

Comparison of the Antifungal Activity of Titanium Dioxide Based Nano-Silver Packaging and Conventional Polyethylene Packaging in Consumed Bread

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Abstract

Using titanium dioxide nano-silver packaging, which is antibacterial and resistance to the diffusion of gases such as oxygen, is increasing in food industry. Therefore, we compared the effect of titanium dioxide-based nano-silver packaging and conventional polyethylene packaging on the fungal flora of consuming bread in order to increase the shelf-life storage of consuming bread. One hundred forty four samples of 6 different types of loaf of bread were randomly obtained from 12 bakeries in District 2 of Tehran. The samples were packaged with 3%, 5% and 10% nano-coatings, and also conventional polyethylene coatings as the control group. The bacterial examination and monitoring of samples, according to the national standards of Iran, was carried out 3 times, on the days 1, 3, 7, 14 and 28 of the study period. This study showed that the film type and storage period were the main factors, which significantly influenced the fungal flora of bread. The lowest rate of various fungi growth (%14) was observed in the 10% nano-film, while the highest rate of various fungi growth (47%) was observed in the conventional polyethylene coating ($P < 0.001$). With the increase of the storage period, the number of various fungi increased; however, this correlation was not similar in most of the breads and fungi types, and there were significant difference between them in this regard ($P = 0.001$). According to the results of the present study, due to increasing population growth and in order to improve food security, the use of packages with nano-silver particles, which are based on titanium dioxide, prevails over the polyethylene packages. Therefore, using such packages is highly recommended in the bakery industry.

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1. Introduction

Increasing the shelf-life of food products such as bread is very important. The quality and nutritional value of bread are important, because they provide our high percentage of energy, protein and other nutrient requirements. Bread and other grain products are cheap sources of energy and protein, so they are vital in the diet of the majority of population around the world. In

recent years, Iran has been facing with large percentage of bread spoilage (up to 30%); of which, 7-8% is due to poor packaging. Considering the high consumption of bread in Iran, the figure will be significant. Factors such as poor quality, short shelf-life and staling are the main causes of bread spoilage in Iran [1].

In the baking industry, the quality and sustainability of products are reduced by mold

and bacteria. These changes begin after staling process, and result in reduced acceptability of the products by consumers [2]. In addition, these changes may pose the public health at risk through mild, severe, or fatal fungal induced toxic. The study conducted by Mikaeili [3] showed that there are different fungal infection on the bread, and only 10% of samples were without contamination. The genus *Aspergillus*, *Penicillium* and *Rhizopus* were the most common contaminations, respectively, in the analyzed breads [3]. Currently, there are several methods to increase the shelf-life of food (canning, freezing, etc); however, few of them are applicable for bread due to the specific conditions of production, supply and consumption of bread. Today, one of the new technologies considered by researchers for preserving foods is antimicrobial packaging that can be used in the baking industry products. In the manufacture of these materials, the nano-scale materials such as silver nano-particle, which is the most effective one [4]. In the study conducted by Emamifar and colleagues [5], the shelf-life of orange juice was increased by using silver nano-particles and zinc. Also Foroughi and colleagues improved the shelf-life of meat products by using silver nano-particles and titanium dioxide (TiO₂) [6]. In the researches carried out by Binesh and Moradi [7] and Piromuosavi and colleagues [8], the use of silver nano-particles increased the shelf-life of dates without reducing their quality. The study of Valipour Motlagh and colleagues [9] demonstrated improving in the quality and durability of Barberry when nano-silver particles were employed in the packing. Other studies also showed the effects of nano-particles on reducing the growth of different fungal culture media *in vitro* [10, 11].

