P-ISSN: 2305-6622; E-ISSN: 2306-3599
International Journal of Agriculture and Biosciences
www.ijagbio.com; editor@ijagbio.com
Review Article https://doi.org/10.47278/journal.ijab/2022.025

Physical factors affecting the antibacterial activity of Silver (Ag) and Zinc Oxide (ZnO) Nanoparticles (NPs), their application in edible and inedible food packaging, and regulation in food products

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Article History: 22-287 Received: 18-June-2022 Revised: 29-Jul-2022 Accepted: 23-Sep-2022

ABSTRACT

Nanochemistry has its diverse applications in the field of food science and technology. Various nano particle acts as anti-bacterial agents in active packaging of food. The application of these active packaging containing nanoparticles in it plays a vital role in the protection, preservation and enhancement in the shelf life of food. Silver nano particles (AgNPs) and Zinc Oxide nanoparticles (ZnO NPs) can be easily mixed with bio-polymer and synthetic polymers used for packaging materials. These nanoparticles act as anti-microbial agent to enhance the shelf life of food products. This review presents the effect of various physical factors including size, concentration, morphology, pH, surface charge and strain specific activity upon the anti-bacterial activity of AgNPs and ZnO NPs against five gram positive *Staphylococcus aurerus*(*S. aurerus*), *Listeria monocytogenes (L. monocytogenes), Streptococcus pneumoniae(S .pneumoniae), Enterococcus faecalis* (*E. faecalis), Bacillus subtilis* (*B.subtilis*) and five gram negative *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa (P. aeruginosa), Salmonella typhimurium (S. typhimurium), Klebsiella pneumonia (K. pneumoniae), Klebsiella aerogenes (K. aerogenes)* strains are presented. Furthermore, practical approaches for addition of AgNPs and ZnO NPs in edible and inedible coating of various food products to enhance the shelf life of food materials is also presented. It is important to address all possible health effects caused by nanoparticles to ensure food safety. Therefore, this review also presents regulation and limitation of different regulating bodies' sets for Ag and ZnO NPs.

Key words: Nanochemistry, Inedible Food Packaging, Anti-Microbial Agent

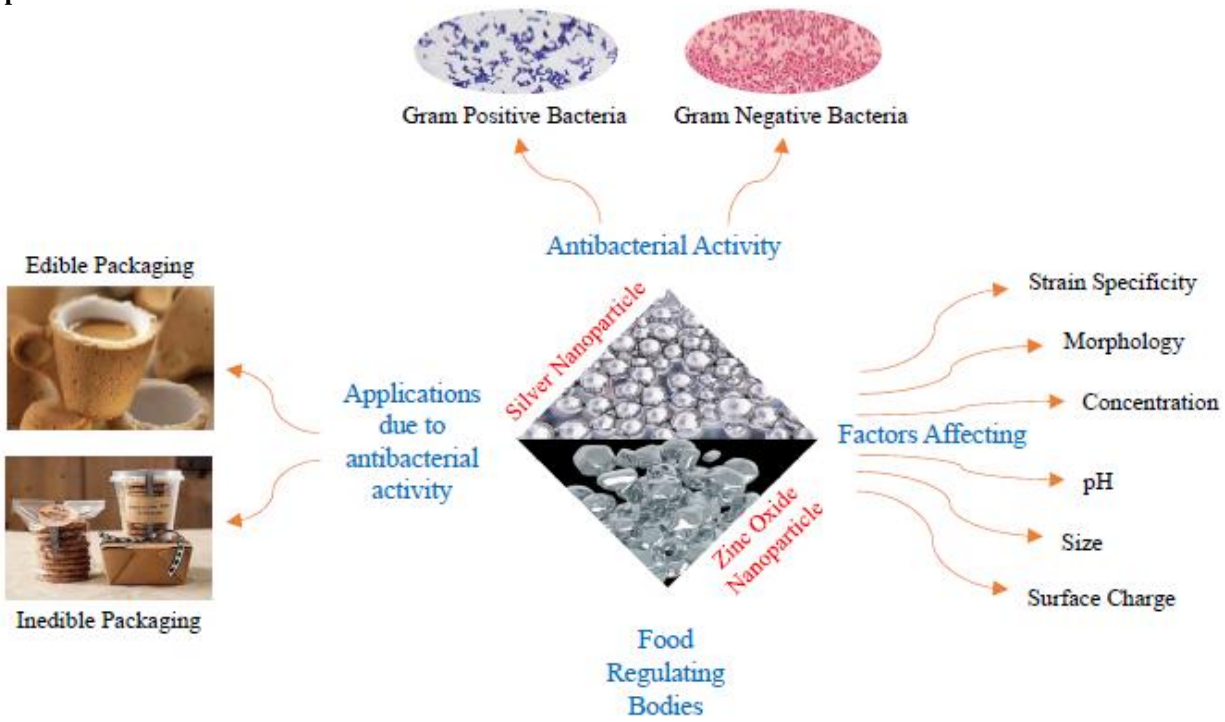
INTRODUCTION

The word "Nano"is derived from the Greek letter άνος (Latin nános), meaning "dwarf". The word "nano" means miniature size or very small. It is usually combined with a noun to form different words like nanotechnology, nanometer, nanomaterial or nanorobot. In the area of nanotechnology we deal with the items with size (10^{-9} m). Nanotechnology is the manipulation of individual atoms, molecules, or compounds into structures to produce special properties (Usha et al., 2018). Nanotechnology has the ability to be used in food packaging industry as a tool for delivery systems of various nanoparticles, for the detection of pathogens, for treatment of diseases, for packaging materials, and for the delivery of bioactive chemicals to specific target sites. Application of nanotechnology helps modern food system by providing good nutritional value to food items and by improving the safety level of foods (Rashidi& Khosravi-Darani, 2011).

