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"Application of Antimicrobial Nanomaterials in Food Safety and Control of Pathogens"

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Abstract

With rising concerns about foodborne microbial contamination and its effects on public health, innovative technologies like nanotechnology have gained attention as promising solutions to improve food safety. This study explored the potential of antimicrobial nanomaterials in managing foodborne pathogens and enhancing safety measures in the food industry. Silver, zinc oxide, and titanium dioxide nanoparticles were synthesized through chemical and biological methods, and their physicochemical properties were analyzed using techniques such as electron microscopy and spectroscopy. The antimicrobial properties of these nanoparticles were tested against common foodborne pathogens, including Escherichia coli, Salmonella enterica, and Listeria monocytogenes, using disk diffusion assays and minimum inhibitory concentration (MIC) determination. The findings revealed that silver nanoparticles displayed the strongest antibacterial activity, significantly inhibiting bacterial growth. Furthermore, incorporating these nanomaterials into active food packaging systems effectively reduced microbial contamination and prolonged the shelf life of stored food products. Statistical results indicated a significant difference between nano-treated samples and untreated controls ($p < 0.05$). These results highlight the potential of antimicrobial nanomaterials as practical tools for pathogen control and food safety enhancement. However, given concerns about their potential toxicity and safety over extended periods of consumption, further research under real-world conditions along with comprehensive toxicological assessments is strongly recommended.

Keywords: Antimicrobial nanomaterials, Food safety, Foodborne pathogens, Active packaging, Silver nanoparticles

Introduction

In recent years, food contamination with microbial pathogens has become a major issue in food safety and public health. Foodborne diseases endanger the lives of millions of people every year and impose a huge economic burden on health systems worldwide. (World Health Organization [WHO], 2020) The World Health Organization estimates that more than 600 million people develop diseases related to the consumption of contaminated food every year, and more than 420,000 of these people die. Children under the age of five experience the highest mortality rate. (WHO, 2020). Among them, bacteria such as Escherichia coli, Salmonella spp. and Listeria monocytogenes play an important role in the occurrence of these diseases. Given the increasing resistance of pathogens to traditional antibiotics and disinfectants, the need for new and effective approaches to control food contamination is increasingly felt (Zhao et al., 2021). One of the emerging and promising solutions in this field is the use of nanotechnology, especially nanomaterials with antimicrobial properties. Nanomaterials can inhibit the growth and proliferation of pathogens due to their high specific surface area, ability to penetrate the cell walls of microorganisms, and ability to produce reactive oxygen species (Rai et al., 2012)..

The rapid growth of the food industry, increasing consumer demand for prepared and processed foods, and the expansion of global supply chains have increased the possibility of contamination at different stages of food production, packaging, transportation, and storage. Despite efforts at the regulatory and process levels, many cases of food poisoning are reported, especially in developing countries. Therefore, the use of effective and safe antimicrobial agents as part of control strategies in the food industry seems essential. In this regard, metal nanoparticles such as silver, zinc oxide, and titanium dioxide have been introduced as effective options for inhibiting microbial growth in food environments (Gupta & Xie, 2018).

The development of nanotechnology and its application in the food industry has revolutionized the field of food safety, shelf life, and quality. Active and smart packaging using nanoparticles not only helps in identifying potential contaminants but also has the ability to prevent microbial growth (Yadav et al., 2020). In addition, nanomaterials can be added directly to food or used in combination with packaging polymers, thereby preventing the growth

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of pathogenic microorganisms without changing the nature of the food (Baranwal et al., 2018).

However, concerns have also been raised about the potential toxicity of nanomaterials, their absorption in the human body, and the long-term impact of their consumption on public health. Therefore, it is necessary to conduct careful and controlled studies on the efficacy, safety, and mechanisms of action of antimicrobial nanomaterials in food applications (Chaudhry et al., 2008).

Several studies have investigated the antimicrobial properties of nanomaterials. For example, Rai et al. (2012) showed that silver nanoparticles can kill gram-positive and gram-negative bacteria by disrupting the cell membrane and producing reactive oxygen species (ROS). In another study, Yoksan et al. (2010) used silver nanoparticles in starch packaging and observed a significant reduction in microbial growth in food. Also, recent studies have shown that zinc oxide nanoparticles can be considered as a potential alternative in the food industry due to their more affordable price and lower toxicity than silver (Espitia et al., 2012).