In relation to the types of fungus growth and its flora in bread, Leines and colleagues found that fungal growth was not observed at +1°C during the 28 days period, while fungal growth started from day 9 when the breads were stored at 7°C [12]. Similarly, Leuschner and colleagues reported that fungus growth started to grow on the day 15 when the bread was stored at 4°C [13]. Also Najafabadi and colleagues showed that the use of nano-films (silver nano-particles and clay) was effective in reducing the number of bacteria and molds, and maintaining the bread's freshness, and extending its shelf-life [2, 14]. Research has shown reduction of microbial growth in packages containing silver nano-particles and titanium dioxide. This is due to the resistance against the penetration of gases such

as oxygen [6, 7]. In an *in vivo* test carried out on fresh pears packaged in TiO₂ nano-composite film and stored under illumination by a fluorescent light lamp at 5°C for 17 days, the number of mesophilic bacteria and yeast cells decreased significantly as compared to the samples stored in LDPE film. The greatest effects were recorded by combining UVA illumination and active film. It was also proven that the photocatalyst thin film prepared by extrusion could be effectively used in fruit packaging applications [15]. With regard to the effects of nano-particles on the microbes and subsequently, increasing the shelf-life of food and increasing the resistance of microorganisms to antibiotics and preservatives in food, the use of this new technology seems very essential. Therefore, we aimed to compare the effect of titanium dioxide nano-silver (3, 5, and 10%) and conventional polyethylene packaging on the fungal flora of consuming bread in order to increase the shelf-life storage of consuming bread.

2. Materials and Methods

In this experimental study, using extruder, three types of Non-transparent packaging were built in the Chemistry Lab., and the results were compared with conventional polyethylene film (without nano-particles). One hundred and forty-four of Six types of most commonly consumed breads (Barbari, Taftoon, Sangak, Baguette, Barley and Toast) were taken randomly from the bakeries of the District 2 of Tehran and were packed by electric sewing machine [1, 16].

The breads were packed with nano-film and polyethylene, stored and examined on the days 1, 3, 7, 14, and 28 in the Lab temperature [2, 12, 13].

The structure of the type of film used in this study or the range of nano-silver particles varied from 3% to 10%. In the treatment of nano-particles, nano-silver was added to the films' compound during the extruding process. The conventional LDPE (Low Density Poly Ethylene) was used as a control film.

Identification of fungal properties was performed using Iranian National Standard protocol (No. 10899) [17]. Data were analyzed using Chi-square test and One-way ANOVA by SPSS software (version 16), and post-hoc tests (Tukey) were used to compare the difference of growth rate of the fungal flora between coverages, days of storage and the type of bread.

3. Result

3.1 Effect of film type on the fungal flora of bread

The overall effect of nano-silver packaging on the growth of various fungi rated at 0.99 level was significant ($P < 0.001$). As expected, the effect of silver nano-particles was well visible.

With the rise of nano-silver to 10%, fungal type counts dropped. But this effect was not significant between the 3% and 5% nano-films ($P = 0.313$) (Table 1).

Table 1. Comparison of fungi growth during the storage period in terms of packaging

Storage time/Day	Cover	Mean	Standard deviation	ANOVA Test
1	PE	0.0741	0.26311	df=428,3
	NS3	0.0648	0.24765	F=0.832
	NS5	0.0648	0.24735	P-Value=0.477
	NS10	0.0278	0.16510	R=3
3	PE	2.0741	1.68946	df=428,3
	NS3	0.0278	0.96978	F=109.370
	NS5	0.0093	0.16510	P-Value=0.000
	NS10	0.0278	0.09623	R=3
7	PE	2.1111	1.65949	df=428,3
	NS3	1.2963	1.48046	F=17.059
	NS5	1.1389	1.28586	P-Value=0.000
	NS10	0.8333	0.97156	R=3
14	PE	2.6296	1.29406	df=428,3
	NS3	1.75	1.29791	F=34.008
	NS5	1.6019	1.26742	P-Value =0.000
	NS10	1	0.88603	R=3
28	PE	2.7685	1.22746	df=428,3
	NS3	1.8704	1.46033	F=26.055
	NS5	1.8056	1.33576	P-Value =0.000
	NS10	1.2953	1.02648	R=3
Total	PE	1.9315	1.64270	df=428,3
	NS3	1.0667	1.38880	F=26.055
	NS5	0.9278	1.25887	P-Value =0.000
	NS10	0.6259	0.90805	R=3

As Table 2 shows, there are significant differences in different fungi counts during the storage days ($P < 0.001$) except in the first day ($P = 0.447$). But in the 3rd and 7th days, this difference was not significant between the nano-films and in the 14th and 28th days there was no significant difference between the 3% and 5% nano-films even at 0.95 level.

