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RESEARCH ARTICLE

Antifungal activity of nano-composite films-based Poly Lactic Acid

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ABSTRACT

Objective(s): Nanocomposite active packaging systems were used to prepare antimicrobial and antifungal properties. This study was to investigate the physical and antimicrobial activity of prepared films against three types of aflatoxin producing fungi Aspergillus *Flavus*.

Methods: For investigating the effect of antibacterial nano-covers, the direct contact of 0, 1%, 3% and 5% zinc oxide nanoparticles was contaminated with standard strains of three types of Aspergillus *Flavus* (PTCC 5004), Aspergillus *Parasiticus* (PTCC 5286) and Aspergillus *Parasiticus* (PTCC5018) provided. Pistachios were coated by edible films then peroxid index gradient were measured during the time for coating Pistachios containing different concentrations of 0, 1 and 3% of "nano-ZnO". Then coating pistachios were preserved inside sealed Polyethylene bags for six months and the effect of preventing fungal growth during the time were investigated.

Results: The study of antifungal properties of films on three Aspergillus spp. showed that all four percent of nano zinc oxides in this study has inhibitory effect by increasing the percentage of nano-materials significantly ($P < 0.05$). Poly lactic acid edible films Containing 5% nanoparticles has appropriate coating with anti-oxidation agent. Nano-coating Pistachios were observed any growth of mold, however, growth was observed in all control samples.

Conclusions: Poly lactic acid films containing nano-zinc oxide show a high potential for antifungal packaging applications to enhance the shelf life of this products.

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INTRODUCTION

Edible films and coatings are thin layers of food items that used on the food products and employ an important role in protection during distribution and marketing of foodstuffs. Edible coatings protect the product against mechanical damage and microbial, physical and chemical activity. Edible films and coatings are types of packaging material, these films are biodegradable, non-toxic and harmless to the environment [1-3]. During the past two decades, extensive studies have been done on biodegradable materials derived from protein and carbohydrates, these macromolecules can be an alternative for synthetic polymers which are non-biodegradable [3-7].

A variety of edible films and coatings include polysaccharides, proteins, lipids coatings and compound coatings. To improve the properties of the films that have been produced from one component, composite films were developed [4, 8].

PLA has been studied in combination with polymers such as chitosan, gelatin, starch, glucomannan, sodium caseinate, pullulan, PVA, polyvinyl amine and other various materials such

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as sunflower oil and oleic acid. Despite its unique ability of PLA in film and coating production and also its availability and its low cost, this coatings is its relatively weak inhibition against water vapor [8-11].

Deterrence capability against water vapor is the ability of film to reduce moisture exchange between the product and environment. Most of biopolymer films due to polar nature of their constituent units had hydrophilic nature and have high permeability to moisture. This issue limits their use as packaging material. Also PLA film isn't exception. Extensive studies have been done in conjunction with the reduction of permeability of edible films and various solutions have been proposed by researchers. The use of composite coatings is one of the most widely used methods. Composite films were developed to improve the properties of films that have been produced from a compound. The most films which were studied are compounds of lipid - hydrocolloids. The mixing of biopolymers is improved properties of resulting films. Many studies have shown that edible films that produced from the compound of choice biopolymers have better properties in comparison to single-component films [9, 11, 12 and 13].

Essences in polymers act as an emollient and reduce tensile strength of polymers and increase their elasticity. Essences are liquid at room temperature, easily deformed and can change the film elasticity [13, 15]. Essences are placed between the polymer chains and caused their flexibility, since the nature of essences is oily, adding essence to film constituent solution changes the steady structure of film and thus reduce film resistance to destruction (amount of changes is depends on concentration of essences).

