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Applications of Antimicrobial Polymer Nanocomposites in Food Packaging

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1. Introduction

The demand for minimally processed, easily prepared and ready-to-eat 'fresh' food products, globalization of food trade, and distribution from centralized processing pose major challenges for food safety and quality. Recently, extensive studies have been conducted to develop non-thermal processing techniques (PEF, HHP, IR, UV, US) as replacements for thermal processing in order to keep the freshness of food along with extending its shelf life. Although some of these technologies are capable of decontaminating of food, they are energy-intensive and require costly equipment; hence, their yet relatively limited commercial applications. However, the use of proper packaging technology to minimize food losses and provide safe and sound food products has always been the focus of food packaging. Nanotechnology has potential to influence the packaging sector greatly. Nanoscale innovations in the forms of pathogen detection, active packaging, antimicrobial packaging and barrier formation are poised to elevate food packaging to new heights. This chapter lays foundations for the role of antimicrobial polymer nanocomposites in food packaging (Emamifar et al., 2010; Brody et al., 2008). This chapter briefly describes new food packaging and focus mainly on the applications of nanotechnology in food packaging. Effort will be made to considerable extent to explore the potential of using antimicrobial polymer metal nanocomposites for food packaging industry. Moreover, the antimicrobial performance of the most common nanoparticles based on zinc oxide, titanium oxide and nanosilver applied directly to plastic films, will be described. This chapter also demonstrates the effect of nanocomposite packaging containing Ag and ZnO on the shelf life of fresh orange juice and on inactivation of *Lactobacillus plantarum* in orange juice as a case study.

2. Innovative food packaging

Traditional food packaging is meant for mechanical supporting of otherwise non-solid food, and protecting food from external influences. This principal function of packaging involves retardation of deterioration, extension of shelf-life, and maintenance of quality and safety of packaged food. Packaging protects from environmental influences causing deterioration of foods and beverages such as heat, light, the presence or absence of moisture, oxygen, pressure, enzymes, spurious odors, microorganisms, insects, dirt and dust particles, gaseous emissions, and so on. Prolonging shelf-life involves application of various strategies such as

temperature control; moisture control; addition of chemicals such as salt, sugar, carbon dioxide, or natural acids; removal of oxygen; or a combination of these with effective packaging (Restuccia et al., 2010). In recent years, new food-packaging systems have been developed as a response to trends in consumer preferences towards mildly preserved, fresh, tasty and convenient food products with a prolonged shelf-life. In addition, changes in retail practices, such as centralization of activities (e.g. preparation of retail packs of fresh meat and sliced vegetables) and the globalization of markets resulting in longer distribution distances, present major challenges to the food-packaging industry to develop packaging concepts that extend shelf-life while maintaining the safety and quality of the packaged food. Traditional systems are reaching their limits with regard to further extension of shelf-life of packaged food. To provide this shelf-life extension, and to improve the quality, safety and integrity of the packaged food, innovative active and intelligent packaging concepts are being developed. Active and intelligent packaging may be defined as follows:

Active packaging changes the condition of the packaged food to extend shelf-life or improve food safety or sensory properties, while maintaining the quality of the packaged food.

Intelligent packaging systems monitor the condition of packaged foods to give information about the quality of the packaged food during transport and storage (De Kruijf et al., 2002).

Important examples of active packaging include oxygen scavengers, carbon dioxide emitters/absorbers, moisture absorbers, ethylene absorbers, ethanol emitters, flavor releasing/absorbing systems and antimicrobial containing films and for Intelligent packaging include time-temperature indicators, leakage indicators and freshness indicators (Ozdemir et al., 2004).

2.1 Antimicrobial food packaging

Antibacterial agents are of relevance to a number of industrial sectors including environmental, food, synthetic textiles, packaging, healthcare, medical care, as well as construction and decoration. They can be broadly classified into two types, organic and inorganic. Organic antibacterial materials are often less stable particularly at high temperatures and/or pressures compared to inorganic antibacterial agents. This presents a potential obstacle for the product formulation. As a consequence, inorganic materials such as metal and metal oxides have attracted lots of attention over the past decade due to their ability to withstand harsh process conditions (Zhang et al., 2007). Antimicrobial packaging is a form of active packaging (Appendini & Hotchkiss, 2002). Antimicrobial food packaging materials have to extend the lag phase and reduce the growth rate of microorganisms in order to extend shelf life and to maintain product quality and safety. The need to package foods in a versatile manner for transportation and storage, along with the increasing consumer demand for fresh, convenient, and safe food products presages a bright future for AM Packaging. However, more information is required on the chemical, microbiological and physiological effects of these systems on the packaged food especially on the issues of nutritional quality and human safety. So far, research on AM packaging has focused primarily on the development of various methods and model systems, whereas little attention has been paid to its preservation efficacy in actual foods. Research is essential to identify the types of food that can benefit most from AM packaging materials (Suppakul et al., 2003). Antimicrobial packaging can take several forms including:

1. Addition of sachets pads containing volatile antimicrobial agents into packages.
2. Incorporation of volatile and non-volatile antimicrobial agents directly into polymers.

3. Coating or adsorbing antimicrobials onto polymer surfaces.
4. Immobilization of antimicrobials to polymers by ion or covalent linkages.
5. Use of polymers that are inherently antimicrobial (Appendini & Hotchkiss, 2002).

Among the active packaging applications, the incorporation of antimicrobials is receiving considerable attention as a means of extending the bacterial lag phase, slowing the growth rate of micro-organisms and maintaining food quality and safety.

Direct addition of antimicrobials (organic acids or their respective acid anhydrides, spice extracts, chelating agents, metals, enzymes, bacteriocins, etc.) could result in some loss of activity because of leaching into the food matrix, and cross-reaction with other food components such as lipids or proteins. Therefore, the use of packaging films containing antimicrobial agents could be more efficient, by a controlled migration of the compound into the food, not only allowing for initial inhibition of undesirable micro-organisms, but also residual activity over time, during the transport and storage of food during distribution (Mauriello et al., 2005). Combinations of more than one antimicrobial incorporated into packaging have also been investigated. For example, it is hypothesized that compounds active against Gram-positive bacteria (i.e. lysozyme. Combined with chelating agents (i.e. EDTA. can target Gram-negative bacteria. Addition of EDTA to edible films containing nisin or lysozyme, however, had little inhibition effect on *E. coli* and *Salmonella typhimurium*. The rationale for incorporating antimicrobials into the packaging is to prevent surface growth in foods where a large portion of spoilage and contamination occurs. For example, intact meat from healthy animals is essentially sterile and spoilage occurs primarily at the surface. This approach can reduce the addition of larger quantities of antimicrobials that are usually incorporated into the bulk of the food. Many antimicrobials are incorporated at 0.1-5% w/w of the packaging material, particularly films. Antimicrobials may be incorporated into polymers in the melt or by solvent compounding. Thermal polymer processing methods such as extrusion and injection molding may be used with thermally stable antimicrobials such as metals (Appendini & Hotchkiss, 2002). Antimicrobials that cannot withstand the processing temperatures of the polymers are often coated onto the materials, which require surface functionalisation to improve the adhesion of the coatings. Surface immobilization requires the presence of functional groups on both the antimicrobial and the polymer and also spacer molecules that link the polymer surface to the active agent (Radheshkumar & Münstedt, 2006).