As can be seen in Fig 1, growth of different types of fungus between the first and third days was not significant in the breads packaged with nano-films ($P_{10} = 0.316$, $P_5 = 0.204$). But difference in the fungal growth rate between the

3% polyethylene and nano-composite coverings was significant during the first to the third days ($P < 0.001$). Also the difference between the rate of fungal growth on nano-packaging packed with polyethylene was significant in the next days (7, 14 and 28) as compared with the first day ($P < 0.001$), and as can be seen, the count type of fungi in the nano-packs declined towards polyethylene on the days 7, 14 and 28.

3.2 Effect of storage time on fungal flora bread

As Fig 2 shows, there is a significant difference between the days of maintenance with the conjunction kinds of fungal growth ($P < 0.001$). According to Chart 1, the difference was significant between the growth rates of various fungi on the first, third and seventh days ($P < 0.001$) but it was not significant on the 14th and 28th days ($P = 0.193$).

3.3 Effect of bread on the fungal flora

Fig 3 shows that type of bread is effective in bread fungal flora ($P < 0.001$). However, there is no significant difference between the fungal flora of Toast and Tafton ($P = 0.976$), as well as between Baguette and Barlay ($P = 0.179$).

The effect of type of bread on selected fungal flora was significant for all grown fungi ($P < 0.001$) (Fig 4). However, *Aspergillus*'s fungal growth was not significant between Sangak and Barbari and also between Taftoon and Toast breads.

Cladosporium fungi grew significantly more on Baguettes bread than on other types of breads. *Penicillium* fungi growth was not significant in comparing the Sangak, Barbari, Baguettes and Taftoon breads with Toast bread. Comparison of the growth of different types of fungi in different breads is shown in Table 2.

3.4 Growth of different fungi type

Among the different types of fungi, *Aspergillus* had the highest (33.5%) and *Cladophialophora* had the lowest percentage (1.3%) counts. The growth percentage of other types of fungi is shown in Fig 5.

This study demonstrated that the antifungal effect of silver nano-particles was well visible, and with increasing of nano-silver to 10%, the different fungal counts dropped significantly, and the highest count of fungal microflora was observed in polyethylene packaging. The results revealed no significant difference in microflora counts between the 3% and 5% nano-films and also between the nano-films on the third and seventh days of storage. The study further

showed that the quality of the breads did not change on the third day of storage using 5% and 10% nano-silver films, and that the type of bread was effective in the growth of fungal flora.

Our study was supported by Pirmuosavi study [8] in which the packages containing silver nano-particles and titanium dioxide increased the shelf-life of meat products.

Table 2. Comparison of fungi growth on bread types

Type of fungus	Type of bread	Mean	Standard deviation	ANOVA Test
Aspergillus	Sangak	0.5861	0.49321	df =2154,5
	Barbari	0.6611	0.47399	F =94.079
	Taftoon	0.1611	0.36815	P-Value =0.000
	Baguettes	0.4639	0.49939	
	Toast	0.1111	0.31471	
Cladosporium	Barlay	0.3083	0.46245	
	Sangak	0.02	0.138	df =2154,5
	Barbari	0.02	0.148	F =26.656
	Taftoon	0.01	0.91	P-Value =0.000
	Baguettes	0.14	0.349	
Penicillium	Toast	0	0.053	
	Barlay	0.05	0.212	
	Sangak	0.34	0.473	df =2154,5
	Barbari	0.53	0.5	F =56.957
	Taftoon	0.09	0.285	P-Value =0.000
Rhizopus	Baguettes	0.29	0.454	
	Toast	0.11	0.318	
	Barlay	0.40	0.491	
	Sangak	0.19	0.396	df =2154,5
	Barbari	0.35	0.477	F =68.933
Cladophialophora	Taftoon	0.06	0.245	P-Value =0.000
	Baguettes	0.33	0.472	
	Toast	0.02	0.156	
	Barlay	0.02	0.138	
	Sangak	0.01	0.074	df =2154,5
Alternaria	Barbari	0	0.053	F =22.584
	Taftoon	0	0.053	P-Value =0.000
	Baguettes	0	0.053	
	Toast	0	0	
	Barlay	0.08	0.264	
Candida	Sangak	0.4	0.491	df =2154,5
	Barbari	0.24	0.425	F =82.635
	Taftoon	0	0	P-Value =0.000
	Baguettes	0.09	0.281	
	Toast	0	0	
Chrysosporium	Barlay	0.15	0.358	
	Sangak	0.1	0.3	df =2154,5
	Barbari	0.08	0.268	F =26.136
	Taftoon	0	0.053	P-Value =0.000
	Baguettes	0.01	0.074	
Fusarium	Toast	0	0	
	Barlay	0	0	
	Sangak	0.1	0.3	df =2154,5
	Barbari	0.09	0.285	F =22.078
	Taftoon	0	0	P-Value =0.000
	Baguettes	0	0	
	Toast	0.01	0.074	
	Barlay	0.1	0.304	
	Sangak	0	0	df =2154,5
	Barbari	0.1	0.3	F =39.899
	Taftoon	0	0	P-Value =0.000
	Baguettes	0	0	
	Toast	0	0	
	Barlay	0	0	