Aflatoxins are natural toxins that are produced by Aspergillus flavus and Aspergillus parasiticus and are grown in many food products by providing their own favorable conditions. Keeping in adverse circumstances and moisture absorption from the environment cause mold growth in the nuts [16-18]. Iran is one of the largest manufacturers and exporters of pistachios in the world. Currently, the most important problem of pistachio exports is aflatoxin in relatively high levels and Pistachio quality loss during storage that in recent years it has been highly regarded. Although many efforts have been done to reduce aflatoxin and enhance the qualitative characteristics of pistachios, but still these values are not according to international standards. Considering the foregoing, one of the ways to prevent these problems is the use of edible coatings. Edible coating creates a barrier against moisture, oxygen and dissolved substances in pistachios and has capability of disturbed or eaten by the consumer. Edible coatings increase the quality and shelf life of pistachios and preventing them from oxidation. In recent years, numerous studies have been conducted to evaluate the effects of antimicrobial extracts and spices. Due to customer interest towards the natural antimicrobial agents, in this study PLA films was used as a film-forming biopolymer containing nano zinc oxides as an antifungal agent and then its antioxidant and antimicrobial properties were studied. In the following, films was used as active coat on pistachio and properties of pistachios coated with this film were investigated.

MATERIAL AND METHODS

Poly (lactic acid) (PLA) films with 0%, 1%, 3% and 5% zinc dioxide nanocomposite were a commercial grade supplied by NatureWorks LLC, Minneapolis, USA by extrusion method which were made of (PLA (with M_n (PLA) = 88 500, index of polydispersity, $M_w/M_n = 1.8$, D isomer <2% and a specific gravity of 1.25 g/cm^3 (210 °C, 2.16 kg) and a melting temperature of 170 °C and the nano ZnO from Wacker-Chemie GmbH (Hydrophilic Wacker HDK® T40, with particle size of about 30 nm, bulk density: 280 g/L, specific surface area (BET): 25 - 35 m^2/g, ZnO content ~99.5%. Aspergillus *flavus* (PTCC 5004), Aspergillus *parasiticus* (PTCC5286) and Aspergillus *parasiticus* (PTCC 5018) and collection center of microbial species of scientific and industrial research organization, physiology serum, fungal culture medium of Potato dextrose agar from Merck were obtained.

Microorganisms' activation

To activate the lyophilized culture, ampoules containing fungi in sterile conditions were broken and dissolved in saline then were cultured in potato dextrose agar (PDA) medium culture that is slant and were placed in an incubator at a temperature of 20-25°C. After 7 days that fungus were grown completely and produce spores and tubes were washed with a solution of 0.1%Tween 80 in order to extract spores. 10 ml of this solution was vigorously stirred with crystal balls for several minutes. This allows spores were plucked from colony surface and enter into the solution, then the solution was passed through number 1 filter paper in sterile conditions and mold and fungus mycelium and plucked particles remained on filter paper. The spores were collected under the filter paper. At this stage, the collected solution was diluted with a solution of Tween 80 (0.1%) and its light absorption was measured using UV-VIS light beam of 300 nm up to 1100 nm wavelength generated from the instrument spectrophotometer Hitachi (Japan) and a suspension of 104 spores in ml was obtained.

The antimicrobial activities of films

Inhibition zone test on potato dextrose agar medium culture was used to determine the antimicrobial effects of prepared films against fungi Aspergillus *flavus* (PTCC 5004), Aspergillus *parasiticus* (PTCC5286) and Aspergillus *parasiticus* (PTCC 5018). 0.1 ml of each fungus spore suspension (104 spores per ml) were cultured in potato dextrose agar medium culture. Disc shape films with diameter of 10 mm were placed in sterile conditions at the center of plate containing medium culture. Plates were placed in the oven at 25 °C for 48 hours then composed zone diameter was measured using a caliper with 0.01 precision, composed zone diameter was considered as an index of antimicrobial activity of films. In cases that halo was not formed means there was no antimicrobial activity, instead of reporting 10 mm (primary diameter of disc), its equivalent size was considered zero. But when the antimicrobial activity was observed, with respect the disc diameter, the composed halos diameter always was more than 10 mm. To ensure the steady growth of fungus on the surface of plate, a cultured plate without film was used as control, also a plate lacking fungi was used to ensure that no fungus contamination there is.