Food packaging material used for packaging of food should be safe, has ability to temper resistance, and should meet specific chemical, physical or biological standards. Now a day's packaging materials of food utilize different application of nanoparticles, to develop high barrier properties, to capable of releasing nanoparticles as preservatives for the enhancement of food' s shelf life in containers, to repair torn packaging, to improve safety of food, and to alert customers when food is contaminated or rotten (Chellaram et al., 2014). Results of different studies suggest that nanoparticle optical assays could be used as a new and efficient way for real-time evaluation of the nanoscale mode of action of antimicrobial agentin living bodies(Kyriacou et al., 2004).

Cite This Article as: Azam SE, Yasmeen F, RashidMS and Latif MF, 2022. Physical factors affecting the antibacterial activity of Silver (Ag) and Zinc Oxide (ZnO) Nanoparticles (NPs), their application in edible and inedible food packaging, and regulation in food products. Int J Agri Biosci, 11(3): 181-193. https://doi.org/10.47278/journal.ijab/2022.025

Graphical Abstract



Graphical demonstration of Abstract

In food sector there are two main types of nanoparticle. Organic nanoparticles and metallic nanoparticle .Organic nanoparticles are mostly used in the synthesis of active food items where metallic nano particle act as antimicrobial agent (Peters et al., 2011). Nanoparticles has high level of anti-bacterial activity due to their larger surface area to volume ratio (Agarwal et al., 2018). This review mainly focuses to describe the effect of various physical factors like size, morphology, dose, pH, surface charge and strain specificity upon the antibacterial study of Ag and ZnO nanoparticles against different strain of gram-positive and gram-negative bacteria. The reviews also discuss the practical application of Ag and ZnO nanoparticle in edible and inedible coating of various food materials to enhance their shelf life. Regulations and limitation of Ag and ZnO nanoparticles for their use in food products or in food packaging also reviewed.

A. Role of silver nanoparticle

Silver nanoparticle could be easily mixed in biopolymer films of starch and chitosan as they are added in petroleum-based thermoplastic films of nylon, polystyrene, polypropylene and polyethylene (Rhim et al., 2013). Various natural compounds such as essential oil or plant extract used in edible packaging for food preservation but because of their high level of fragrance and taste they may strongly contribute to alter the organoleptic characteristics of food product. Therefore use of essential oils and plant extracts in food packaging are limited. Besides the good anti microbial activity, using silver nanoparticle in edible packaging has the advantage that it has very low effect on the sensory attributes of food. Therefore the use of Ag NPs in food packaging is more reliable for consumers (Kraśniewska et al., 2020).

A.1 Factors affecting anti microbial activity of silver nanoparticles

Shape, size, concentration and surface charges are the important factors that affect the antimicrobial properties of silver nanoparticles. Regarding shape (i.e., platelet, cubic , decahedron, triangular, spherical) and in many others shapes the spherical and the triangular shapes show highest antimicrobial activity (Zorraquín-Peña et al., 2020).

A.1 (a) Regarding morphology

A study revealed that the anti-bacterial activity of Ag NPs is shape depended and the results of the study shows that the interaction of gram negative bacteria *Escherichia coli* is also in shape-dependent manner, when interact with silver nanoparticles (AgNPs). Although the findings for antibacterial behavior of silver nanoparticles towards *Escherichia coli* were same in all nanoparticles but with nanoparticles with distinctively different shapes have different results for inhibition of *E. coli*. The percentages of active faces found in nanoparticles of various shapes can be used to explain the observed differences in inhibition of *E. coli*. The study shows a high reactivity of the truncated triangular nanoparticles is expected, when compared to other spherical or rod-shaped particles (Pal et al., 2007).

In a similar study assessment of the anti-microbial activity of nano silver shapes; Ag-nanoplates (AgNPls), Ag-nanorods (AgNRds) and silver nano-particles (AgNPs) was studied. The result demonstrates that AgNPls have an excellent anti-bacterial activity for *Staphylococcus aurerus (S. aurerus*) and *Escherichia coli* (*E. coli*) when compared with AgNRds and AgNPs. Finding shows that AgNPls compared with AgNRds and

AgNPs, requires a minimum amount to inhibit the strains development of *S.mutants*and *E. coli* and this is because of an increase in surface area of AgNPls compared with AgNRds and AgNPs(Sadeghi et al., 2012).

In an in vitro study the antibacterial properties of synthetic AgNPs against *Pseudomonas aeruginosa* and *E. coli* were evaluated by using the Kirby-Bauer disc diffusion-sensitive technique. Compared with large spherical and triangular AgNPs, the smallest-sized spherical silver nanoparticles exhibited superior antibacterial activity over both *P. aeruginosa* and *E. coli* strains. Although the larger size spherical silver nanoparticles had lower bactericidal activity over both bacterial types than the triangular silver nanoparticles, the smaller sized spherical silver nanoparticles exhibited better antiseptic properties than the triangular silver nanoparticles (Raza et al., n.d.).