2.2 Nanotechnology applications for food packaging

Nanotechnology focuses on the characterization, fabrication, and manipulation of biological and nonbiological structures smaller than 100 nm. Structures on this scale have been shown to have unique and novel functional properties. Consequently, interest and activities in this research area have greatly increased over the past years. According to the National Nanotechnology Initiative (2006), Nanotechnology is the understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where unique phenomena enable novel applications. Encompassing nanoscale science, engineering and technology, nanotechnology involves imaging, measuring, modeling, and manipulating matter at this length scale (Weiss et al., 2006). Nanotechnology recently introduced in the food packaging industry can potentially provide solutions to food packaging challenges such as short shelf life (Emamifar et al., 2010). Nanotechnology derived food packaging materials are the

largest category of current nanotechnology applications for the food sector. The main applications for food contact materials (FCMs) including:

1. FCMs incorporating nanomaterials to improve packaging properties (flexibility, gas barrier properties, temperature/moisture stability).
2. "Active" FCMs that incorporate nanoparticles with antimicrobial or oxygen scavenging properties.
3. "Intelligent" food packaging incorporating nanosensors to monitor and report the condition of the food.
4. Biodegradable polymer-nanomaterial composites (Chaudhry et al., 2008).

2.2.1 Nanocomposites food packaging

Polymer composites are mixtures of polymers with inorganic or organic fillers with certain geometries (fibers, flakes, spheres, particulates). The use of fillers which have at least one dimension in the nanometric range (nanoparticles) produces polymer nanocomposites. Three types of fillers can be distinguished, depending on how many dimensions are in the nanometric range. Isodimensional nanoparticles, such as spherical silica nanoparticles or semiconductor nanoclusters, have three nanometric dimensions. Nanotubes or whiskers are elongated structures in which two dimensions are in the nanometer scale and the third is larger. When only one dimension is in the nanometer range, the composites are known as polymer-layered crystal nanocomposites, almost exclusively obtained by the intercalation of the polymer (or a monomer subsequently polymerized) inside the galleries of layered host crystals (Azeredo, 2009). There are three common methods used to process nanocomposites: solution method, *in situ* or interlamellar polymerization technique, and melt processing. The solution method can be used to form both intercalated and exfoliated nanocomposite materials. In the solution method, the nanocomposite clay is first swollen in a solvent. Next, it is added to a polymer solution, and polymer molecules are allowed to extend between the layers of filler. The solvent is then allowed to evaporate. The *in situ* or interlamellar method swells the fillers by absorption of a liquid monomer. After the monomer has penetrated in between the layers of silicates, polymerization is initiated by heat, radiation, or incorporation of an initiator. The melt method is the most commonly used method due to the lack of solvents. In melt processing, the nanocomposite filler is incorporated into a molten polymer and then formed into the final material (Brody et al., 2008). Nanocomposite packages are predicted to make up a significant portion of the food packaging market in the near future. Research on use of nanocomposites for food packaging began in the 1990s. Most of the research has involved the use of montmorillonite clay as the nanocomponent in a wide range of polymers such as polyethylene, nylon, polyvinyl chloride, and starch. Many nanocomposite food packages are either already in the marketplace or being developed. The majority of these are targeted for beverage packaging. In large part, the impetus for this predicted growth is the extraordinary benefits nanoscience offers to improve food packages. (Brody et al., 2008). Recent advances in the field of nanotechnology, particularly the ability to prepare highly ionic metal oxide nanoparticulates of any size and shape, may lead to the development of new antibacterial agents (Jones et al., 2008). Antimicrobially active packaging based on metal nanocomposites is a new generation of nano food packaging which are made by incorporating metal nanoparticles into polymer films. The high performance of nanoparticles is due to their high surface area/volume ratio, which is the main reason for increasing antimicrobial activity of metal nanoparticles (Emamifar et al.,

2010). The metal and metal oxide nanomaterials commonly used as antimicrobial agents are silver (Ag), gold (Au), zinc oxide (ZnO), silica (SiO₂), titanium dioxide (TiO₂), alumina (Al₂O₃) and iron oxides (Fe₃O₄, Fe₂O₃). The antimicrobial properties of nano-zinc oxide and magnesium oxide have recently been discovered. Compared to nanosilver, the nanoparticles of zinc oxide and titanium dioxide are expected to provide a more affordable and safe food packaging solutions in the future (Chaudhry et al., 2008).

2.2.2 Antimicrobial mechanisms of nanomaterials

The antimicrobial activity of nanoparticles may be related to several mechanisms. The nanoparticles can either directly interact with the microbial cells, e.g. interrupting transmembrane electron transfer, disrupting/penetrating the cell envelope, or oxidizing cell components, or produce secondary products (e.g. reactive oxygen species (ROS) or dissolved heavy metal ions) that cause damage (Fig 1.) (Li et al., 2008). Antimicrobial mechanisms of the major nanomaterials are discussed as followed:

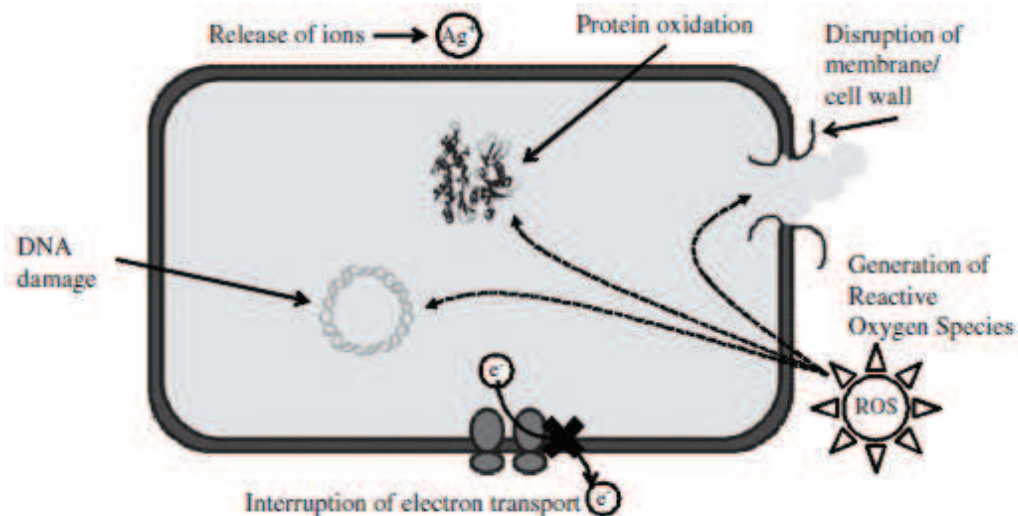


Fig. 1. Various mechanisms of antimicrobial activities exerted by nanomaterials

TiO₂

Titanium dioxide (TiO₂) is non-toxic and has been approved by the American Food and Drug Administration (FDA) for use in human food, drugs, cosmetics and food contact materials. Currently there is considerable interest in the self-disinfecting property of TiO₂ for meeting hygienic design requirements in food processing and packaging surfaces. Bactericidal and fungicidal effects of TiO₂ on *E. coli*, *Salmonella choleraesuis*, *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Diaporthe actinidiae* and *Penicillium expansum* have been reported. Application of TiO₂ photocatalytic disinfection for drinking water production was investigated. The development of TiO₂-coated or incorporated food packaging and food preparing equipment has also received attention. Chawengkijwanich & Hayata, 2008 concluded that the TiO₂-coated film could reduce the microbial contamination on the surface of solid food products and thus reduce the risks of microbial growth on fresh-cut produce (Chawengkijwanich & Hayata, 2008). Adams et al., 2006 studied the toxic effects associated with TiO₂ water suspensions using two model bacterial species, Gram-negative *E. coli* and Gram positive *B.*