Similarly Foroghi [6] found that the mentioned packaging increased the shelf-life of Mozafati fresh date. Emamifar and colleagues also showed that the shelf-life of orange juice

was increased to 28 days using packaging containing silver and zinc oxide nano-particles [5]. In relation to storage period of bread, Najafabadi et al [14] showed the positive effect

of silver nano-particles on suppressing the fungal growth of bread. However, this study revealed that the extent of this effect is different on different types of fungi and bread [2]. Here, we

found that *Penicillium* and *Aspergillus* fungi had the highest percentage of flora counts among the studied fungi.

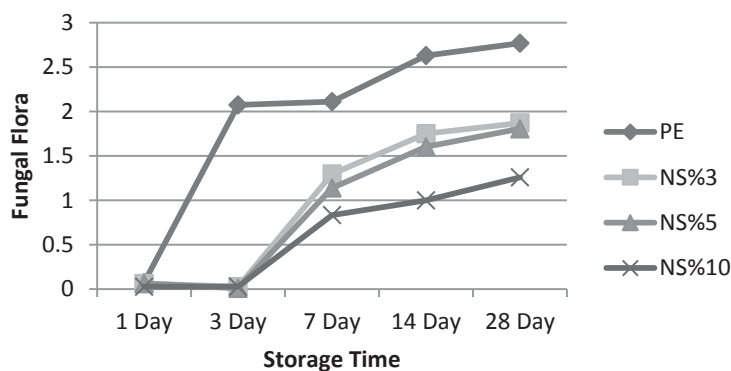


Fig 1. Comparison of fungus types in different covering

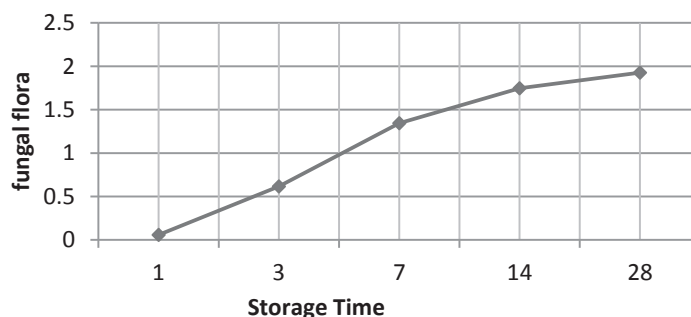


Fig 2. Comparison of the overall mean of fungal flora during the storage period



Fig 3. Comparison of bread types and fungal flora

The same results were reported by Mikaeili [3] in examining the fungal growth in bread. These findings indicate that *Aspergillus* and

Penicillium fungi are the main fungi of food industry, especially bread products [18].