Pistachio coating by edible films

To determine the effects of antifungal coverage on total count of molds and yeasts in pistachio during maintenance two methods were done: In the first method coated pistachio samples were prepared and uncoated samples were used as control. Pistachio were immersed for 2 minutes in soluble of prepared edible films and were dried for 24 h at 35 °C before the tests. After drying, pistachios were stored in polyethylene bags for 6 months at room temperature and mold count tests were performed every two weeks. Mold and yeast test was conducted in accordance with national standards 218, 8923, 10899. In other method, Pistachio were immersed for 2 minutes in soluble of prepared edible films and were dried for 24 h at 35 °C before the test, then coated pistachios with the control sample (uncoated pistachio) were placed in plates with fungus medium culture, after that they were placed in an oven at 25 °C for 48 h and then the number of molds were counted [20, 21].

Humidity assessment test

Pistachios moisture measurement is performed using Iranian National standard methods (Standard 672, methods of measuring the moisture of dried fruit). For this purpose, 10 g samples were grind and are dried in an oven with air flow at a temperature of 100±2°C until sample weight to be constant. Reducing the weight of sample before and after drying is reported as the moisture content of pistachio [22].

Measurement of oxygen permeability

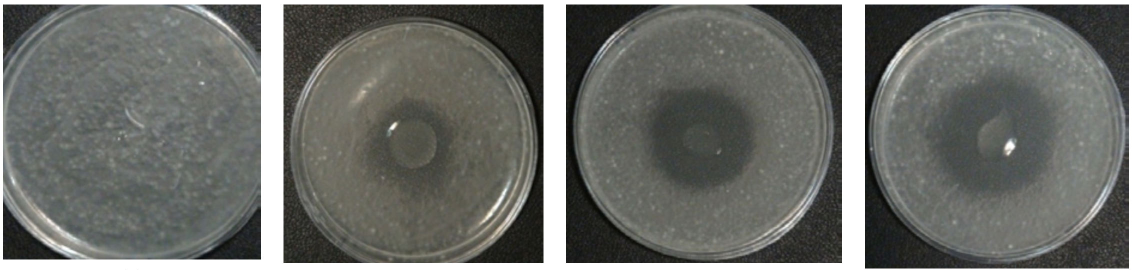
Oxygen permeability was measured by indirect method [18, 19]. This method is based on the number of fresh oil oxidation. Fresh sunflower oil and antioxidants free was used as a control and its peroxide number were measured. Fresh sunflower oil was poured in wineglasses and their cup openings were coated by studied film. Wineglasses were kept for 15 days at a temperature of (25±2 °C) and 75% relative humidity.

RESULTS AND DISCUSSION

The antifungal properties of nano-composite films

The inhibitory activity of films was measured based on measurements of composed halo diameter around the disc-shaped film [23, 24]. The more anti-microbial property of film is, the more formed halo diameter around the film is. In this test, the diameter of inhibition zone of control films (films without nano-particles) was zero which showed that these films have no antimicrobial activity. Halo was formed around the films with Cinnamon essence which show their antimicrobial activity. Antimicrobial activity of films against three types of fungi which produced aflatoxin has been shown in 1, 2 and 3 figures. About all three types of fungi, the more Cinnamon essence concentration is, the more inhibition zone diameter that formed around the film is. It means that the antibacterial property of film is increased by increment of Cinnamon essence concentration.

In other method, coated pistachios were placed in fungus cultivation with the control sample

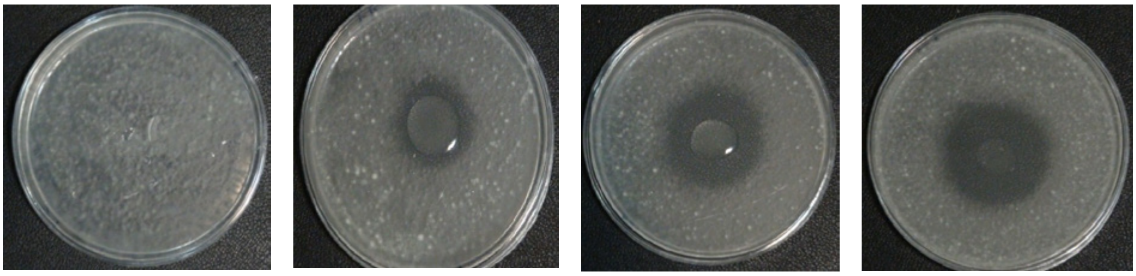


(a) (b) (c) (d)