subtilis. They suggested that the antibacterial activity of TiO₂ towards both Gram negative and Gram positive bacterial species is significantly greater ($p < 0.05$) in the presence of light than in the dark, and this difference was more pronounced for *B. subtilis*. Specifically, the degree of inhibition for *B. subtilis* was 2.5-fold greater in the presence than in the absence of light, compared to 1.8-fold for *E. coli*. The greater inhibition in the presence of light supports the notion that the antibacterial activity of TiO₂ was related to photocatalytic ROS production. While cell death with TiO₂ was less pronounced in the dark, it still occurred, indicating that an additional mechanism is involved. Similar results have been reported from mammalian cytotoxicity studies, where TiO₂ exerted oxidative stress in the dark under non-photocatalytic conditions (Adams et al., 2006).

ZnO

The antibacterial activity of ceramic powders has attracted attention as a new technique that can substitute for conventional methods using organic agents. Ceramic powders of zinc oxide (ZnO), calcium oxide (CaO) and magnesium oxide (MgO) were found to show marked antibacterial activity (Yamamoto, 2001). The advantage of using these inorganic oxides as antimicrobial agents is that they contain mineral elements essential to humans and exhibit strong activity even when administered in small amounts (Sawai & Yoshikawa, 2004). Jin et al., 2009 studied several approaches (powder, film, PVP capped and coating) for the application of nano-ZnO in food systems and concluded that nano-ZnO exhibits antimicrobial effects against *L.monocytogenes* and *S.enteritidis* in liquid egg white and in culture media. ZnO has found many applications in daily life such as in drug delivery, cosmetics, and medical devices due to its strong antimicrobial effect on a board spectrum of microorganisms. Moreover, it is currently listed by FDA as a generally recognized as safe (GRAS) material. Antimicrobial effects of ZnO nanoparticles may be attributed to several mechanisms: 1) induction of oxidative stress due to ROS generation especially interior or out of cell H₂O₂ which leads to interaction with proteins, DNA, and lipids causing death ; 2) membrane disorganization due to accumulation of ZnO nanoparticles in the bacterial membrane and also their cellular internalization; 3) release of Zn ions that may be responsible for antimicrobial activity by binding to the membrane of microorganisms. However, the toxicity of ZnO nanoparticles is not directly related to their entering into the cell, rather their intimate contact onto the cell causes changes in the microenvironment in the vicinity of the organism-particle contact area to either increase metal solubilization or to generate ROS, that may ultimately damage cell membrane . Moreover, the toxicity of ZnO nanoparticles is not only affected by the light via ROS production, but may also happen in the dark although its mechanism is not yet defined (Emamifar et al., 2010). The generation of highly reactive species such as OH⁻, H₂O₂ and O₂²⁻ is explained as follows. Since ZnO with defects can be activated by both UV and visible light, electron-hole pairs (e⁻h⁺) can be created. The holes split H₂O molecules (from the suspension of ZnO) into OH⁻ and H⁺. Dissolved oxygen molecules are transformed to superoxide radical anions (O₂⁻), which in turn react with H⁺ to generate (HO₂[·]) radicals, which upon subsequent collision with electrons produce hydrogen peroxide anions (HO₂⁻). They then react with hydrogen ions to produce molecules of H₂O₂. The generated H₂O₂ can penetrate the cell membrane and kill the bacteria. Since, the hydroxyl radicals and super oxides are negatively charged particles, they cannot penetrate into the cell membrane and must remain in direct contact with the outer surface of the bacteria; however, H₂O₂ can penetrate into the cell (Padmavathy & Vijayaraghavan, 2008). It can be assumed that the concentration of H₂O₂ generated from the

surface increases with decreasing particle size, because the number of ZnO powder particles per unit volume of powder slurry increases with decreasing particle size. Based on the above, the increase in antibacterial activity is assumed to be due to the increase in H₂O₂ generated from the surface of ZnO on reducing the particle size of the powder samples. For ZnO powders, the influence of particle size on *S. aureus* was less than that on *E. coli*. The structures and chemical compositions of the cell surface of the bacteria used in this study are quite different. Thin layers of lipid A, lipopolysaccharide and peptidoglycan are present on the cell surface of *E. coli*, whereas there is only a peptidoglycan layer for *S. aureus*. However, the differences in antibacterial action towards *S. aureus* and *E. coli* are assumed to be due to the different sensitivities towards H₂O₂ (Yamamoto, 2001). Overall, the preliminary findings suggest that ZnO nanoparticles can be used externally to control the spreading of bacterial infections. It would be interesting to determine if any derivatives of ZnO nanoparticles with various chemical groups or bioagents are more effective at eliminating various microorganisms. In the prevention and control of bacterial spreading and infections, the main target is the cell wall structure. The cell wall of most pathogenic bacteria is composed of surface proteins for adhesion and colonization, and components such as polysaccharides and teichoic acid that protect against host defences and environmental conditions. These components are charged macromolecules; therefore, specific interactions to disrupt their main function and location may be triggered by introducing specific groups on the surface of the nanoparticles. It has been reported that certain long-chain polycations coated onto surfaces can efficiently kill on contact both Gram-positive and Gram-negative bacteria (Jones et al., 2008).

Ag

Silver has been in use since time immemorial in the form of metallic silver, silver nitrate, silver sulfadiazine for the treatment of burns, wounds and several bacterial infections. But due to the emergence of several antibiotics the use of these silver compounds has been declined remarkably. Nanotechnology is gaining tremendous impetus in the present century due to its capability of modulating metals into their nanosize, which drastically changes the chemical, physical and optical properties of metals. Metallic silver in the form of silver nanoparticles has made a remarkable comeback as a potential antimicrobial agent. The use of silver nanoparticles is also important, as several pathogenic bacteria have developed resistance against various antibiotics. Hence, silver nanoparticles have emerged up with diverse medical applications ranging from silver based dressings, silver coated medicinal devices, such as nanogels, nanolotions, etc (Rai et al., 2009). Silver has also been long known to have microbial inhibition. The antimicrobial activity of these nanoparticles may be related to several mechanisms including, induction of oxidative stress due to generation of reactive oxygen species (ROS) which may cause the degradation of the membrane structure of the cell, release of ions from the surface of nanoparticles that has been reported to cause bacterial death due to binding to cell membrane. However, the mechanism of toxicity is still only partially understood (Emamifar et al., 2011). Silver nanoparticles can damage cell membranes of microorganisms by forming "pits" on their surfaces. Moreover, they may penetrate into the cells to cause DNA damage (Morones et al., 2005). Silver ions released from the surface of these nanoparticles can interact with thiol groups in protein to induce bacterial inactivation, condensation of DNA molecules, and loss of their replication ability (Emamifar et al., 2010). Based on electron spin resonance (ESR) measurements, Kim et al., 2007 observed that the antimicrobial mechanism of Ag nanoparticles is related to the