A.1 (b) Regarding Size and Concentration

In a study the size- and concentration-dependent antimicrobial activities of silver nanoparticles were thoroughly investigated. AgNPs with average sizes of 5, 7, 10, 15, 20, 30, 50, 63, 85, and 100 nm were prepared in high yield and monodispersity. It was noticed that the minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of silver nanoparticles is size and dose dependent. The experimental results show that all AgNPs are extremely harmful to various bacterial types and the antibacterial effect of these nanoparticles increase with a corresponding decrease in their particle size. Compared with 7 nm and 10 nm silver nanoparticles at their particular minimum bactericidal concentration doses, 5 nm AgNPs exhibited the fastest bactericidal effect. This effect is significantly amplified as the nanoparticle size approaches the sub-10 nm range. *S. aurerus, E. coli,* and *B. subtilis* are the three bacterial strains used in the study. Interestingly, 5, 7 and 10 nm AgNPs has shown the same level of bacteriocidal activity regardless of these strains (Agnihotri et al., 2014).

In another work, stable Silver nanoparticles with markedly diverse sizes (5, 15 and 55 nm average) were prepared. Their antibacterial activity was examined by colony counting assay growth inhibition curve method, and equivalent MIC method against aerobic (*E. coli*) bacteria and five oral infectious anaerobic bacteria (*S. sanguis, S. mutans, S. mitis, F. nuceatum and A. actinomycetemcomitans*).

The findings of study showed that all three types of AgNPs (various diameters of 5, 15 and 55 nm averaged) considerably slowed the development of all anaerobic and aerobic species and the bacterial growth gradually slowed down with increasing nanoparticles concentration. Particles of different sizes exhibited different rates of growth inhibition. At a concentration of 25 g/mL, 5-nm silver nanoparticle totally prevented the growth of *F. nuceatum*; for 15- and 55-nm Ag, the concentrations were 50 and 100 g/mL, respectively. The parallel Minimum Inhibitory Concentration values for 5, 15, and 55 nm of silver nanoparticles for *A. actinomycetemcomitans*were 25, 50, and 200 g/mL respectively. In contrast anaerobic oral pathogens were less resistant to Ag NPs than aerobic bacteria. During the study Ag NPs exhibited a clear size-dependent antibacterial effect against anaerobic bacteria. The most potent antibacterial activity was observed with 5nm silver nanoparticles (Lu et al., 2013).

A.1 (c) Regarding pH

A study was conducted to understand how pH affects the antimicrobial properties of AgNPs. *Pseudomonas fluorescens* was exposed to well-characterized AgNPs for 24 hours at pH levels from 6 to 9. pH had little effect on the development of the control, with a little bit slower growth at low pH values. Controls included only bacteria, silver nanoparticles, latex nanoparticles (negatively controlled, comparable in size but with low to zero chemical toxicity), or silver nitrate (positive control) used in the bacterial absence. Only in lack of SRHA (Suwannee River humic acid) silver nanoparticles become toxic at a dose of 2000 ppb at pH 9, resulting90% reduction in bacterial cell density after the 24 hours of contact. There is no apparent explanation for pH-related toxicity (Fabrega et al., 2009).

A.1 (d) Regarding strain specificity

A study was conducted to determine the strain specific anti microbial activity of silver nano particle. *E. coli* (four strains), *S. aureus* (three strains) and *B. subtilis* were utilized to study anti-microbial potential of silver (Ag) and copper (Cu) nanoparticles. Transmission electron microscopy was used to find out the average size of copper and silver NPs, which were 3 nm and 9 nm respectively. Different bacteria have different extent of sensitivity to nanoparticles, which depends on the species of microbes. Silver nanoparticles were more effective than copper nanoparticles in disc diffusion assays of *E. coli* and *Staphylococcus aureus*. Compared to other strains, *B. subtilis* was the most susceptible to nanoparticles and copper nanoparticles affects them prominently. *S. aureus* has minimal strain-specific variation in MIC and MBC, but *E. coli* exhibits some strain-specific variation (Ruparelia et al., 2008).

A.1 (e) Regarding surface charge

Surface charge also has a significant role in the bactericidal activity of silver nano particles. The work has been reported in various studies. In a study AgNPs with different surface charges (negative, positive and neutral) were prepared. To check the anti-bacterial activity, all synthesized Ag NPs were tested against gram-positive (i.e., *Streptococcus mutans, Streptococcus pyogenes* and *Staphylococcus aureus)* and of course against gram-negative (i.e., *Proteus vulgaris* and *Escherichia coli)* bacteria. The result of the study show that positively charged AgNPs has the most efficient antimicrobial activity against all gram positive and gram negative bacteria. Whereas the neutral Ag NPs showed intermediate level of antibacterial activity, while the negative chargedAg NPs showed the least level ofantibacterial activity(Abbaszadegan et al., 2015).

A.2 Antibacterial activity against gram positive and gram negative bacteria

Silver nano particle are reported as a good anti bacterial agents in edible coating of food. In the Table 1 bacteriostatic and bacteriocidal activity of silver nano particles against various strain of gram positive bacteria

Table 1: Antibacterial activity of various biopolymer-based materials containing Ag NPs.

