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Inhibition of *Staphylococcus aureus* growth in fresh calf minced meat using low density Polyethylene films package promoted by titanium dioxide and zinc oxide nanoparticles

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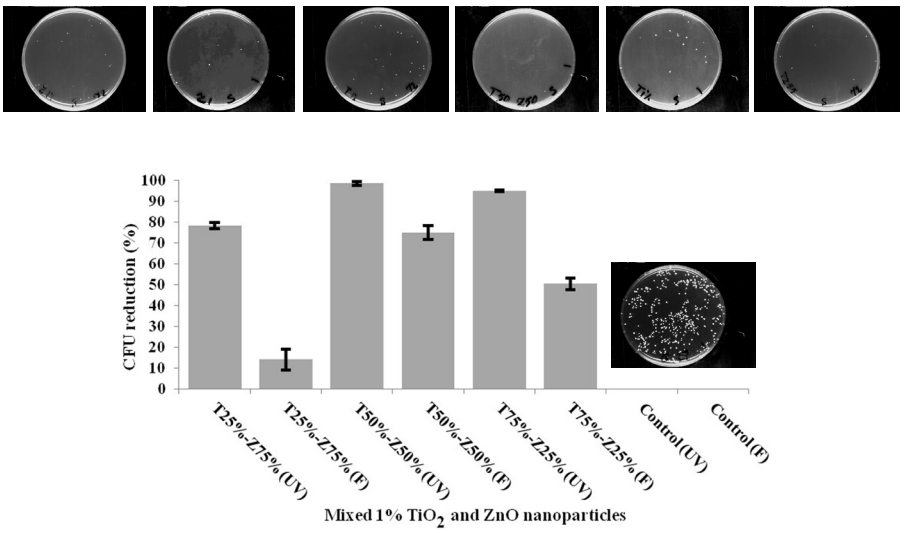
HIGHLIGHTS

- ZnO nanoparticle showed 100% bactericidal effect against *Staphylococcus aureus*.
- TiO_2 nanoparticle showed 96% bactericidal effect against *Staphylococcus aureus*.
- Mixed TiO_2-ZnO showed 98% bactericidal effect against *Staphylococcus aureus*.

GRAPHICAL ABSTRACT



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ABSTRACT

Antibacterial properties of TiO_2, ZnO as well as mixed TiO_2-ZnO nanoparticles coated low density polyethylene films on *Staphylococcus aureus* PTCC1112 were investigated. Bactericidal efficiency of 0.5, 1 and 2 wt% for TiO_2 and ZnO nanoparticles and also 1 wt% mixed TiO_2-ZnO nanoparticles with TiO_2:ZnO ratios of 25:75, 50:50 and 75:25 were tested under UV and fluorescent lights exposure at two different states: films alone (Direct effect) and fresh calf minced meat packed inside the films. The ZnO nanoparticle showed good antibacterial properties against *Staphylococcus aureus* PTCC1112. Maximum CFU reduction of 99.59% and 97.07% were obtained using 2 and 1 wt% ZnO nanoparticle coated LDPE film under UV light for films alone as well as 62.43% and 59.57% for fresh calf minced meat packed. The best antibacterial functionalities of 96.25% and 77.11% CFU reduction were recorded for 1 wt% TiO_2 nanoparticle coated LDPE films in the presence of UV light at direct contact with bacteria and fresh calf minced meat packed, respectively. In the case of mixed TiO_2-ZnO, maximum CFU reductions of 98.37% and 97.84% were obtained using 50:50 ratio of TiO_2: ZnO nanoparticles at the presence of UV light for direct effect and fresh calf minced meat packed, respectively. The 2 wt% ZnO nanoparticle as well as 1 wt% mixed TiO_2-ZnO nanoparticles in ratio of 50:50 coated LDPE films were identified as the best case to improve shelf life and prevent *Staphylococcus aureus* PTCC1112 growth in fresh calf minced meat.

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1. Introduction

Today, the presence and propagation of pathogenic bacteria in food industrial products are known as one of the most worldwide serious menaces for public human health. Due to antibacterial resistant subject, development of new methods in bacterial infectious diseases control seems necessary.

Use of active and antibacterial packaging technologies to better protection of spoilable food products has been attended in the recent years [1]. Adding an antimicrobial agent to packaging material causes to extended food shelf life and accepted microbial quality. In this field of knowledge, some metal nanoparticles (NPLs) such as zinc oxide (ZnO), silver doped titanium dioxide (Ag-TiO_2), copper oxide (CuO) and silver (Ag) are introduced as applicable agents with good antibacterial characteristics [2-4]. Metal NPLs have higher diffusivity and increased biological impacts such as bactericidal properties in compare to normal size ones [5]. For ZnO NPLs, it may be relating to the dependence of produced H_2O_2 amount on ZnO surface area [6,7].