formation of free radicals and the subsequent free radical-induced membrane damage. Treatment with nano-Ag destabilized the outer membrane of bacteria. This indicates that nano-Ag can disrupt the outer membrane barrier components such as lipopolysaccharide or porins, culminating in the perturbation of the cytoplasmic membrane. Although the detailed mechanism by which nanoparticles with a diameter of 10 nm can penetrate and disrupt the membranes remains to be determined, electron microscopy and optical imaging results suggest that nano-Ag penetrate the outer and inner membranes of the treated Gram negative bacteria, with some nanoparticles being found intracellularly. Nano-Ag elicited a rapid collapse of proton motive force. This was also indicated by the observation that nano-Ag induced a massive loss of intracellular potassium. As expected, nano-Ag also decreased the cellular ATP levels, apparently resulting from the collapse of membrane potential. The rapid and complete depletion of ATP may be also indicative of a stimulation of hydrolysis of residual ATP. It is conceivable that this dissipation of bacterial membrane potential and reduction of ATP levels by nano-Ag may culminate in loss of the cell viability. In addition, nano-Ag and Ag⁺ ions in the form of AgNO₃ appear to share a similar membrane-targeting mechanism of action. The effective concentrations of nano-Ag and Ag⁺ ions are at nanomolar and micromolar levels, respectively. Nano-Ag appear to be significantly more efficient than Ag⁺ ions in mediating their antimicrobial activities (Lok et al., 2006). Yoon et al., 2007 observed that *B. subtilis* was more sensitive than *E. coli* to the nanoparticles, meaning that *E. coli* was more resistive to nanoparticles than *B. subtilis* was. They concluded that the lower sensitivities of *E. coli* as compared to *B. subtilis* is that the outer membrane of Gram-negative bacteria such as *E. coli* is predominantly constructed from tightly packed lipopolysaccharide (LPS) molecules, which provide an effective resistive barrier against nanoparticles (Yoon et al., 2008).

2.2.3 Polymer nanocomposites with enhanced photocatalytic antimicrobial properties

Modification of polymeric matrices to prevent growth or reduce adhesion of detrimental microorganisms is a highly desired objective. Hence, there is a significant interest in the development of antimicrobial biomaterials for application in the health and biomedical devices, food, and personal hygiene industries. Among several possibilities currently explored, titania (TiO₂) can be spot out as a potential candidate for polymer modification with a significant number of advantages. TiO₂ works under UV light excitation with energy above the corresponding band gap (ca. 3.2 eV) forming energy-rich electron-hole pairs. Once at the surface of the material, such charge carriers are able to interact with microorganisms rendering biocidal properties to the corresponding polymer-based nanocomposite films. A point of relevance is the control of the TiO₂ polymorphism ensuring the presence of the anatase form, the one with biocidal capability, as well as to control primary particle size in the nanometer range, a fact that would limit scattering events among other things. Novel hybrid or nanocomposite organo-inorganic materials that combine attractive qualities of dissimilar oxide and polymer components are not simply physical blends (vide supra) but can be broadly defined as complex materials having both organic and inorganic constituents intimately mixed. The scale of mixing or, in other words, the degree of homogeneity would influence or even command the properties of the nanocomposite solid materials when the component mixture is adequately reached, typically at the nanometer range. In particular, the optimization of the component contact has been shown to be crucial in order to render TiO₂-containing polymer nanocomposites with outstanding biocidal properties. Another

point of importance to improve the performance of TiO₂-containing nanocomposite systems concerns the optimization of light absorption and the adequate handling of subsequent charge (electron-hole) pair creation and annihilation processes. This task has been typically attempted by controlling the morphological-structural-defect characteristics of the oxide and/or by extending its absorption power into the visible region through a doping process (Kubacka et al., 2009). Metal doping has long been known to be one of the most effective ways to change the intrinsic band structure of TiO₂, and consequently, to improve its visible light sensitivity as well as increase its photocatalytic activity under UV irradiation. Among various dopants, noble metals (especially Ag) have received much attention for this purpose. It is generally believed that Ag nanoparticles enhance the photoactivity of TiO₂ by lowering the recombination rate of its photo-excited charge carriers and/or providing more surface area for adsorption. Visible light absorption by surface plasmon resonance of Ag nanoparticles is also thought to induce electron transfer to TiO₂ resulting in charge separation and thus activation by visible light (Akhavan, 2009). A route to simultaneously influence both light absorption and charge handling is based on the so-called plasmonic photosystems. As detailed above, TiO₂ is excited by near-UV irradiation and a metal such as Ag shows a very intense localized surface plasmon (LSP) absorption band in the near-UV-visible region. Adequate handling of the LSP resonance can allow extending the absorption light into the visible region of the electromagnetic spectrum and, due to the enhancement of the electric near-field in the vicinity of the Ag, would allow boosting the excitation of electron-hole pairs. An overall improvement of the oxide-polymer nanocomposite performance upon excitation on a region ranged from the near-UV (above ca. 280 nm) to the visible light (below ca. 500-525 nm) can be thus envisaged through a plasmonic effect. This would yield highly efficient systems, with improved performance with respect to TiO₂-alone nanocomposites, and having the potential of working under sunlight and/or diffuse artificial light typical of human environments. A last point to mention is the concomitant degradation of the polymer matrix by effect of the charge carriers; this has been proved to be limited by addition of small amounts of titania, typically below 5 wt. % (Kubacka et al., 2009). On the other hand high release level of silver, especially for silver based bulk materials, leads to shortening the effective life of antibacterial activity. If Ag nanoparticles and nanostructures with high antibacterial activities are immobilized on porous matrixes, the release time of silver can be delayed for a long time so that these kinds of silver-supported materials will be of great potentials for bactericidal application (Akhavan, 2009). Yao et al., 2007, demonstrated that Ag, as a typical antimicrobial metal, could be homogeneously deposited onto the surface of TiO₂ photocatalyst thin films under UV light illumination. The amount of Ag deposited was controllable as desired by controlling only the UV irradiation time. The Ag/TiO₂-coated silicon catheters possessed significant bactericidal activity against *E. coli*, *P. aeruginosa*, and *S. aureus* under dark conditions. In addition, the self-cleaning property of Ag/TiO₂ thin films was confirmed on decomposition of MB dye with a certain amount of deposited Ag under UV light illumination. Thus, Ag/TiO₂ coating of silicon catheters is believed to be useful and reusable as an antimicrobial coating for medical devices against nosocomial infections. We are currently examining the photocatalytic antibacterial effect of TiO₂-coated and Ag/TiO₂-coated catheters and their safety for clinical applications (Yao et al., 2008).