Polymer Matrices	Approach	Size of Silver Nanoparticles (nm)	Concentration of AgNO3/AgNPs in Film-Forming Solution	Tested Strains	Gram (-) Pathogens	Gram (+) Pathogens	Antibacterial Effect of Nanocomposite Films	Reference
Chitosan/ Hydroxyethylcellulose (HEC)	in-situ	–	–	*S. aureus*, *E. coli*, *S. Typhimurium*, *L.monocytogenes*, *B. cereus*	*S. Typhimurium*, *E. coli*	*B. cereus*, *L.monocytogenes*, *S.aureus*	Inhibition zone (mm): *S. Typhimurium*: 13–15 *E. coli*: 12–14 *S. aureus*: 10–11 *B.cereus: 12* *L.monocytogenes:* 10–11	(Youssef et al., 2015)
Hydroxypropyl methylcellulose (HPMC)/ Tragacanth/ beeswax	ex-situ: Silver Nanopartilces were purchased from US Research Nanomaterials Inc.	8–10	AgNPs: 2%, 4% and 8%	*K. pneumoniae* ATCC10031, *P. aeruginosa* ATCC 9027, *S. Typhimurium* ATCC 14028, *E.coli* ATCC 8739, *S. pneumoniae* ATCC 49615, *B. cereus* ATCC 1247, *S. aureus* ATCC 25923, *L.monocytogenes*ATCC 7644	*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. Typhimurium*	*B. cereus*, *S. aureus*, *L. monocytogenes*, *S. pneumonia*	Inhibitory effect was observed against all tested strains of bacteria; The high percentage concentration of silver nanoparticles into biopolymer matrix become cause of larger inhibitory zones diameter	(Arash et al., 2018)
Zein	ex-situ	___	Ag-MTT NPs (a) 10 mg; (b) 15 mg; (c) 20 mg	Pseudomonas spp.	Pseudomonas spp.	___	(a) 1.53 log CFU/g (b) 1.83 log CFU/g (c) 2.12 log CFU/g	(Incoronato et al., 2010)
Chitosan	ex-situ: Silver Nanoparticles were synthesized by biological methods	10–25	AgNPs: (a) 0.5% (b) 1% (c) 2%	*Candida albicans*, *A. niger* *P. aeruginosa*, *S. aureus*	*P. aeruginosa*	*S. aureus*	Inhibition zone (mm): *A. niger* (a) 10; (b) 8; (c) 9 *C. albicans* (a) 12; (b) 12; (c) 19 *P. aeruginosa* (a) 11; (b) 12; (c) 15 *S. aureus* (a)9; (b) 10; (c) 18	(Youssef et al., 2014)
Chitosan/ sodium alginate	ex-situ: Silver Nanoparticles were synthesized by biological methods	5–21	–	*P. aeruginosa* MTCC 2488 *E. aerogenes* MTCC 2822, *E.coli*,	*E. aerogenes*	*E. faecalis*	Inhibition zone (mm): *P.aeroginosa*:3.1 *E. aerogenes*: 1.5 *E. coli*: 1.9 *E. faecalis*: 5.1	(Sharma et al., 2012)

				E. faecalis MTCC 439, *B.cereus* MTCC 1305			*B. cereus*: 6.0	
Agar	ex-situ	___	Ag-MTT NPs (a) 10 mg; (b) 15 mg; (c) 20 mg	Pseudomonas spp.	Pseudomonas spp.	___	Reduction level of bacteria count (a) 1.88 log CFU/g (b) 2.09 log CFU/g (c) 3.59 log CFU/g	(Incoronato et al., 2011)
Commercial Nano Silver film	ex-situ	___	4.5 µg Ag/g film	*Enterobacteriaceae* and *Pseudomonas*	*Pseudomonas*, *Enterobacteriaceae*	___	Film having Ag NPs shows no significant difference in the antibacterial activity when compared with conventional film	(Deus et al., 2008)
Chitosan	in-situ	___	___	*Staphylococcus aureus* *Escherichia coli*	*Escherichia coli*	*Staphylococcus aureus*	Film compromised of chitosan, laponite and Silver nanoparticles show less antibacterial activity when compared with chitosan film. The reason behind low antibacterial of film is may be because laponite decreases the release of silver nanoparticles. The inhibition of gram positive bacteria is greater than gram negative bacteria.	(Wu et al., 2018)
PVA-montmorillonite K10 clay nanocomposite	In-situ	___	___	*Salmonella typhimurium* *Staphylococcus aureus*	*Salmonella typhimurium*	*Staphylococcus aureus*	Film having silver nanoparticles show antibacterial activity against both types of bacteria. When the film was used to store chicken sausages at 4◦C for 4 days, a low level of microbial growth was observed compared to local films.	(Mathew et al., 2019)
Cellulose naofibrils /AgNP Composite	In-situ	8–15 nm	30, 75, 150, and 300 µg Ag/mL	*Listeria monocytogenes* *Escherichia coli* O157:H7	*Escherichia coli* O157:H7	*Listeria monocytogenes*	The material having silver nanoparticlesshow high level of antibacterial activity against E. *coli* than against L. *monocytogenes* because of the greater wall thickness of the gram-positive bacteria.	(Yu et al., 2019)

Dose-dependent antimicrobial activity of silver nanoparticles on polycaprolactone fibers showed that Gram-negative bacteria are more susceptible to polycaprolactone/Ag complexes than Gram-positive bacteria (Pazos-Ortiz et al., 2017).

{Listeria monocytogenes (L. monocytogenes), Staphylococcus aurerus (S. aurerus), Streptococcus pneumoniae (S. pneumoniae), Bacillus subtilis (B.subtilis), Enterococcus faecalis (E. faecalis)} and gram negative bacteria {*Escherichia coli (E. coli), Klebsiella pneumonia (K. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa), Salmonella typhimurium (S. typhimurium, Klebsiella aerogenes (K. aerogenes)*}have been discussed.