Zinc oxide NPLs showed high antibacterial efficiency against *Salmonella typhimurium* and *Staphylococcus aureus*, two important foodborne pathogenic bacteria [8,9]. Panea et al. (2014) reported highly accepted sensorial and microbial characteristics of chicken breast meat packed in low density polyethylene (LDPE) films loaded by ZnO NPLs [10]. Different mechanisms were proposed for antibacterial activity of ZnO NPLs, though the exact mechanism is unknown yet. Direct impact of ZnO on the bacterial cell wall and its destructive effect is one of the most accepted theories [11, 12].

Titanium dioxide (TiO_2) has identified photocatalitic activities upon UV irradiation caused to break down some organic vital molecules such as polyunsaturated phospholipids of the microbial cell membrane and finally cell death [13]. Also, dependence of the bactericidal properties of nitrogen-doped TiO_2 films on temperature and time duration of exposure to visible artificial light has been proved [14]. However, so far no study has been done to evaluate the antibacterial effects of ZnO, TiO_2 and especially mixed ZnO-TiO_2 NPLs on *Staphylococcus aureus* in fresh calf minced meat.

In the present work, for the first time, antibacterial effects of low density polyethylene (LDPE) films loaded with ZnO, TiO_2 and mixed ZnO-TiO_2 NPLs were evaluated against pathogenic *Staphylococcus aureus* PTCC1112 in fresh calf minced meat under UV and fluorescent lights exposure.

2. Experimental

2.1.Materials

Lyophilized ampoule of *Staphylococcus aureus* PTCC1112 (ATCC 6538) was purchased from Iranian Research Organization for Science and Technology (Persian Type Culture Collection, PTCC, IRAN). Baird-Parker Agar as the selective culture medium for isolation, identification and counting *Staphylococcus aureus* colonies was prepared from Merck Millipore Co. (Germany). All chemicals for analysis and culture media were prepared from Merck Millipore Co. (Germany) too.

Low density polyethylene (LDPE) 020 granules (melt flow rate of 2 g per 10 min, density of 0.92 g ml-1) were funded from Bandar Imam Petrochemical Co. (IRAN).

Zinc oxide (ZnO) nanopowder (molecular weight of 81.39 g mol-1, 15-25 m2 per gram surface area, about 80% zinc basis and <100 nm particle size) and Titanium(IV) oxide (TiO_2) nanopowder, anatase (molecular weight of 79.87 g mol-1, 45-55 m^2 per gram specific surface area, 99.7% trace metal basis and < 25 nm particle size) were prepared from Sigma Aldrich Co. (USA).

Fresh calf minced meat certified by Iran Veterinary Organization was purchased as without fat and bone meat from Refah Chain Store in Sari, Iran.

2.2. NPLs loaded LDPE films manufacturing

LDPE granules were mixed separately with required amounts of TiO_2 and ZnO NPLs to make LDPE containing 0.5, 1 and 2 wt% ratios of each NPL and also 1% of the combined two NPLs with ZnO-TiO_2 ratios of 25:75, 50:50 and 75:25. Twin screw Extruder (Coperion, ZSk, Screw diameter of 50 mm, Germany) at different temperature profiles applied for melted LDPE nanogranules preparation from each sample, separately. At the next stage, LDPE films coated by different composition of NPLs were made by using Film Blowing Apparatus (Brabender, Screw diameter of 45 mm, Germany). Thickness assessment of the films was performed by using Digital micrometer (Mitutoyo, JAPAN) and determined lower than 50 μm. The images

of NPLs coated LDPE films were prepared using Scanning Electron Microscope (SEM, KYKY Technology Development LTD, China).

2.3. Initial culturing of S. aureus

The lyophilized PTCC1112 was revived in Trypticase Soy broth (containing g. L-1 of: pancreatic digest of casein, 17; NaCl, 5; papaic digest of soybean meal, 3; K_2HPO_4, 2.5; and glucose, 2.5) with pH= 7.2 at 25°C for and 150 rpm and 24 h. Then, remained bacterial cake from centrifugation at 4000×g for 20 min, was suspended in sterile distilled water. Number of *S. aureus* cells in suspension was counted through colony count method using Baird-Parker Agar [15]. Initial cell number of *S. aureus* in prepared suspension was estimated equal to 5.95 log CFU/ml.

2.4. Evaluation of bactericide effects of NPLs loaded LDPE films

NPL loaded films were sterilized by using alcohol 80% and placed inside the sterile testing tubes. Then, 1 ml of initial culture containing *S. aureus* was added to each testing tube. For each NPL dosage, six separate testing tubes were prepared. All tests were repeated for three times and the mean values were determined as final result. All inoculated testing tubes were incubated for 72 hr at 25°C in a dark cabinet under UV light (Spectroline, 8 W, 365 nm) or fluorescent light (Osram, Germany). Actually, for each NPL dosage, three testing tube were evaluated under UV light and other three testing tubes investigated under fluorescent light. Counting of viable cells was conducted after 72 hr incubation. For this, 10 ml sterile distilled water was added to each testing tube, and then the tubes were shacked strongly and diluted if necessary. Cell counting was conducted through Most Probable Number (MPN) method by using Petri dishes containing Baird-Parker Agar medium at 25°C for 24 hr [16]. The percent of cell growth inhibition for each testing tube was calculated based on the mean cell numbers in control. Control was LDPE film inside three testing tube without any types of NPL.