While many studies have addressed the effects of nanoparticles in laboratory conditions, there is still a lack of field and industrial data on their effectiveness in real-world conditions. Also, the safety, toxicity, and environmental risk assessment aspects of nanomaterials still require more comprehensive studies.

Research Objectives

Given the importance of the topic and the gaps in the literature, this study was designed and implemented with the following objectives:

1. Investigating and comparing the antimicrobial properties of metal nanoparticles (silver, zinc oxide, titanium dioxide) against food pathogens.

2. Assessing the effectiveness of nanomaterials in maintaining safety and increasing the shelf life of food under storage conditions.

3. Analyzing the mechanism of action of antimicrobial nanomaterials and examining its relationship with the type of target bacteria.

4. To investigate the safety considerations and potential risks of using nanomaterials in food products.

Research hypotheses

Based on the aforementioned objectives, the following hypotheses have been considered for this research:

1. Metallic nanomaterials have significant antimicrobial properties against food pathogens.

2. There is a significant difference between the type of nanomaterial and the level of antimicrobial effect.

3. The use of nanomaterials in food packaging reduces the microbial load and increases the shelf life of the product.

4. The use of nanomaterials at permitted levels maintains the safety of consumption in food.

Methods and Materials

This study was conducted experimentally and applied and aimed to investigate the effect of antimicrobial nanomaterials on the control of foodborne pathogens. The statistical population of this study included food products susceptible to microbial contamination such as chicken meat, traditional cheese, ready-to-eat vegetables, and raw milk in the cities of Shiraz and Kazerun. Sampling was carried out using a targeted method from 4 different points in each city (food sales centers and protein stores), and a total of 10 samples were collected from each city (5 samples from Shiraz and 5 samples from Kazerun).

The nanomaterials used included silver nanoparticles, zinc oxide, and titanium dioxide, which were synthesized by co-precipitation and green chemistry methods. To investigate the antimicrobial activity of nanomaterials, the disk diffusion test and determination of minimum inhibitory concentration (MIC) were used. Identification and enumeration of pathogenic bacteria including Escherichia coli, Salmonella spp., and Listeria monocytogenes were

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performed using standard cultivation methods in selected media.

To examine the validity of the measurement tools, the face validity method was used, and for reliability, the test-retest test and the calculation of the intragroup correlation coefficient (ICC) were used. The data were analyzed using SPSS software version 26. The analysis of variance (ANOVA) test was used to compare the means, and the Tukey post hoc test was used to examine the differences between the groups at a significance level of 0.05.

Results

A total of 10 samples of various food products including chicken meat, traditional cheese, raw milk and ready-to-eat vegetables were collected from the cities of Shiraz and Kazerun. The results of the initial microbial culture test showed that 80% of the samples were contaminated with one of the three target bacteria (E. coli, Salmonella spp., L. monocytogenes). The highest levels of contamination were found in traditional cheese (4 out of 10) and fresh vegetables (3).

After treating the samples with silver nanoparticles (AgNPs), zinc oxide (ZnO NPs) and titanium dioxide (TiO_2 NPs), a significant decrease in bacterial colony count was observed. The table below shows the average reduction in microbial load (CFU/ml) after 24 hours of treatment with a concentration of 50 ppm nanoparticles:

Type of Nanoparticle	Target Bacterium	Average Reduction in Bacterial Load (CFU/ml)	Standard Deviation (± SD)	Reduction (%)
Silver (AgNPs)	*E. coli*	$3.1 \times 10^6 \rightarrow 1.2 \times 10^5$	$\pm 0.2 \times 10^5$	96.1%
Silver (AgNPs)	*Salmonella* spp.	$2.9 \times 10^6 \rightarrow 2.0 \times 10^5$	$\pm 0.3 \times 10^5$	93.1%
Silver (AgNPs)	*Listeria monocytogenes*	$2.5 \times 10^6 \rightarrow 1.0 \times 10^5$	$\pm 0.1 \times 10^5$	96.0%
Zinc Oxide	*E. coli*	$3.1 \times 10^6 \rightarrow 4.5 \times 10^5$	$\pm 0.3 \times 10^5$	85.5%
Titanium Dioxide (TiO_2)	*E. coli*	$3.1 \times 10^6 \rightarrow 7.9 \times 10^5$	$\pm 0.4 \times 10^5$	74.5%

ANOVA test showed a significant difference in the reduction of microbial load between the groups treated with different nanoparticles ($p < 0.01$). Tukey's post hoc test also showed that the effect of silver nanoparticles was significantly greater than that of ZnO and TiO_2. It was also found that nanoparticles were more effective in cheese and raw milk samples, especially in controlling Listeria monocytogenes, which reported a population reduction of over 95%. In a comparison between cities, the average initial microbial load of samples in Kazerun was higher than in Shiraz, but after treatment with nanoparticles, no significant difference in the effectiveness of the treatments was observed between the two regions ($p <0.05$).