A.3 Role of silver nanoparticles in packaging to enhance shelf life

Edible coating of food material is an emerging research area in food safety and quality. It is now necessary to create protective coatings with antibacterial properties as they help to enhance the shelf life of foods. Researchers have been going on to evaluate the risks, mechanism and the advantages of utilizing nano particles in edible coating to increase the shelf life. Silver nano particles are reported with good bactericidal activity during in vitro studies. Practical applications are going on to check the anti bacterial activity in edible coating.

A.3 (a) Role of silver nanoparticles in edible packaging to enhance shelf life

A research was performed to estimate the performance of anti-microbial packaging of a film having AgNPs in it to prevent the quality of Fior di Latte cheese. The film was prepared by adding silver montmorillonite embedded in agar. At 10 °C, tests were conducted to evaluate the organoleptic quality of cheese, the cellular load of dairy and pathogenic bacteria that can cause spoilage. The results of this study showed an extended shelf life for all Fior di Latte samples packed actively and confirm that silver cations may have a negative effect on the growth of pathogenic bacteria and remained ineffective on the functional dairy microbiota and organoleptic quality. The active packaging technology prepared in this work can be utilized to increase the shelf-life and increases distribution of Fior di Latte beyond local market boundaries (Incoronato et al., 2011).

In several studies the packaging film loaded with mixture of nano particles and essential oil used for challenge study and proved as anti microbial packaging. In a study, pullulan films having the compounds (essential oils of oregano and rosemary, silver 100 nm and ZnO 110 nm nanoparticles) effectively inhibited the pathogens *Staphylococcus aurerus (S. aurerus*), *Listeria monocytogenes (L. monocytogenes), Escherichia coli* (*E. coli* O157:H7), and *Salmonella typhimurium (S. typhimurium)* in plate overlay assays when poultry products and meat store for 3 weeks at 4 °C under vacuum conditions rather than control films. The results of this research suggest that edible films of pullulan containing nanoparticles or essential oils can improve the safety of fresh, processed or poultry and frozen meat products (Morsy et al., 2014).

In another study, research was performed to examine the antibacterial effects of carboxymethyl cellulose (CMC) and guar gum based coatings having AgNPs. Film was applied on kinnow citrus (Citrus reticulata cv. Blanco) and its postharvest storage durability was analyzed for four months at 4°C and 10°C with 85%-90% relative humidity. After every 15 days of storage, microbiological and physicochemical properties of Kinnow citrus were monitored. All samples stored at 4°C and 10°C contain total aerobic psychrophilic bacteria, yeasts and molds; however their development was limited at 4 °C. The control samples kept at 10°C showed severe fruit rot, but have no adverse effects from freezing. The result of study showed that coated Kinnow fruit could be kept fresh for two months at 10°C and for four months at 4°C (Wasim et al., 2015).

A.3 (b) Role of silver nanoparticles in, inedible packaging to enhance shelf life

A research was performed to find out the effect of nano silver in Polyvinylpyrrolidone (PVP) coating about the physical, chemical and anti microbial characteristic in asparagus spear stored at 2 and 10 °C. After the comprehensive comparison and evaluation the result of the study shows that asparagus spear coated by silver nano particle could be maintained in excellent quality for 20 days at 10°Cand 25 days at 2°C and thus enhancing the shelf life for 10 days at 2 °C (An et al., 2008).

A research was carried out to find the effect of packaging containing nano-silver to enhance the shelf life of nuts. The special effects for various concentrations (0, 1, 2, and 3%) of nano-silver on the chemical and biological characteristics of 432 nut samples having almonds, hazelnuts, walnuts and pistachios were examined for 2 years at every 3 months interval. The packaging with different concentrations of nano-silver (1%, 2%, and 3%) have markedly decrease mold, coliform and microbial counts in most samples when compared with control group. Nano-silver packaging contains 3 % silver concentration show excellent results. This packaging produced antioxidant effects, especially at nano-silver concentrations of 2% and 3%. According to the results of biological and chemical examination, AgNPs significantly affected the shelf life of nuts. Pistachios, almonds, hazelnuts and walnuts have the longest shelf lives of 20, 19, 18 and 18 months respectively. The amount of AgNPs has an effect on shelf life. The best antibacterial effect was seen when 3% concentration of nano-silver was used in packaging of pistachios. The average shelf life of the control group stored under comparable settings was determined to be 13 months. The results of studies suggest that packaging of nuts with nano-silver can extend their shelf life. Therefore it is recommended that food packaging companies should adopt nano-silver packaging (Tavakoli et al., 2017).

B. Role of Zinc Oxide nanoparticle

A commonly used inorganic substance in daily life is zinc oxide (ZnO). The U.S. Food and Drug Administration currently lists ZnO as a Generally Recognized As Safe (GRAS) material and is frequently used by food manufacturing companies as a food additive. The advancement of nanotechnology has facilitated the formulation of materials with novel characteristics so that it could be used as antimicrobial agents. Nano-scale ZnO has exhibit strong antibacterial properties and can be used for food preservation. Therefore, ZnO nanoparticles can be easily added to polymer matrices to enhance packaging properties and impart antimicrobial activity to packaging materials (Judith& Espitia, 2012).

B.1 Factors affecting anti microbial activity of Zinc Oxide (ZnO) nanoparticles

The anti-bacterial activity of Zinc Oxide nanoparticles is affected by many physical parameters, just like silver and many other nanoparticles. The effect of ZnO on its biological function depends on its shape, particle size, pH, concentration, exposure time, and biocompatibility (Siddiqi et al., 2018) .