2.5. Antibacterial effects of NPL loaded LDPE films on fresh calf minced meat

Similar to the previous tests, each NPL dosage tested in three separate tests, too. Fresh calf minced meat in 100 g samples were packed inside 15×15 cm^2 LDPE films (containing different NPL dosage) at clean and sterile condition. The packages were stored at 4°C for 72 hr. Some packages were stored under UV light and some others under fluorescent light. Then, after incubation time of 72 hr, number of viable cells was counted and the percent of cell growth inhibition calculated as mentioned in the previous section [16].

2.6. Statistical method for results analysis

Obtained experimental results were analyzed by using SPSS 22 software. The mean calculated percent of cell growth inhibition (bactericidal effects of NPLs) for all tests were compared by two ways analysis of variance (ANOVA) and Fishers Least Significant Difference (LSD) test. The value of $P \leq 0.05$ was considered as significant difference between each test and other tests.

3. Results

Antibacterial effect of various LDPE films containing different dosages of ZnO and TiO_2 NPLs was investigated based on the designed experimental procedure. These tests were including LDPE films without any types of NPL, LDPE films loaded with 0.5, 1 and 2 wt% of ZnO NPLs, LDPE films loaded with 0.5, 1 and 2 wt% of TiO_2 NPLs, and LDPE films coated by growth inhibition Antibacterial test of different films (uncoated LDPE films, TiO_2 NPLs coated LDPE films with ZnO-TiO_2 in ratios of 25:75, 50:50 and 75:25. Antibacterial properties of each type of films were evaluated both in the presence of UV and fluorescent light. Also, all experiments were repeated both for free *S. aureus* inside the testing tubes and *S. aureus* in fresh calf minced meat.

3.1. NPLs loaded verification

To verify the exact loading of ZnO and TiO_2 NPLs in LDPE films, SEM analyses was conducted (Figure 1). This image shows the presence of ZnO and TiO_2 NPLs, separately at 1 wt% dosages (b,c) and also mixed 1 wt% of combined ZnO-TiO_2 NPLs (d) in compare to control (LDPE film without any NPL). The part “a” of Figure 1 presents an image of control sample.

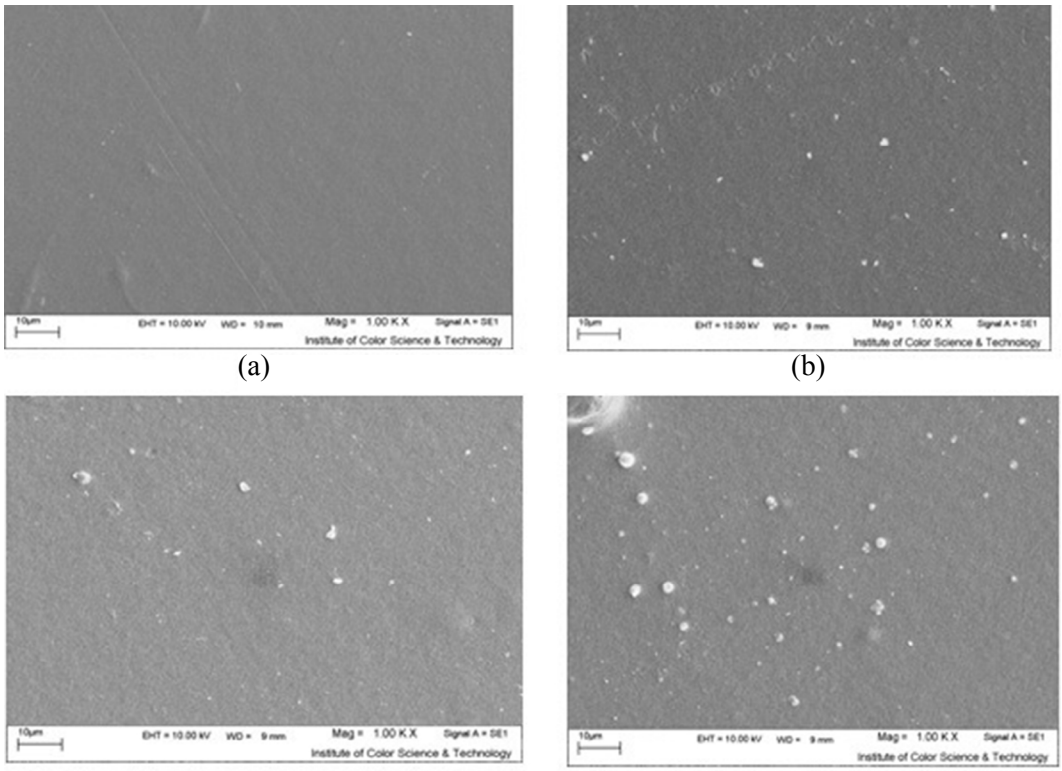

Fig. 1. SEM of LDPE film without any NPL (a), LDPE film loaded by 1% ZnO NPLs (b), LDPE film loaded by 1% TiO_2 NPLs (c), LDPE film loaded by 1% mixed of TiO_2 and ZnO NPLs (d)

3.2. Direct inhibition of S. aureus growth by LDPE films coated with TiO_2 NPLs

Results showed unconsidered direct bactericidal impacts of LDPE films loaded by TiO_2 NPLs on *S. aureus* in the presence of fluorescent light. Increase the dosage of loaded TiO_2 had not any perceptible effect on antibacterial properties of the LDPE film (Table 1).