Fig. 1. Antifungal activity of nano ZnO 0, 1%, 3% and 5% (a, b, c and d) particles against Aspergillus flavus (PTCC 5004)

Table 1. Diameter of inhibition zone (mm) in poly lactic acid films containing nano-zinc oxide on Aspergillus flavus (PTCC 5004)

Sample	Diameter of inhibition zone (mm)
	Aspergillus flavus (PTCC 5004)
PLA	0 ± 0
PLA/ZnO1%	3.19 ± 0.05
PLA/ZnO3%	4.35 ± 0.02
PLA/ZnO5%	6.87 ± 0.03

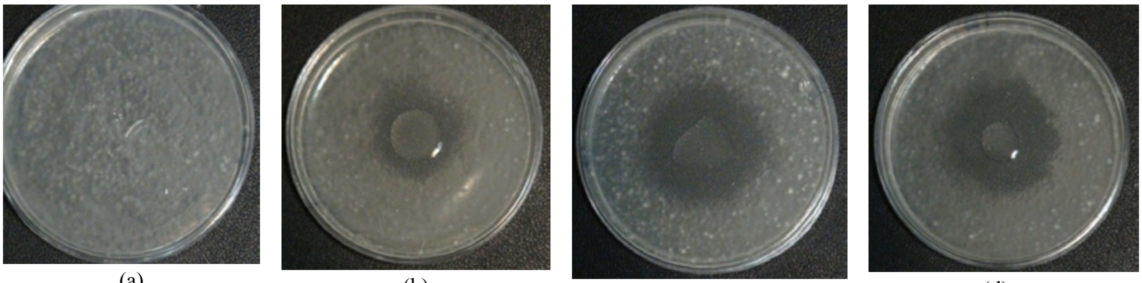


(a) (b) (c) (d)

Fig. 2. Antifungal activity of nano ZnO 0, 1%, 3% and 5% (a, b, c and d) particles against Aspergillus parasiticus (PTCC 5286)

Table 2. Diameter of inhibition zone (mm) in poly lactic acid films containing nano-zinc oxide on Aspergillus flavus (PTCC 5004)

Sample	Diameter of inhibition zone (mm)
	Aspergillus flavus (PTCC 5004)
PLA	0 ± 0
PLA/ZnO1%	5.21±0.01
PLA/ZnO3%	7.31±0.02
PLA/ZnO5%	10.91±0.02



(a) (b) (c) (d)

Fig. 3. Antifungal activity of nano ZnO 0, 1%, 3% and 5% (a, b, c and d) particles against Aspergillus parasiticus (PTCC 5018)

Table 3. Diameter of inhibition zone (mm) in poly lactic acid films containing nano-zinc oxide on Aspergillus parasiticus (PTCC 5018)

Sample	Diameter of inhibition zone (mm)
	Aspergillus parasiticus (PTCC 5018)
PLA	0 ± 0
PLA/ZnO1%	3.32±0.01
PLA/ZnO3%	5.25±0.01
PLA/ZnO5%	7.34±0.02

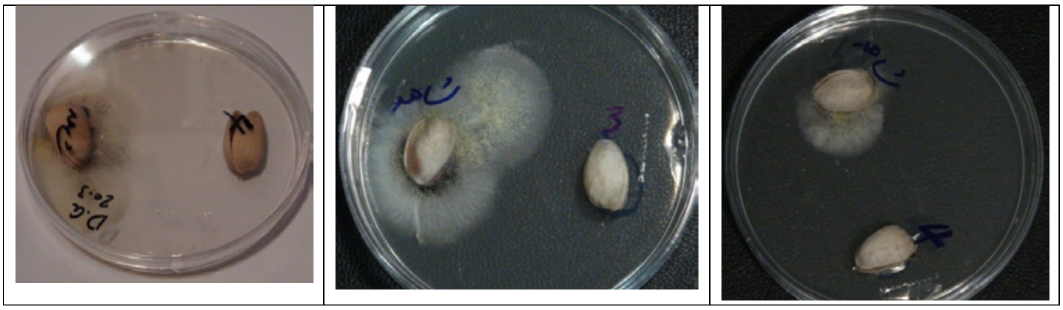


Fig. 4. Antimicrobial activity of coated PLA nano-composites containing ZnO 1%, 3% and 5% (a, b and d) and uncoated pistachios against Aspergillus flavus (PTCC 5004)