B.1 (a) Regarding morphology

In a controlled study the research focuses the controlled preparation of Zinc Oxide NPs and its morphology-dependent antibacterial and optical studies. Scanning electron microscopy (SEM) imaging revealed that ZnO with rod-like, spherical and flower-like shapes was prepared. Inactivation of *E. coli* and *S. aurerus* was observed using ZnO NPs in MilliQ water. The antibacterial effect of ZnO nanoparticles shows that antibacterial activity of Zinc Oxide flower-shaped nanoparticles is significantly better than that of Zinc Oxide rod-shaped and spherical nanoparticles (Talebian et al., 2013).

In a study sphere and rod shape ZnO nanoparticles were prepared. Their antibacterial activity was examined by using diverse Gram-positive and Gram-negative bacteria. According to result of studies, rods and wires shaped nanoparticles penetrate bacterial cell walls with more ease and rapidly compared to the spherical ZnO-NPs. This result shows that the shape of ZnO NPs affects the entry mechanism of ZnO NPs and show morphology dependent antibacterial activity (Yang et al., 2009).

In another study ZnO NPs was synthesized in different forms, including multi-petal, rod, and spherical shape. Then these NPs were applied to cotton fabrics for Ultra Violet blocking and antibacterial studies. The Zinc Oxide nanoparticles were studied by using SEM and X-ray diffraction analysis (XRD). Atomic absorption spectroscopy (AAS) and SEM analyses clearly reveals that Zinc Oxide NPs was successfully deposited on the cotton surface and remained adhere after ten washing cycles. In all treated samples, *S. aureus* was successfully eradicated with excellent antibacterial activity. The result of study shows that shape of ZnO NPs did not affects its antibacterial ability(Sricharussin et al., 2011).

B.1 (b) Regarding size

In a study on the size-dependent inhibition of bacterial growth by ZnO NPs, nanoparticle of 12, 25, 30, 88,142, 212, 307 nm has been prepared. The anti bacterial activity was tested again *Staphylococcus aureus* (*S. aureus*) with a fixed concentration of 6 mM. The anti-bacterial activity of ZnO nanoparticles was found to be inversely correlated with their size against Staphylococcus aureus. The smallest ZnO particle size (12 nm) not only restricted the growth of S. aureus, but also killed them effectively (Raghupathi et al., 2011).

In another study, powders with different particle sizes having range from 0.1 to 0.8 mm were used to examine the possible effect of particle size upon the antimicrobial activity of Zinc Oxide powders. The variations in the electrical conductivity were monitored with changes in bacterial growth development and it was noticed that the antibacterial activity of Zinc Oxide increased with increasing powder concentration and decreasing particle size. There was resemblance in the antibacterial effect of *S. aureus* and *E. coli*. However, *S. aureus* had less effect on particle size than *E. coli*(Yamamoto, 2001).

Another study examined the antibacterial properties of ZnO nanoparticles of different particle sizes such as 12nm, 31nm and 45nm against *E. coli*. The nanoparticles were characterized by using X-ray diffraction (XRD), transmission electron microscopy (TEM) and photoluminescence (PL) spectroscopy. According to bacteriological results, the bactericidal effect of ZnO nanoparticles gradually decreases by increasing the particle size. The reason for the greater bactericidal activity of small particles is believed to be their high level of surface area to volume ratio. ZnO nanoparticles of smaller size were used in a large amount to cover a bacterial colony of 2µm. This produces a larger amount of reactive oxygen species from Zinc oxide on the colony surface which enhance their bactericidal activities (Padmavathy& Vijayaraghavan, 2008).

B.1 (c) Regarding concentration

In a study the antibacterial activity of ZnO NPs at various concentrations (20-100 g/ml) was determined against the pathogens *K. pneumoniae, E. faecalis, S. aureus* and *E. coli*. The results showed that due to the bacteriostatic of ZnO nanoparticles growth of all the tested pathogens suppressed effectively. The result of study shows that antibacterial activity of ZnO enhances by increasing the concentration and decreasing the size of nanoparticles (Narayanan et al., 2012).

A research was conducted to examine the effect of zinc oxide nanoparticle concentration on its antibacterial activity. MIC, MBC, population growth and changes in the values of the optical density (OD) at 620 nm were used to observe the bacteriocidal effects of ZnO NPs. The result of study shows that zinc oxide nanoparticles have an antibacterial effect on *Escherichia coli* K88 and their bacteriocidal effect increased with increasing ZnO NP concentration (Chao Wang, 2012).

B.1 (d) Regarding strain specificity

A research was performed to examine the strain-specific activity of ZnO NPs. In the study qualitative and quantitative experiments were used to determine the susceptibility of several bacterial strains to ZnO nanoparticles. Two Gram-positive bacteria (*B. cereus* and *S. aureus*) and seven Gram-negative bacteria (*E. cloacae, E. coli O157:H7*, E. coli O157:H7, *P. fluorescens*, *P. aeruginosa*, *S. typhimurium* and *S. enteritidis* are used in the study. ZnO NP of 50 nm was synthesized. The results of the study showed that *Bacillus cereus* was most susceptible to ZnO nanoparticles. The MICs of *Pseudomonas* shows that it has higher resistant against ZnO NPs compared to other strains of bacteria used in the study (Tayel et al., 2011).