3. Preparation antimicrobial nanocomposite films containing Ag and ZnO

As a case study a laboratory trial by Emamifar et al., 2010, was conducted to producing antimicrobial nanocomposite films containing Ag and ZnO. Film grade LDPE resin pellets were directly mixed with each of the antimicrobial agents including P105 powder (a combination of 95% TiO₂ powder, with an average particle diameter of about 250 nm, which acts as a base for doping of nanosilver plus 5% metal nanosilver with particle diameters of about 10 nm) and ZnO nanoparticle powder with an average particle diameter of about 70 nm (Figs 2a, b) separately and the mixture was fed into a twin-screw extruder machine to be cut into masterbatch nano-granules. Proper amounts of masterbatch resins were then added to pure LDPE resin pellets into a single-screw blowing machine to fabricate the final nanocomposite film (50 μm thick) with the desired nanomaterial concentrations (0.25 and 1% for nano-ZnO & 1.5 and 5% for P105). Dispersion quality of nanomaterials into the polymer matrix film was monitored using the Transmission Electron Microscope (Philips CM 200 kV, The Netherlands). Figs (3a, b, c, d) show TEM images of nanocomposites LDPE containing different concentrations of P105 and ZnO nanoparticles. As shown in Figs (3a, b), P105 particles are well distributed in the polymer matrix. However, a slight agglomeration is observed by increasing the concentration of particle powders to 5%. The TEM image of nanocomposite LDPE+0.25% nano-ZnO indicates that the particles are well dispersed in the polymer matrix exhibiting nanometer-scale aggregates ranging from 10 to 200 nanometers with an average size of 70 nm (Fig 3. c). As the nano-ZnO content increases to 1%, the quantity of the agglomerates increases and their size becomes more uneven (Fig 3. d).

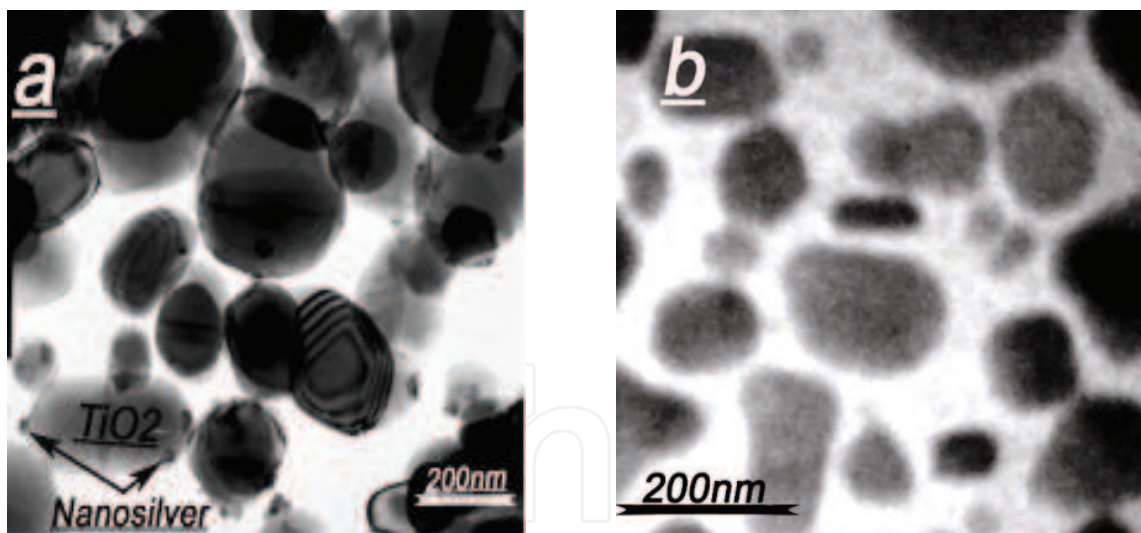


Fig. 2. TEM micrograph of a: P105 and b: nano-ZnO

4. Applications of antimicrobial polymer nanocomposites for fresh orange juice packaging

Orange juice is one of the most globally accepted fruit products. Demand for natural orange juice with high quality in terms of nutritional value, physicochemical properties and sensory characteristics with minimal or no heat treatment has increased considerably (Bull et al., 2004; Souza et al., 2004). Natural orange juice, even kept under refrigeration, has a short shelf-life due to increasing microbial spoilage (Emamifar et al., 2010). Therefore, Emamifar

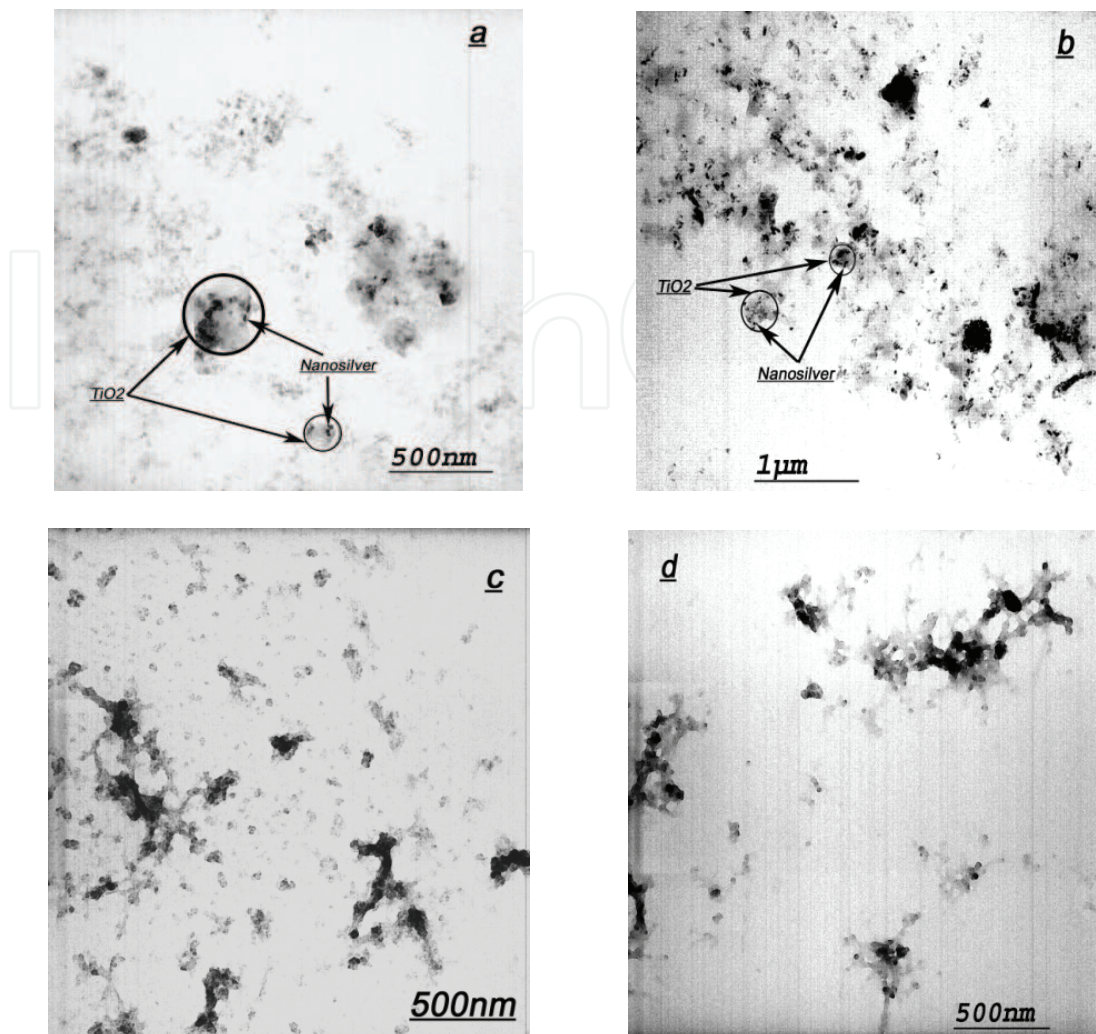


Fig. 3. TEM micrograph of antimicrobial nanocomposites LDPE film. **a:** LDPE+1.5% P105, **b:** LDPE+5% P105, **c:** LDPE+0.25% nano-ZnO, **d:** LDPE+1% nano-ZnO.