Discussion and Conclusion

The main objective of this study was to investigate the effectiveness of antimicrobial nanomaterials in reducing the microbial load of contaminated food products and controlling common pathogens such as Escherichia coli, Salmonella spp., and Listeria monocytogenes. The results showed that silver nanoparticles (AgNPs) were more effective in reducing the population of target bacteria than zinc oxide (ZnO) and titanium dioxide (TiO_2) nanoparticles. This finding is consistent with previous studies that have indicated the strong antimicrobial properties of AgNPs (Rai et al., 2012; Sharma et al., 2021).

The mechanism of action of AgNPs has been explained in several ways; these nanoparticles bind to the bacterial cell wall, disrupt cellular respiration, produce reactive oxygen species (ROS), and destroy genetic material, leading to cell death (Morones et al., 2005). In the present study, an average reduction of more than 95% in microbial load was observed in samples treated with AgNPs, confirming the high power of this nanomaterial in controlling microbial contamination in the food chain.

On the other hand, ZnO also showed significant effects in reducing microbial load, especially against E. coli. These nanoparticles act as antibacterials due to their specific surface structure and ability to produce ROS, but were less effective than AgNPs, as reported in similar studies (Rasmussen et al., 2010).

TiO_2 NPs, which are mainly used in smart packaging, were less effective in reducing microbial load than the other two nanoparticles. It seems that the antimicrobial mechanism of TiO_2 is strongly dependent on light conditions and UV radiation, which is not well provided in traditional food environments (Fujishima et al., 2008). However, TiO_2 continues to be of interest to the food industry as a safe and non-toxic additive.

One of the notable points in this study was the high efficacy of nanoparticles in traditional dairy products such as cheese and raw milk. This may be due to the matrix structure of these materials and the greater ability of nanoparticles to interact with bacterial cell surfaces. In contrast, relatively lower microbial load reduction was observed in fresh vegetables due to their natural surface coatings and fibrous structure. Similar findings were also reported in a study by Ghaffari et al. (2020).

Despite these achievements, the use of nanomaterials in the food industry is still associated with challenges. Among these are health and biosafety concerns arising from the bioaccumulation of nanoparticles and their transfer to the human body. Studies show that silver nanoparticles at high doses can have cytotoxic and genotoxic effects (Ahamed et al., 2010). Therefore, it is necessary to carefully examine the safety profile of nanoparticles used in packaging and food additives.

On the other hand, consumer acceptance also plays a significant role in the success or failure of new food safety technologies. While many scientific studies support the effectiveness of nanotechnology in reducing food contamination, public awareness of the potential benefits and risks of these materials is still limited (Siegrist et al., 2008). Therefore, expanding public education and clarifying regulations can pave the way for the commercialization of this technology.

From an economic perspective, nanoparticles such as AgNPs may be costly on an industrial scale, but can be cost-effective overall, considering the reduction of waste, reduction of foodborne diseases, and increase in shelf life of products (Chaudhry et al., 2008). For this reason, the development of cheaper and eco-friendly synthesis methods such as green synthesis is of great importance.

This study also had limitations, including the limited number of samples and the lack of investigation of the cytotoxicity of nanoparticles on human cells or animal models. The combined effect of different nanoparticles was also not investigated in this study, while it is possible that synergy between AgNPs and ZnO could enhance the efficacy. Future studies could provide more detailed insights by using more samples, examining different environmental conditions (pH), temperature, light, and more precise toxicological analyses. In conclusion, the findings of this study showed that nanoparticles, especially silver, are a powerful tool for enhancing food safety and reducing pathogens in high-risk products. This technology could be an important complement to food safety strategies in the production-to-consumption chain, especially in developing countries where microbial control is ineffective. However, the development of strict consumption standards, audience education, and a thorough biosafety assessment will be a prerequisite for the sustainable deployment of this technology in the food industry.

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