However, a remarkable direct antibacterial potency of 96.25% was observed for LDPE film loaded by 1 wt% TiO_2 NPLs in the presence of UV light. This value was obtained very lower and just 26.05% reduction in mean colony counts of *S. aureus* with using of LDPE film loaded by 0.5% TiO_2 NPLs. In the case of LDPE film loaded by 2% TiO_2 NPLs, a relatively acceptable bactericidal impact equal to 90.65% CFU reduction was obtained under UV light exposure.

3.3. S. aureus growth inhibition in fresh calf minced meat by LDPE films coated with TiO_2 NPLs

Fairly similar findings to direct antibacterial effects of these types of film on S. aureus were obtained here in the case of fresh calf minced meat (Table 2). Antibacterial properties of TiO_2 NPLs were observed significant when UV radiation is used. The best CFU reduction value of 77.11% was obtained under UV light exposure when meat packed inside an LDPE film loaded by 1% TiO_2 NPLs, while bactericidal of the same film under fluorescent light was recorded only 18.87% (Figure 3).

3.4. Direct inhibition of S. aureus growth by LDPE films coated with ZnO NPLs

Based on the obtained results, a very high CFU reduction of 99.59% was obtained using LDPE film loaded by 2 wt% ZnO NPLs under UV light (Figure 4). Increase ZnO concentration from 0.5 to 2%, showed a positive effect on antibacterial characteristics of the LDPE film. Unlike TiO_2 NPLs, LDPE films loaded by ZnO NPLs showed an acceptable bactericidal activity in the absence of UV light. In this case, the higher CFU reduction of 74.51% was recorded for direct effect of LDPE film contained 2% ZnO NPLs on *S. aureus* (Figure 4).

3.5. S. aureus growth inhibition in fresh calf minced meat by LDPE films coated with ZnO NPLs

A mediocre CFU reduction of 62.43% was obtained

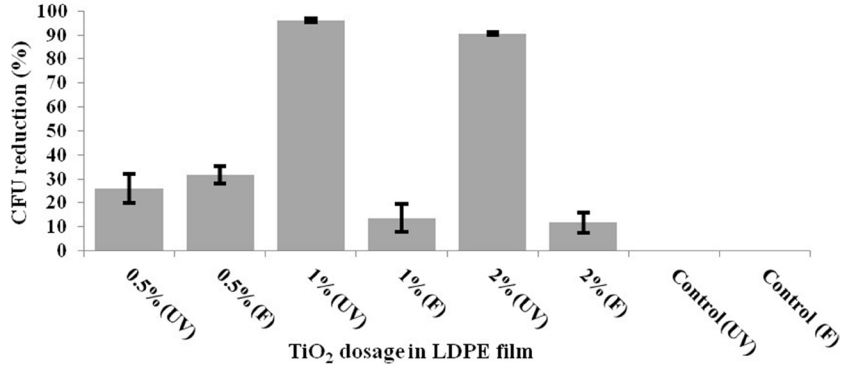

Fig. 2. Direct bactericidal effects of TiO_2 NPLs loaded LDPE films on *S. aureus* after 72 hr in the presence of fluorescent and UV lights at 20°C

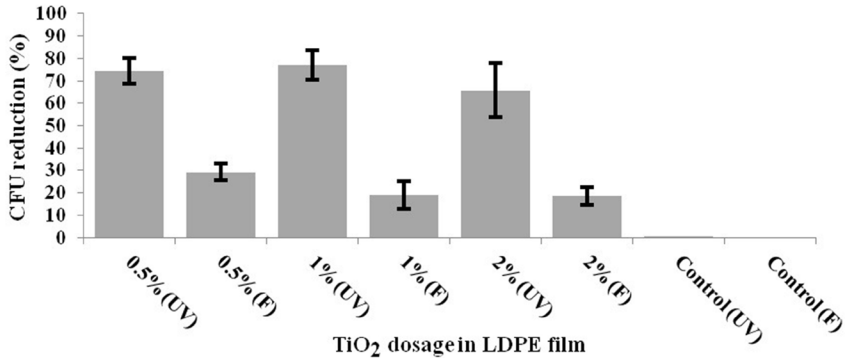

Fig. 3. Bactericidal effects of TiO_2 NPLs loaded LDPE films on *S. aureus i*n fresh calf minced meat after 72 hr in the presence of fluorescent and UV lights at 4°C