et al. 2010 evaluated the capabilities of ZnO and Ag nanoparticles filled LDPE nanocomposite packaging as a new approach to preservation and prolonging shelf life of orange juice by using an antimicrobial nanocomposite (0.25 and 1% for nano-ZnO & 1.5 and 5% for P105) and pure LDPE films 15×10 cm in size, similar to Doypack packaging commonly used for packaging fruit juice. The packages were immediately wrapped in aluminum foil and sanitized at 95°C for 2 min. After cooling and under a sterile laboratory hood, 175 ml of fresh orange juice was poured into each package and sealed by the heat sealer. Packages containing orange juice were stored in dark and cool conditions (4°C). The samples were evaluated in duplicate for their microbiological and sensory characteristics immediately after packaging and after 7, 28, and 56 days of storage.

Mean initial population immediately after packaging was determined to be 4.93 log cfu/ml for yeast and moulds and 4.83 log cfu/ml for total aerobic bacteria in orange juice. The variations in the population of yeast and moulds and total aerobic bacteria are shown in Table 1. In packages made from pure LDPE, the mean population of yeast and moulds increased whereas that of total aerobic bacteria decreased after 7 days of storage. It can be observed that yeast and moulds are better adapted to orange juice under refrigeration than

Film type	Storage time (day)	Fungi (log cfu/ml)	Total Aerobic Bacteria (log cfu /ml)
LDPE pure	0	4.94 ^{ij} ± 0.05	4.84 ^{fg} ± 0.07
	7	5.08 ^h ± 0.08	4.65 ^{hi} ± 0.05
	28	6.26 ^{cd} ± 0.02	5.27 ^d ± 0.06
	56	6.47 ^b ± 0.14	6.35 ^a ± 0.06
LDPE+1.5%P105	0	4.94 ^{ij} ± 0.03	4.83 ^{fg} ± 0.02
	7	4.51 ^k ± 0.07	4.65 ^{hi} ± 0.05
	28	5.74 ^f ± 0.04	4.85 ^{fg} ± 0.05
	56	6.16 ^d ± 0.05	5.76 ^c ± 0.16
LDPE+5%P105	0	4.94 ^{ij} ± 0.05	4.84 ^{fg} ± 0.06
	7	4.36 ^l ± 0.05	4.16 ^k ± 0.05
	28	5.43 ^g ± 0.08	4.54 ^j ± 0.05
	56	6.02 ^e ± 0.02	5.66 ^c ± 0.06
LDPE+0.25% nano-ZnO	0	4.95 ⁱ ± 0.03	4.83 ^{fg} ± 0.05
	7	4.85 ⁱ ± 0.03	4.62 ^{ij} ± 0.05
	28	5.97 ^e ± 0.03	4.90 ^f ± 0.10
	56	6.30 ^c ± 0.04	5.72 ^c ± 0.08
LDPE+1% nano-ZnO	0	4.90 ^{ij} ± 0.03	4.83 ^{fg} ± 0.01
	7	4.93 ^{ij} ± 0.03	4.75 ^{gh} ± 0.05
	28	6.15 ^{sc} ± 0.03	5.10 ^{0e} ± 0.01
	56	6.59 ^a ± 0.12	6.15 ^{0b} ± 0.01

Table 1. Effect of packaging containing Ag and ZnO nanoparticles (mean ± SD) on the fungi, and total aerobic bacteria population, during 56 days storage at 4°C.

bacteria. In the LDPE +5% P105 sample, significance decreases were observed over 7 days of storage in total count and yeast and moulds population compared with LDPE +0.25% nano-ZnO packages and LDPE pure packages containing the same concentration of nano-ZnO . Table 1 shows that the level of population of yeast and moulds and total count increased to 6.47 log cfu/ml and 6.37 log cfu/ml, respectively, after 28 days of storage in LDPE pure packages. The shelf life of fresh orange juice is defined as the time to reach a microbial population of 6 log cfu/ml (Raccach & Mellatdoust, 2007). The mean population of total

aerobic bacteria and yeast and moulds remained below 6 log cfu/ml after identical storage times in all the packages except for the LDPE +1% nano-ZnO one. By increasing nano-ZnO concentration to 1%, the antimicrobial activity of the film decreased (Table 1).

This is due to the agglomeration of nanoparticles during the processing of the film (Fig 3. d). In contrast, by increasing the nanosilver concentration, the antimicrobial activity of the film increased (Table 1). The reduced antimicrobial activity of ZnO powder might be related to the increasing particle size, which might decrease the generation of H₂O₂ from the surface of ZnO powder (Yamamoto, 2001). No significant differences were observed in total aerobic bacteria between LDPE +1.5% P105 and LDPE +0.25% nano-ZnO packages, whereas LDPE +1.5% P105 showed a higher antifungal activity compared with LDPE+0.25% nano-ZnO after 28 days of storage. It appears that antifungal activity of nanocomposites containing nanosilver is significantly ($p<0.05$) higher than that of nano-ZnO. Sawai et al., 2004 have concluded that ZnO, CaO, and MgO powders have satisfactory antimicrobial effects against broad spectrum microorganisms but that ZnO has a poor antimicrobial effect on *Saccharomyces cerevisiae* and other yeast and moulds compared with bacteria.

Based on our results (Table 1), the antimicrobial effect of Ag nanoparticles is much higher than that of ZnO nanoparticles. However, it seems that LDPE+5% P105 has a significantly ($p<0.05$) higher antimicrobial activity compared with other nanocomposites over 28 days of storage for orange juice at 4°C. This is while previous studies have shown a shelf life of up to 14 days for natural, cold orange juice (4°C) (Bull et al., 2004). Yeast, moulds and bacteria exhibit different levels of susceptibility to antimicrobial nanoparticles. The shelf life of natural orange juice has been observed to depend mainly on yeast growth in cold storage (Zanoni et al., 2005). Microbial population increased with increasing storage time to 56 days in all the test packages, indicating the limited effect of long storage time on natural orange juice preservation. However, LDPE +5% P105 packaging has a significantly less loading level at this storage time than other packaging materials. Fig.4. shows the change of sensory attributes of natural orange juice packed in different packages. The high similarity observed in color attribute scores of the packages after 28 days of cold storage ($p<0.05$) indicates that the change in the color of the samples is still invisible. These results correlated well with the values of browning index presented in Table 1. Odor attribute is greatly influenced by microbial growth and may lead to fermentation in orange juice during storage. After 28 days of storage, a significant difference is observed between the odor of orange juice packed in the test packages and that in pure package except for the one containing 1% nanoZnO. Changes in the taste of packed orange juice during 28 days of storage show the positive effect of nanoantimicrobial packaging. It is obvious that there is a significant difference between LDPE +0.25% nano-ZnO packaging and other nanosilver packaging materials, the lowest score being associated with LDPE +1% nano-ZnO and pure LDPE. The sensory panelists recognized LDPE +0.25% nano-ZnO film followed by LDPE +5% P105 and LDPE +1.5% P105 as the best packaging material in terms of overall acceptability. It is noteworthy that changing orange juice flavor during storage is not only due to the growth of microorganisms but also to heating, storage time, and the common chemical interactions that occur in stored juices (Parish, 1998; Haugaard et al., 2002). Souza et al., 2004 reported that lower storage temperatures of unpasteurized orange juice gave rise to a higher sensory acceptance than the higher temperatures for 72h. Leizeron et al., 2005 reported that the sensorial shelf life of orange juice is equal to half its microbial and 2/3 its chemical shelf life.