B.1 (e) Regarding pH

A study was conducted with the aim to understand how temperature and pH affect the antibacterial activity of Zinc Oxide NPs against various pathogenic bacteria. Ammonium citrate was used as a dispersion to produce, characterize, and then disperse ZnO NPs in glycerol. Antibacterial study was performed by monitoring the growth of *E. coli* O157:H7 and *S. aureus* in glycerol by

using different concentrations of ZnO NPs. Different incubation temperatures (25-42°C) and pH levels (4-10 for *E. coli* O157:H7 and 5-10 for *S. aureus*) were used for each experiment. The result of study reveals that the Zinc Oxide nanofluid had antibacterial effect against and *Escherichia coli O157:H7* and *S. aureus*. The inhibition of tested strains was stronger with increasing nanofluid concentration. The experimental result also reveals that temperature and pH had effects upon the antibacterial activity of ZnO NPs. High level of antibacterial activity was observed at acidic pH.ZnO NPs show high level of toxicity for *E. coli O157:H7* at pH 5 and for *S. aureus* at pH 4. The toxicity of ZnO nanofluids increased with increasing temperature, with both bacteria having the greatest antibacterial activity at 42°C compared to the positive control in the same condition. Data analysis showed that culture variables and ZnO NP exposure medium had significant effects on the cytotoxic effect of ZnO nanoparticles (Saliani et al., 2015).

B.1 (f) Regarding surface charge

A study was conducted to find out the anti-bacterial propensity of Zinc Oxide NPs against various strains of Gram positive and Gram negative bacteria. Zinc Oxide nanoparticles with positive and negative surface potentials were tested on various bacteria with surface potentials ranging from 214.7 to 223.6 mV. The chemically developed ZnO NPs with positive surface potential showed a strong antibacterial tendency. The minimum inhibitory concentrations against Gram-negative and gram-positive bacteria were 50 and 100 mg/mL respectively and similar sized ZnO NPs with negative surface potential show negligible antibacterial activity against the studied bacteria. In contrast to positively charged nanoparticles, neither zinc ions (Zn2+) nor negatively charged zinc oxide nanoparticles (ZnO NPs) remarkably inhibit bacterial growth (Arakha et al., 2015).

B.3 Role of Zinc oxide nanoparticles in packaging to enhance shelf life

Zinc oxide nano particles are generally recognized as safe to use as antibacterial agent. Active and edible coating of food material is an emerging research program in food safety and quality. In current era its need of time to develop food packaging that could enhance the shelf life of food products. Researchers have been going on to evaluate the risks, mechanism and the advantages of utilizing nano particles in packaging to extend the shelf life of food commodities. Zinc Oxide nano particles are reported with good bactericidal activity during in vitro studies. Practical applications are going on to check the anti bacterial activity in edible coating.

B.3 (a) Role of Zinc Oxide nanoparticles in edible packaging to enhance shelf life

In a study active film containing zinc oxide nanoparticles shows antibacterial activity in petri dishes against *S. aureus* and *S. typhimurium*. The synthesized film also act as active packaging for the same bacteria in ready-to-eat poultry meat as a challenge study and decreased the number of targeted bacteria within 10 days at 7-9 ºC from log 7 to 0(Akbar& Kumar, 2014).

In another practical application edible film was prepared by using extract of Gracilariavermiculophylla (GVE). ZnO NPs were added in the films to develop antibacterial properties. The antimicrobial efficacy of the films was evaluated prior to their use as packaging for smoked salmon. After 9 h Compared with the films without Zinc Oxide nano particles, the films containing 5% Zinc oxide nanoparticles decreased the number of *Salmonella typhimurium* and *Listeria monocytogenes* by 5.08 and 4.99 log CFU/mL respectively. A high level of antimicrobial activity against *S. typhimurium* and *L. monocytogenes* was seen when smoked salmon was wrapped in GVE sheets containing 3% ZnO NPs. A small amount of lipid oxidation was observed during storage compared to controls. Therefore result of the studies shows that GVE films containing ZnONP can be used as active food packaging materials to preserve the quality of smoked salmon (Baek& Song, 2018).

B.3 (b) Role of Zinc Oxide nanoparticles in, inedible packaging to enhance shelf life

In a study nanocomposite low-density polyethylene (LDPE) films having silver and Zinc Oxide nanoparticles were fabricated using melt mixing in a twin-screw extruder. Fresh juice of orange is then filled in synthesized packaging film and kept at 4°C for storage. The organoleptic qualities and microbial analysis of the juices were carried after 7, 28 and 56 days of storage. Films containing 1% ZnO nanoparticles kept the bacterial load of fresh juice below the microbial shelf life (6 log cfu/ml) for up to 28 days (Emamifar et al., 2010).

In another study, anti bacterial potential of low-density poly ethylene (LDPE) packaging films containing copper oxide (CuO), silver (Ag), and zinc oxide (ZnO) nanoparticles were investigated to quantify the levels of coliform bacteria in ultra filtered cheese. A number of experiments were run with different combination of these nano particles with various time of exposure. In an experiment when only 1 % of (ZnO) was used, after 3 and 4 week of storage the number of coliform increase from 1.31 $x10^6$ is mentioned below. Experimental design andResponse combination of nanocomposite films containingZnO Nanoparticles is shown below.

The LDPE% in all runs is 99. R is the difference in bacteria concentration (expressed in log cfu/cm2) between the non-treated (1.31 10^6cfu/g) and treated test specimens in log (Beigmohammadi et al., 2016).

In another study, to check the practical application of ZnO NPs in food packaging, cooked minced fish meat was wrapped in Poly lactic acid (PLA) and PLA/ZnO NPs composite films and growth of *L. monocytogenes* and *E. coli* were monitored. The PLA film which did not have ZnO NPs in it shows no hindrance to both bacterial

Factors					Responses	
Run	Ag%	CuO%	ZnO%	Time (weeks)	Coliform (cfu/g)	R (cfu/g)
2	0	0	1	3	$8x10^2$	6.1172
18	0	0	1	4	10^2	6.1172

Table 2: Antibacterial activity of various biopolymer-based materials containing Zinc oxide nanoparticles.