Fig. 5. Antimicrobial activity of coated PLA nano-composites containing ZnO 1%, 3% and 5% (a, b and d) and uncoated pistachios against Aspergillus parasiticus (PTCC 5286)

(uncoated pistachio), then molds were counted. After 48 hours, fungal growth was observed in pistachios 105 to 106 grams of pistachios. While in coated pistachios with antimicrobial film solution was observed no growth of mold. Antimicrobial coating can prevent from mold growth in all three nano-composite caoting.

Peroxide activity

Amount of peroxide index change during storage in uncoated pistachios and coated pistachio is presented in Fig. 7. As the figure shows, peroxide value in all subjects significantly increased during storage period but coated samples oxidation has been done with lower gradient and different numbers for the maximum allowable concentration of peroxide index have been listed in nutty seeds. For example, UNICEF for peroxide index O_2 / kg is 5 meq. In Fig. 7, acceptance threshold of 5 meq O_2 / kg is presented for coated pistachios and uncoated pistachios. Oxidation rate in coated pistachio is lower during the time which means that prepared coating in current research is able to reduce the oxidation rate of pistachio. This coating prevents more from pistachio oxidation and pistachio oxidation rate grows with lower slope during the time [20].

The effect 5% ZnO PLA nancomposite on sensory characteristics of pistachio

Coating Pistachios' were measured after drying and humidity was revaluated after 6 months. Pistachio humidity did not change significantly during 6 months, indicating coating can be prevent

Fig. 6. Antimicrobial activity of coated PLA nano-composites containing ZnO 1%, 3% and 5% (a, b and d) and uncoated pistachios against Aspergillus parasiticus (PTCC5018)

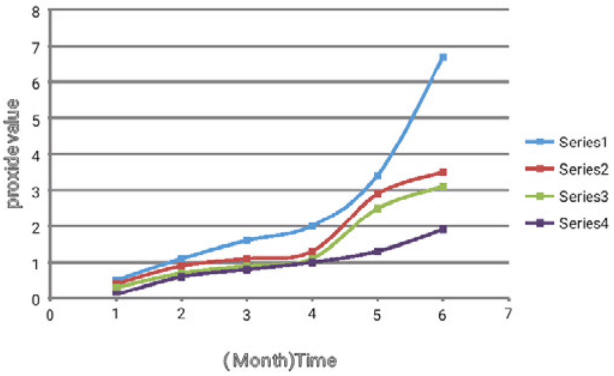


Fig. 7. Peroxide activity during 6 months in PLA based nano-composites of 0, 1%, 3% and 5% ZnO

moisture absorption by pistachios. Hardness of coated pistachios is slightly more in comparison to control samples (uncoated samples) but this amount had no significant difference at the end of 6 months. Evaluation the interaction of coating and storage time on pistachio taste indicate that caoting of pistachio had been no negative impact on taste. Flavor of coated samples did not change after 6 months.

CONCLUSION

This study show antimicrobial effect of nano-particle poly lactic acid on Aspergillus *flavus* (PTCC 5004) Aspergillus *parasiticus* (PTCC5286) and Aspergillus parasiticus (PTCC 5018).

Presented coatage in current research prevent aflatoxin growth in 6 months in terms of antimicrobial properties and in terms of antioxidant properties at the end of 6 months, coated pistachio oxidation rate significantly decreased in comparison to control. In terms of intuitive properties are acceptable by the customer. Therefore, this coating can be properly used to prevent aflatoxin contamination of pistachios and pistachio oxidation.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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