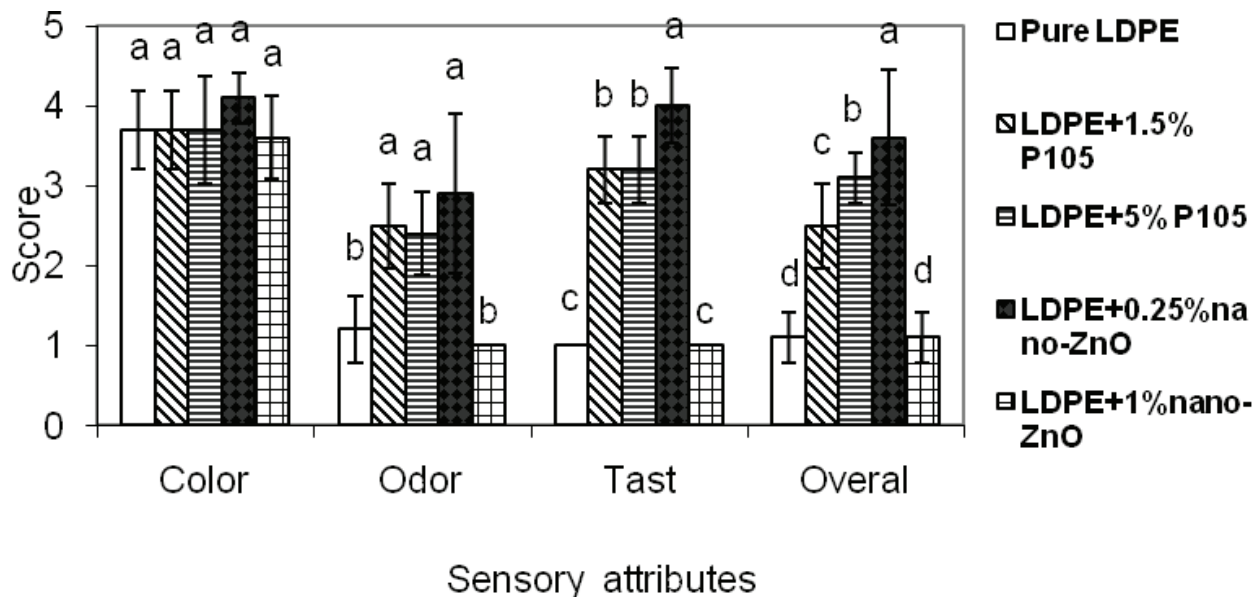


Fig. 4. Sensory attributes of orange juice that is stored in antimicrobial nanocomposite and pure packages

5. Effect of nanocomposite packaging containing Ag and ZnO on inactivation of *Lactobacillus plantarum* in orange juice

Acidophilic microorganisms have been shown to be the major contaminants of citrus juices, especially, lactic acid bacteria and yeasts. *Lactobacillus plantarum* is capable of growing over a wide pH range and spoiling minimally processed or fresh fruit juices owing to its aciduric nature, producing a “butter” off-flavor and swelling of packages. Antimicrobial food packaging materials have to extend the lag phase and reduce the growth rate of microorganisms in order to extend shelf life and to maintain product quality and safety (Emamifar et al. 2011). Emamifar et al. 2011, continued their studied using nanocomposite packaging containing Ag and ZnO on inactivation of *Lactobacillus plantarum* in orange juice. The growth curve of *L. plantarum* was studied and results showed that the stationary phase was reached after 8 h of incubation at 37°C under 5% CO₂ atmosphere (Fig. 5). The survivor curves showed the relationship between microbial population and OD versus time of incubation.

Sterilized orange juice was inoculated with *L. plantarum* at 8.5 log cfu/ml and packaged in antimicrobial and pure LDPE films similar to Section 4. Packages containing inoculated orange juice were stored in dark and cool conditions (4°C) and microbial counts of the samples were evaluated immediately after packaging and after 7, 28, 56, 84 and 112 days of storing. Mean initial microbial population immediately after packaging was determined to be 8.5 log cfu/ml in orange juice. The variations in the *L. plantarum* numbers during storage, are shown in Fig. 5. In all of packages, the mean population increased after 7 days of storage. In the LDPE +5% P105 samples, significance decreases ($p < 0.05$) were observed over 7 days of storage in *L. plantarum* numbers compared with LDPE +0.25% nano-ZnO packages and LDPE pure packages containing the same concentration of nano-ZnO. According to Fig. 5, the level of microbial population increased to 8.82 log cfu/ml after 56 days of storage in