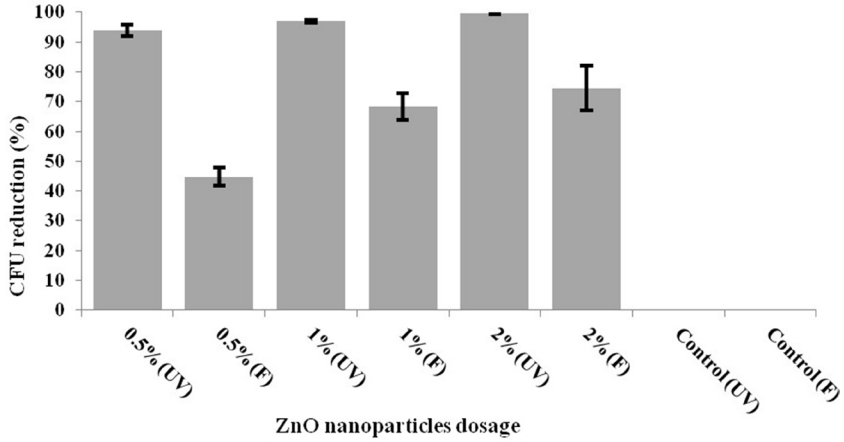

Fig. 4. Direct bactericidal effects of ZnO NPLs loaded LDPE films on *S. aureus* after 72 hr in the presence of fluorescent and UV lights at 20°C

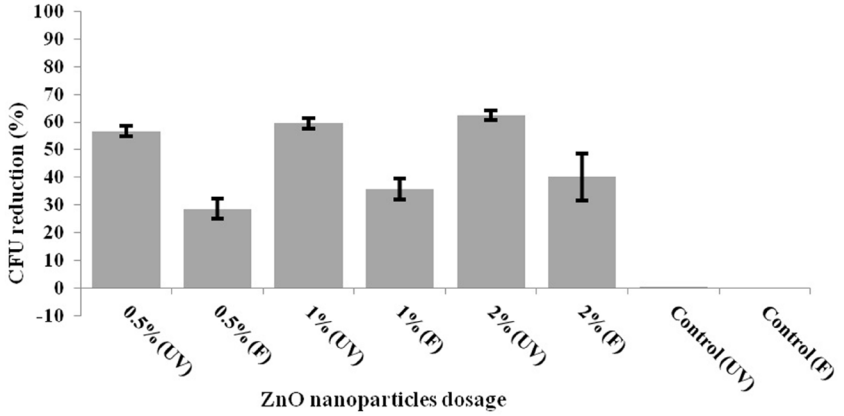

Fig. 5. Bactericidal effects of ZnO NPLs loaded LDPE films on *S. aureus* in fresh calf minced meat after 72 hr in the presence of fluorescent and UV lights at 4°C

Table 1. The mean values of *S. aureus* colony counts (log CFU/g) for direct effect of LDPE films loaded by various dosages of ZnO and TiO_2 NPLs after 72 hr exposed under fluorescent and UV light at 20°C inside the testing tubes.

LDPE films loaded by TiO_2 NPLs					
UV			Fluorescent		
0.5%	1%	2%	0.5%	1%	2%
5.8143 ±0.0308	4.5018±0.1401	4.9167 ±0.0277	5.8418 ±0.0289	5.9426 ±0.0505	5.9533 ±0.0289
LDPE films loaded by ZnO NPLs					
UV			Fluorescent		
0.5%	1%	2%	0.5%	1%	2%
4.6969±0.2198	4.4025±0.1473	3.3004 ±0.3973	5.7493 ±0.0504	5.5007 ±0.1121	5.3687 ±0.2896
LDPE films loaded by 1% mixed TiO_2 and ZnO NPLs					
UV			Fluorescent		
25:75	50:50	75:25	25:75	50:50	75:25
5.2846 ±0.0542	3.9469 ±0.0984	4.6556 ±0.0758	5.9412 ±0.041	5.4010±0.0761	5.7033 ±0.0448
LDPE film without any NPL (Control)					
UV			Fluorescent		
5.9848 ±0.0367			6.0086±0.0003		

T**able 2.** The mean values of *S. aureus* colony counts (log CFU/g) for fresh calf minced meat packed in LDPE films loaded by various dosages of ZnO and TiO_2 NPLs after 72 hr exposed under fluorescent and UV light at 4°C.

