
Polymer	(1%,60µ)	(1%, 80µ)	(1.5%,60µ)	(1.5%,80µ)	(2%,60µ)	(2%,80µ)
HDPE	0.24	0.22	0.36	0.34	0.29	0.26
PP	0.17	0.15	0.30	0.29	0.19	0.18

per cent 80µ HDPE films (Table 1).

Antimicrobial activity :

The maximum inhibition zone of 0.36 cm diameter was found in HDPE based films (1.5%, 60µ) and minimum of 0.15 cm in PP based (1%, 80µ) films (Table 2). In all treatments, the antimicrobial activity decreased with the increase of thickness of films due to more time taken for the diffusion of antimicrobial agent from the films. The non-uniform distribution was found in films with high nanopowder concentration.

Storage studies :

The fresh cut carrots were stored in control films and nanocomposite films (HDPE, PP) and analysed for various quality parameters.

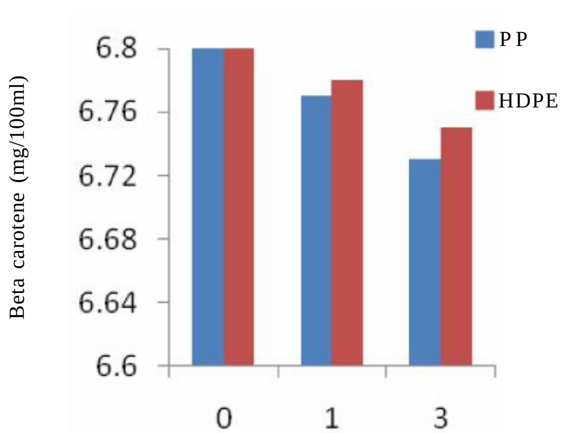
Beta carotene :

Beta carotene content decreased with the increase in storage period in all different storage environments. In the control sample the rate of decrease of beta carotene was more after 3 days of storage and minimum rate of decrease was observed in nanocomposite films after 24 days of storage period. The films with 1.5 per cent of TiO_2 had better retention of beta carotene compared to other films (Fig.1).

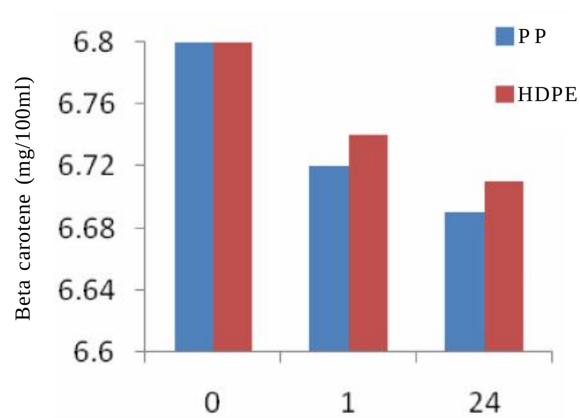
Microbial load :

Bacterial population decreased with the increase of film thickness which might be due to the lesser permeability characteristics. The decrease in bacterial population in HDPE based films was more compared to PP. The maximum reduction of 2 cfu/ml of bacterial population was found in HDPE nanocomposite films after 24 days of storage compared to control films (Fig. 2).

Food processing industry demands packaging material with less permeability and more bearing strength. Nanotechnology is expected to play the greatest role in the

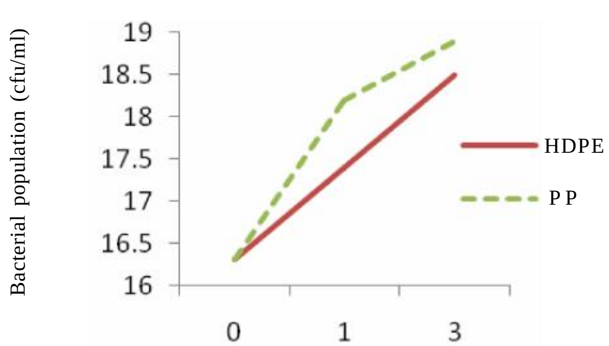


(a) Storage period (days)

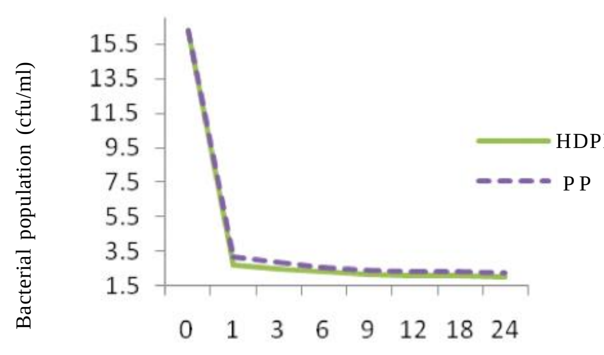


(b) Storage period (days)

Fig.1: Beta carotene of carrot stored in (a) control and (b) 1.5 per cent nanocomposite films



(a) Storage period (days)



(b) Storage period (days)

Fig. 2: Microbial load of carrot stored in (a) control and (b) 1.5 per cent nanocomposite films

development of new food packaging materials with improved mechanical, barrier and antimicrobial properties. Nano magnesium oxide, nano copper oxide, nano titanium dioxide and carbon nanotubes are predicted for future use in antimicrobial food packaging. The nanocomposite films of 1.5 per cent titanium oxide concentration showed better uniform distribution than 2 per cent, in which aggregated structure was observed. Fresh cut carrot in HDPE based nanocomposite films with 1.5 per cent titanium oxide nanopowder was stored for 24 days without spoilage.

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