4. Conclusion

In general, it can be concluded that nano-films due to their effective antifungal effects are more suitable than conventional polyethylene coating in bread products. The 10% titanium dioxide-based nano-silver packaging is much more effective, and the 5% titanium dioxide-based nano-silver packaging is cost-effective. We recommend these two packagings for bread industry. Although there is antifungal effect of

nano-silver particles and nano-particles used in coatings for food packaging; however, the effect of nano-particles on the type of fungus has not been studied. Considering the importance of the type of fungus in food industry, additional studies are needed to determine the effect of nano-silver particles and other nano-particles on the fungal flora to increase the shelf-life of foods during the storage time.

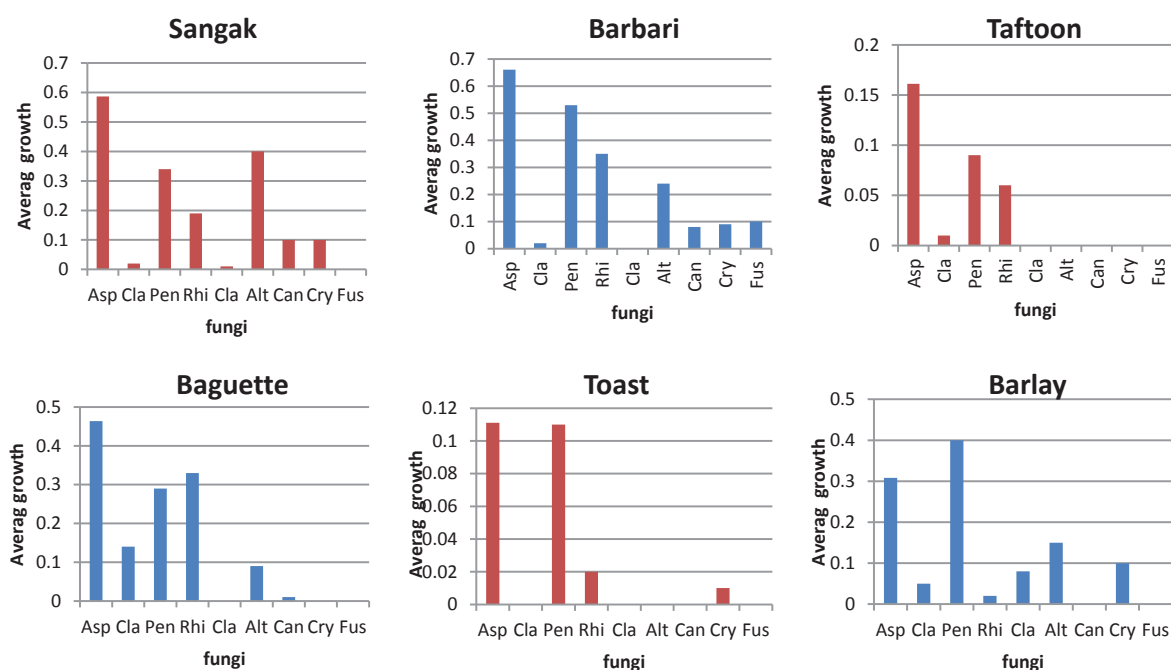


Fig 4. Comparison of effect of bread types on fungal flora

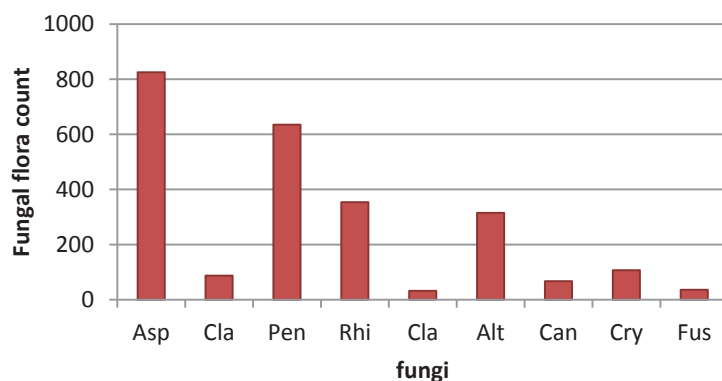


Fig 5. The growth rate of fungus types during the storage period

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References

1. Karimi M. Evaluation of the influence of different levels of sodium stearoyl lactylate (SSL) on dough rheological properties and quality of Taftoon. Tehran: Shahid Beheshti University of Medical Sciences and Health Services College of Nutrition and Food Technology; 2006; 1-10.
2. Sattari Najafabadi M, Minaee S, Azizi MH, Afshari H. Effects of nano-films packaging on organoleptic and microbial properties of bread. *Iran J Nutr Sci Food Technol.* 2010; 4: 65-74.
3. Mikaeili A. Investigation of fungal species in dry bread and flour in Kermanshah. Ninth Congress of Iranian Nutrition, Tabriz University of Medical Sciences. 2003; p4972.
4. de Azeredo HMC. Antimicrobial nano-structures in food packaging. *Trends Food Sci Tech.* 2013; 30: 56-69.
5. Emamifar A, Kadivar M, Shahedi M, Soleimanzadeh S. Effects of nano-composite packaging containing silver and zinc oxide on the shelf-life of fresh orange juice. *Iran J Nutr Sci Food Technol.* 2011; 6: 57-67.
6. Foroughi S, Dabbagh Moghaddam A, Ahari H, Akbarein H, Anvar AA, Aghazadeh Meshki M, et al. A Survey on the Shelf-life Extension of Foods with Nano-films. *J Army Univ Med Sci I.R. Iran.* 2011; 9: 81-86.
7. Moradi O, Binesh M. Effects of silver and titanium dioxide nano-particles in polyethylene packaging used in Mazafati variety of storage on microbial changes during the past 6 months. *J Microb Biotechnol.* 2011; 2: 25-31.