LDPE films loaded by TiO_2 NPLs					
UV			Fluorescent		
0.5%	1%	2%	0.5%	1%	2%
3.3271 ±0.1900	3.2632±0.2495	3.4029±0.3713	2.0182±0.0418	2.0769±0.0494	2.0795±0.0362
LDPE films loaded by ZnO NPLs					
UV			Fluorescent		
0.5%	1%	2%	0.5%	1%	2%
3.7947 ±0.0224	3.7206 ±0.0265	3.7805±0.0945	1.8069±0.0346	1.7759±0.0336	1.7439±0.0422
LDPE films loaded by 1% mixed TiO_2 and ZnO NPLs					
UV			Fluorescent		
25:75	50:50	75:25	25:75	50:50	75:25
3.1017 ±0.0990	2.2746±0.0571	3.3264 ±0.217	2.0378±0.0184	2.0959±0.0226	2.1138 ±0.0405
LDPE film without any NPL (Control)					
UV			Fluorescent		
4.1760 ±0.0002			3.9426±0.0003		

for *S. aureus* in fresh calf minced meat when packed into LDPE film loaded by 2% ZnO NPLs under UV exposure (Figure 5). Increase NPL concentration from 0.5 to 2% caused an increase in bactericidal characteristics of films both in the presence of UV and fluorescent lights (Table 2). Under fluorescent light exposure, at the best condition, 40.12% CFU reduction was obtained using LDPE film loaded by 2% ZnO NPLs (Figure 5).

3.6. Direct inhibition of S. aureus growth by LDPE films coated with mixed TiO_2-ZnO NPLs

For LDPE films contained a mixed of TiO_2 and ZnO NPLs, the best results on CFU reduction of *S. aureus* was obtained when an equal mixing of two NPLs was applied. In this regard, a good antibacterial effect equal to 98.37% direct CFU reduction was recorded by using

LDPE film loaded by 1% mixed TiO_2-ZnO NPLs in ratio of 50:50 under UV light (Figure 6). A meaningful difference was observed between treatments under UV light and the ones under fluorescent lights (Table 1). Under UV light exposure, adding more amounts of TiO_2 NPLs in mixture had positive antibacterial impacts on *S. aureus* in compare to treatments with more portions of ZnO NPLs. So that, CFU reductions of 94.86% and 78.23% were recorded for LDPE films loaded by mixed TiO_2-ZnO NPLs in ratios of 75:25 and 25:75, respectively (Figure 6). Under fluorescent light exposure, the reverse results were observed. So that CFU reductions of 14.07% and 50.34% were recorded for LDPE films loaded by mixed TiO_2-ZnO NPLs in ratios of 75:25 and 25:75, respectively (Figure 6).

3.7. S. aureus growth inhibition in fresh calf minced meat by LDPE films coated with mixed TiO_2-ZnO NPLs

A manifest difference was observed between bactericidal impacts of LDPE film coated with mixed TiO_2-ZnO NPLs under UV and fluorescent lights. The higher obtained CFU reduction in the presence of fluorescent light recorded only 26.20% using 1% mixed TiO_2-ZnO NPLs in ratio of 25:75 (Figure 7). This effect has been decreased even to 11.97% with applying reverse ratio of 75:25. However, a very desirable antibacterial property equal to CFU reduction of 97.84% was recorded for LDPE film loaded by 1% mixed TiO_2-ZnO NPLs in ratio of 50:50 under UV light exposure (Figure 7).

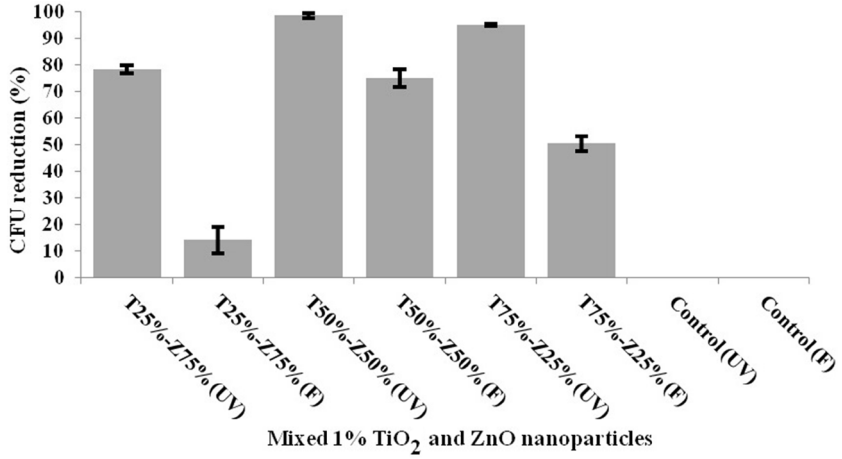

Fig. 6. Direct bactericidal effects of 1% mixed of TiO_2 and ZnO NPLs loaded LDPE films on *S. aureus* after 72 hr in the presence of fluorescent and UV lights at 20°C