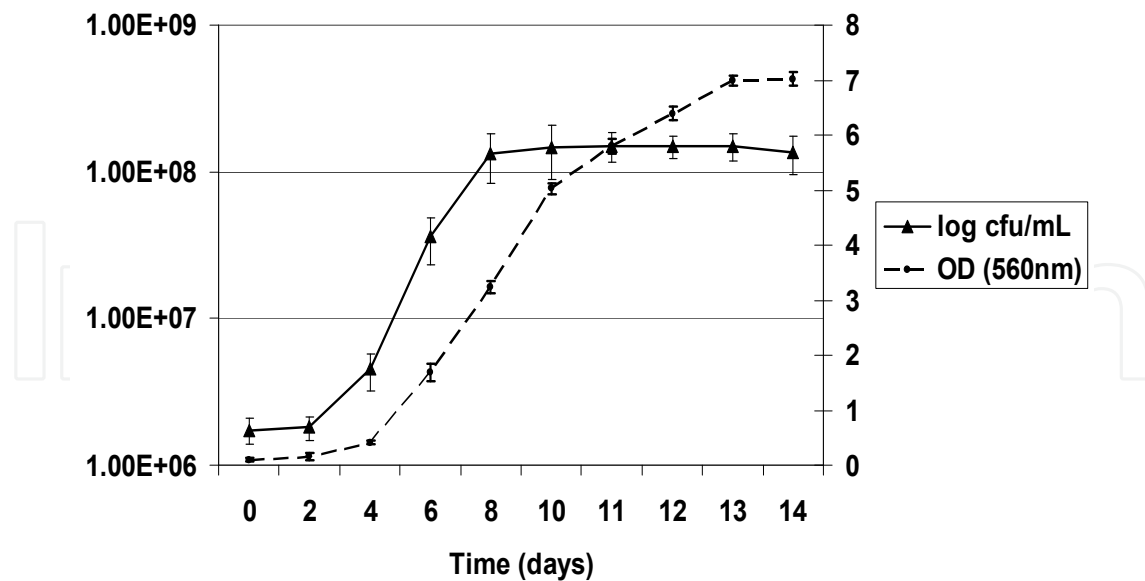


Fig. 5. The survivor curves of *Lactobacillus plantarum*

LDPE pure packages which is higher than that of in LDPE +5% P105 (8.23 log cfu/ml), LDPE +1.5% P105 (8.48 log cfu/ml), LDPE +0.25% nano-ZnO (8.56 log cfu/ml), and LDPE +1% nano-ZnO (8.73 log cfu/ml). No significant differences were observed in *L. plantarum* populations between LDPE +1.5% P105 and LDPE +0.25% nano-ZnO packages, after 56 days of storage. Microbial population increased with increasing storage time to 56 days and then decreased up to 112 days of storage in all the test packages. However, microbial growth in LDPE +5% P105, LDPE +1.5% P105, LDPE+0.25% nano-ZnO, and LDPE+1% nano-ZnO compared with pure LDPE packages, showed a higher reduction up to 112 days storage at 4°C, respectively. Packages containing nanosilver had lower ($P < 0.05$) bacterial populations compared with packages containing nano-ZnO (Fig. 6). The LDPE +5% P105 packages had a significantly less loading level for 112 days of storage than other packages.

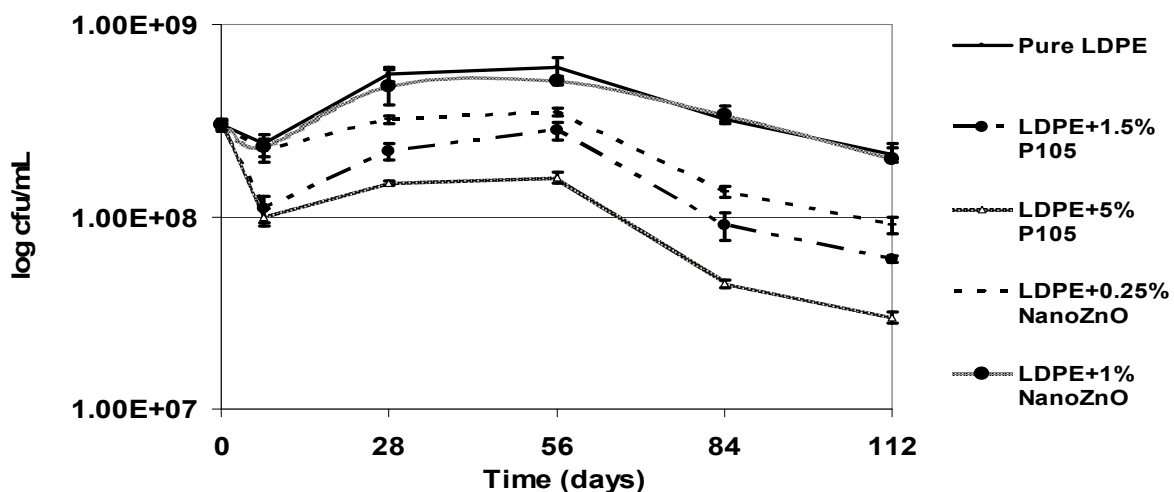


Fig. 6. Effect of packaging containing Ag and ZnO nanoparticles on the population of *L. plantarum* during 112 days of storage at 4°C.

6. Metal ions releasing measurement

The quantities of silver and Zinc ions in orange juice after 112 days of storage are shown in Table 2. The quantity of silver ions migrating into orange juice after 112 days, is less than its allowable concentration (10 ppm). It has been reported that silver ions at as low concentrations as 10^{-9} moles l^{-1} have an antimicrobial effect in water (Damm et al., 2006). Moreover, the quantity of Zinc ions indicated a higher rate of Zn migration than that of silver but as Zinc is proved to be a GRAS compound for food applications, its low concentration is in the acceptable range for food consumers (Jin et al., 2009).

Concentration ions (μgL^{-1})	Storage Time (days)	Film type			
		LDPE+ 1.5% P105	LDPE+ 5% P105	LDPE+ 0.25% nano-ZnO	LDPE+ 1% nano- ZnO
Silver	28	ND ^a	0.1 ± 0.003		
	56	ND	0.11 ± 0.005		
	84	ND	0.13 ± 0.005		
	112	ND	0.15 ± 0.002		
Zinc	28			0.16 ± 0.007	0.11 ± 0.005
	56			0.26 ± 0.006	0.13 ± 0.004
	84			0.48 ± 0.002	0.30 ± 0.005
	112			0.68 ± 0.002	0.54 ± 0.005

Table 2. The quantity of Ag and Zn ions (mean \pm SD) released from nanocomposite LDPE films containing Ag and ZnO nanoparticles in orange juice after 112 days of storage at 4°C.

7. Conclusion

This study showed that application of LDPE nanocomposite packaging materials containing Ag and ZnO nanoparticles is a new approach for preserving and extending the microbial shelf life of fresh orange juice at 4°C. The quality of the packaging film including good dispersion of nanomaterials in the polymer matrix free from agglomeration was shown to be very effective on the antimicrobial effects of these packaging materials. Application of packages containing nano-ZnO prolonged the shelf life of fresh orange juice up to 28 days without any negative effects on sensorial parameters. Nanosilver had a higher antimicrobial activity on *L. plantarum*, yeast and moulds compared with ZnO nanoparticles, especially for

longer storage times. These results would help the industry for combining pasteurization with antimicrobial nanocomposite packages that resulted in developing of cost-effective pasteurization method to control microorganisms in orange juice and to preserve the desirable qualities of it.

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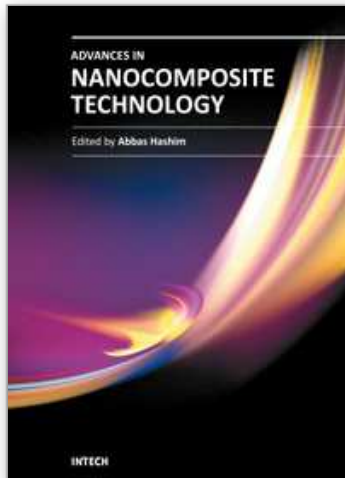
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The book “Advances in Nanocomposite Technology” contains 16 chapters divided in three sections. Section one, “Electronic Applications”, deals with the preparation and characterization of nanocomposite materials for electronic applications and studies. In section two, “Material Nanocomposites”, the advanced research of polymer nanocomposite material and polymer-clay, ceramic, silicate glass-based nanocomposite and the functionality of graphene nanocomposites is presented. The “Human and Bioapplications” section is describing how nanostructures are synthesized and draw attention on wide variety of nanostructures available for biological research and treatment applications. We believe that this book offers broad examples of existing developments in nanocomposite technology research and an excellent introduction to nanoelectronics, nanomaterial applications and bionanocomposites.

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