8. Piro Mousavi F, Heydari Nasab A, Hashemipoure Rafsanjani H, Rajabalipour CHashmegaz AA. Films containing silver nano-particles effect on shelf-life of fresh date Mazafati. *J Food Technol Nutr.* 2013; 10: 65-72.
9. Valipour Mtlagh N, Hamed Mousavian MT, Mortazavi A. Packages containing nano-silver impact on biological and physical properties of barberry with conventional polyethylene packages. *Iran Food Sci Technol Res J.* 2009; 5: 75-82.
10. Haghighi F, Rodbar Mohammadi S, Mohammadi P, Eskandari M. Evaluation of antifungal activity of Titanium Dioxide nano-particles and Ethylene Diamine Tetra Acetic Acid on the growth inhibition of Standard Strain of *Candida albicans*. *Armaghane-danesh.* 2011; 15.
11. Naghsh N, Doudi M, Soleimani S, Torkan S. The synergic effect of alcoholic eucalyptus and nano-silver on colony count of *Aspergillus*. *J Gorgan Univ Med Sci.* 2013; 14.
12. Lainez E, Vergara F, Bárcenas ME. Quality and microbial stability of partially baked bread during refrigerated storage. *J Food Eng.* 2008; 89: 414-418.
13. Leuschner R, O'Callaghan M, Arendt E. Moisture distribution and microbial quality of part baked breads as related to storage and rebaking conditions. *J Food Sci.* 1999; 64: 543-546.
14. Najafabadi M, Minaee S, Sharif Nasab H, Afshari H, Sattari F. Effect of films containing nano-particles on prevention of moisture loss packaged loaf bread. *Iran J Biosyst Eng.* 2012; 42: 141-149.
15. Bodaghi H, Mostofi Y, Oromiehie A, Zamani Z, Ghanbarzadeh B, Costa C, et al. Evaluation of the photocatalytic antimicrobial effects of a TiO₂ nano-composite food packaging film by in vitro and in vivo tests. *Food Sci Technol-Elb.* 2013; 50:702-706.
16. Nikouzadeh H. Effects of the Addition of Oat Barn and Psylliu Husk on the Rheological Characteristics of Dough and Quality and Shelf-life Stability of Sangak Bread. Tehran: Shahid Beheshti University of Medical Sciences and Health Services, College of Nutrition and Food Technology; 2007; 13-19.
17. ISIRI 10899. Microbiology of food and animal feeding stuffs: Horizontal method for the enumeration of yeasts and moulds. Tehran. 2008.
18. Alhendi A, Choudhary R. Current Practices in Bread Packaging and Possibility of Improving Bread Shelf-life by Nanotechnology. *INT J Food SCI Nutr.* 2013; 3: 55-60.