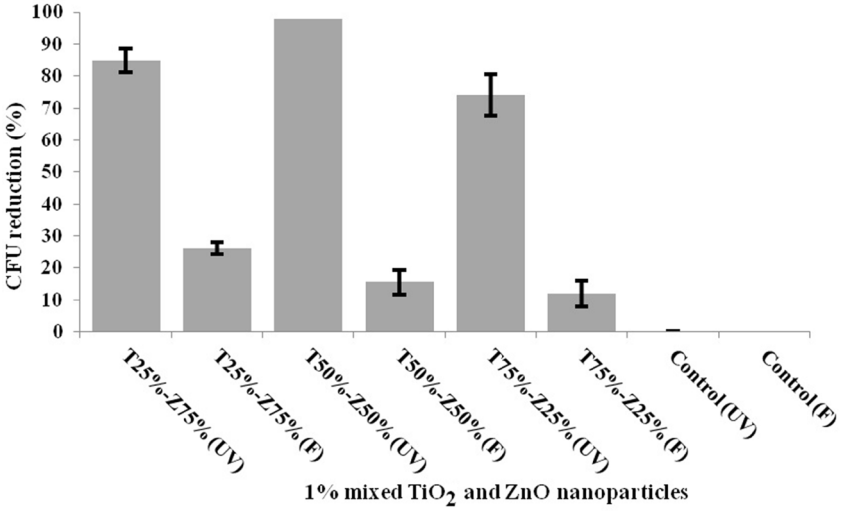

Fig. 7. Bactericidal effects of 1% mixed of TiO_2 and ZnO NPLs loaded LDPE films on *S. aureus* in fresh calf minced meat after 72 hr in the presence of fluorescent and UV lights at 4°C

4. Discussion

4.1. Direct bactericidal effect of LDPE films coated with TiO_2 NPLs

Figure 8 showed *Staphylococcus aureus* PTCC 1112 colonies in direct treatments with LDPE films loaded by ZnO, TiO_2 and also mixed ZnO-TiO_2 NPLs in the present work.

Ibrahim (2015) reported high bactericidal efficiency of 95% for Ag-TiO_2 films against *Staphylococcus aureus* after 3 hours incubation, a little less than our result (96.25% for LDPE film loaded by 1% TiO_2 NPLs in the presence of UV light) [13]. Also, Xing et al. (2012) findings showed 95.2% inhibition of *S. aureus* with using TiO_2 NPLs loaded poly ethylene films irradiated by ultraviolet light for 1 hour [17]. As respects to antibacterial standards, a powerful antibacterial agent must cause a bacterial CFU reduction more than 70% [18]. Then, based on our results, TiO_2 NPLs at 1 wt% under UV light is known as a good antibacterial substance against to *S. aureus* PTCC1112. However this NPL showed low bactericidal properties under visible light irradiation (maximum about 32% obtained CFU reduction).

Based on our obtained results as well as some other reported researches, TiO_2 requires photo-activation to display antibacterial properties [19-20]. Antibacterial feature of UV light by itself isn't enough to prevent microbial growth with an acceptable efficiency [21]. Under ultraviolet light exposure, TiO_2 NPLs are taking part in some photo-related reactions lead to excited electrons and hallow pair formation and then, their diffusion to the surface of TiO_2 NPLs. In the following, hydroxyl radicals and superoxide ions with high performance in destroying the bacterial cell membrane and bactericidal properties are formed [22].

4.2. Bactericidal effect of LDPE films coated with TiO_2 NPLs in fresh calf minced meat

Evaluation of antibacterial effects of this category of films confirmed again the important role of ultraviolet rays in bactericidal properties of TiO_2 NPLs. The same as direct bactericidal studies, in the case of fresh calf minced meat, results showed significant difference between *S. aureus* growth inhibition by TiO_2 NPLs in the presence of UV and fluorescent lights, too. The antibacterial efficiency of TiO_2 NPLs on *S. aureus* in fresh calf minced meat was obtained lower than its direct effect in testing tubes. This may be related to the faster and easier diffusion of hydroxyl radicals and superoxide ions to bacterial cell membrane in direct status. However, according to the standard, an agent

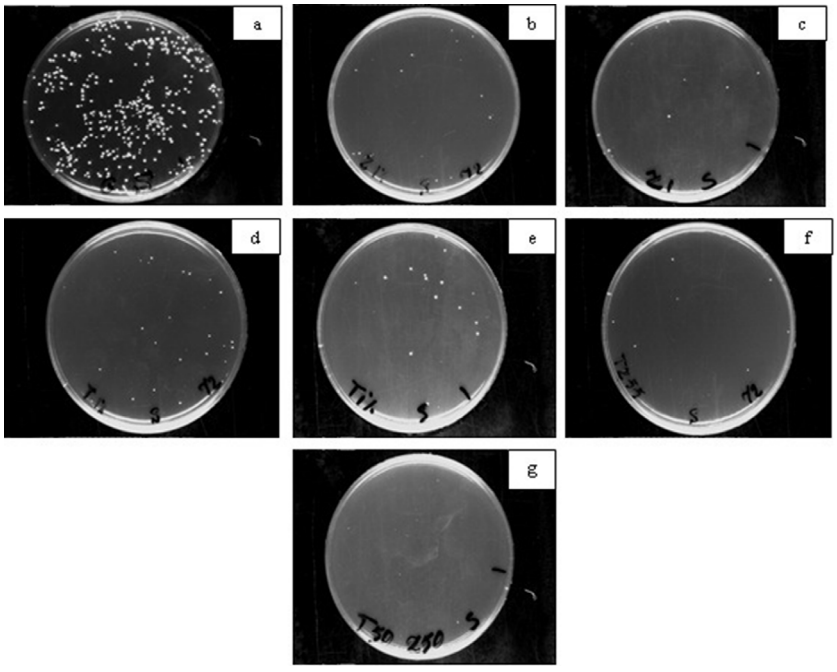

Fig. 8. *S. aureus* colonies in direct treatments with LDPE films loaded by any NPLs in control sample (a), 1% ZnO NPLs under fluorescent light (b), 1% ZnO NPLs under UV light (c), 1% TiO_2 NPLs under fluorescent light (d), 1% TiO_2 NPLs under UV light (e), 1% mixed of 50-50 TiO_2 and ZnO NPLs under fluorescent light (f), 1% mixed of 50-50 TiO_2 and ZnO NPLs under UV light (g).

with CFU reduction less than 20% isn't known as a bactericide material. The values between 20-50% indicate low antibacterial property. CFU reduction between 50-70% and more than 70% show expressive and high bactericidal properties, respectively [18]. Therefore, the results showed that TiO_2 NPLs at dosage of 1 wt% have high bactericidal efficiency of 77% CFU reduction against *S. aureus* PTCC1112 in fresh calf minced meat under UV light exposure. While, isn't introduced as a bactericidal agent in the presence of fluorescent light.

4.3. Direct bactericidal effect of LDPE films coated with ZnO NPLs

Antibacterial effects of ZnO NPLs on *S. aureus* have been reported by some researchers [23]. This agent showed better antibacterial properties against gram positive bacteria such as *S. aureus* than gram negative bacteria such as *E. coli*. Different internal antioxidant content and detoxification agents in gram positive and gram negative bacteria have been mentioned as the main reason of this phenomenon [24]. The actual mechanism of bactericidal properties of ZnO is unknown. However some mechanisms includes release Zn^{2+}, production of reactive oxygen species such as hydroxyl radicals and hydrogen peroxide, as well as destruct the bacterial cell wall have been proposed [25-27]. Results showed about 100% bactericidal efficiency for ZnO NPLs at 2 wt% against *S. aureus* PTCC1112 under UV light (Figure 4). Also, ZnO NPLs at 2 wt% is introduced as a powerful antibacterial agent in the absence of UV light with about 75% CFU reduction. Thus, bactericidal function of ZnO seems to not be depended to ultraviolet rays. Emami-Karvani and Chehrazi (2011) reported compelet inhibition of *S. aureus* growth applying 1.56 mg ml^{-1} ZnO NPLs [28]. They also understood that ZnO NPLs is more effective against gram-positive bacteria than gram-negative ones, may be due to different cell wall structure and metabolism [28, 29].

4.4. Bactericidal effect of LDPE films coated with ZnO NPLs in fresh calf minced meat

The results of Akbar and Anal (2014) showed that use an active package containing calcium alginate film loaded by ZnO NPLs decreased the number of *Salmonella typhimurium* and *Staphylococcus aureus* in ready-to-eat poultry meat from log seven to zero at a period of 10 days incubation at 8±1°C [8]. Panea et al. (2014) reported good antibacterial effects of LDPE films blended with a combination of 5% ZnO and 10% Ag NPLs applied for chicken breast meat packaging [10]. However, our results indicated expressive and low antibacterial effect of ZnO NPL on *S. aureus* PTCC1112 in fresh calf mined meat under UV and fluorescent lights exposure, respectively (Figure 5).

4.5. Direct bactericidal effect of LDPE films coated with mixed TiO_2-ZnO NPLs

An equal parts mixture of TiO_2 and ZnO NPLs under UV light showed high direct antibacterial functionality (near to 100% CFU reduction) at 1 wt% against *S. aureus* PTCC1112 (Figure 6). According to our knowledge, until now, there is not any research work noted the antibacterial characteristics of this mixture on *S. aureus*.

4.6. Bactericidal effect of LDPE films coated with TiO_2-ZnO NPLs in fresh calf minced meat

Mixture of TiO_2-ZnO NPLs in ratio of 50:50 is a powerful bactericidal option with high bacterial growth inhibitory function against *S. aureus* PTCC1112 in fresh calf minced meat. With a CFU reduction of 98%, this mixture is introduced as a favorable antibacterial agent for *S. aureus*. Results showed significant improve in safety and shelf life of fresh calf minced meat by using mixed TiO_2-ZnO NPLs instead of each of these two NPLs separately.

5. Conclusion

Based on the results of this work, antibacterial activity of TiO_2 NPLs is depend to UV light presence while ZnO NPLs are independent from this. Under UV light exposure, TiO_2 NPLs showed high antibacterial properties against *S. aureus* in fresh calf minced meat. ZnO NPLs showed a complete direct bactericidal efficiency against *S. aureus* under and also in the absence of UV. However, low antibacterial effect of ZnO NPL on *S. aureus* in fresh calf mined meat was recorded. TiO_2-ZnO NPLs (50:50) under UV light is introduced as a powerful direct and also in fresh calf mined meat antibacterial agent against *S. aureus*.

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