Polymer Matrices	Approach	Size of Silver Nanoparticles (nm)	Concentration of ZnO-NPs in Film-Forming Solution/ Concentration of ZnO-NPs used for the anti microbial activity	Tested Strains	Gram (-) Pathogens	Gram (+) Pathogens	Antibacterial effect of Nanocomposite Films containing ZnOnps	Reference
Chitosan/HEC	in-situ	_	_	*L.monocytogenes,* *B. cereus,* *E. coli,* *S. Typhimurium* *S. aureus,*	*S. Typhimurium* *E. coli*	*B. cereus,* *L.monocytogenes,* *S. aureus,*	Inhibition zone (mm): *L.monocytogenes:* 10–16 *S.aureus*: 13–15 *B.cereus*: 13-18 *E. coli*: 15–18 *S. Typhimurium*: 17–19	(Youssef et al., 2015)
—	—	(a)Rod shaped Lenghth less then 500nm, Width less then 100nm (b) Rod shape Less then100 nm (c) sphere shape Less then 100nm	Different concentration ranging from 10µg to 50µg for each bacterial strain	*S. aureus* *S. typhi* *P. aeruginosa* *K. pneumoniae* *E. coli* *B. subtilis*	*S. typhi* *P. aeruginosa* *K. pneumoniae* *E. coli*	*S. aureus* *B. subtilis*	Inhibition zone (mm): *B. subtilis* (a) 5.83± 0.95 (b) 15.17± 0.40 (c) 12.17± 0.91 *E. coli* (a) 7.00± 0.37 (b) 15.17± 0.40 (c) 14.17± 0.54 *K. pneumoniae* (a) 8.33± 1.87 (b) 15.17± 0.97 (c) 16.17± 0.57 *P. aeruginosa* (a) 7.67± 0.21 (b) 16.00± 0.26 (c) 19.00± 0.68 *S. typhi* (a) 7.33± 0.33 (b) 15.17± 0.40 (c) 15.83± 0.17 *S. aureus* (a) 4.50± 0.34 (b) 15.17± 0.40 (c) 11.17± 0.40	(V et al., 2012)
Bentonite clay	in-situ	15-70nm	0.5 g	*E. faecalis* ATCC 14506 *E. coli* ATCC 11775	*E. coli* ATCC 11775 *E. coli* ATCC 25922	*E. faecalis* ATCC 14506	Inhibition zone (mm): *E. faecalis* ATCC 14506 (a) 9 (b) 9	(Motshekga et al., 2013)

				E. coli ATCC 25922			*E. coli* ATCC 11775 (a) 5 (b) 8 *E. coli* ATCC 25922 (a) 7 (b) 7	
—	—	spherical agglomerated into polycrystalle structures Smallest size 8nm Average size 15nm		*S. pneumoniae*	___	*S. pneumoniae*	Result of the study revealed that Zinc Oxide NPshas shown good antibacterial activity against S.*pneumoniae*, with an Minimum Inhibitory Concentration value of 40 µg/ml.	(Bhattacharyya et al., 2018)
—	—	Spherical	(a) ZnO (200 µg/40 µL) (b) ZnO (400 µg/80 µL)	*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella aerogenes*	*Escherichia coli, Pseudomonas aeruginosa, Klebsiella aerogenes,*	*Staphylococcus aureus*	Values are the mean ± SE of inhibition zone in mm *Klebsiella aerogenes,* (a) 1.00 ± 0.00 (b) 2.33 ± 0.09* *Escherichia coli* (a) 1.67 ± 0.00 (b)2.67 ± 0.03** *Staphylococcus aureus* (a)2.00 ± 0.00 (b)3.67 ± 0.03** *Pseudomonas aeruginosa,* (a)2.33 ± 0.06* (b)6.33 ± 0.12* * Symbols represent statistical significance, * P\0.05, ** P\0.01 as compared with the control group	(Lingaraju et al., 2016)

strains. Film having ZnO NPs exhibits significant antibacterial activity against both types of bacteria. The active PLA film reduced growth of bacteria to zero within just 10 days of study. The result of the study shows that active packaging of minced fish paste by PLA/ ZnO NPs composite film could enhance its shelf life through its antibacterial actions. Thus the study shows that PLA film with ZnO NPs could be used as food packaging to enhance shelf life of food commodities (Shankar et al., 2018).

Food Authorities Regulation

Although use of nano particles as anti-microbial agent in food packaging is an emerging technology which provides excellent results in its application, however there are multiple factors which need proper attention to ensure health safety of consumers. The major issue faced by food technologist is the migration of silver ions from food packaging to packed food and drinks. Therefore food safety organizations has a prudent type approach for these issues(Cushen et al., 2012).

The European Food Safety Authority (EFSA) panel on Nutrient Sources added to Food and Food Additives, stated its incapability to evaluate the safety of silver hydrosol and for shelf life extended products for food supplements and food packaging that includes silver nanoparticles are not allowed in the European Union until they get certified (Committee, 2011), (Shenir, 2014), (Bumbudsanpharoke& Ko, 2015). EFSA mentioned s safe limit of AgNPs migration form food packaging material to foods. According to EFSA recommendations it is not allowed to exceed 0.05 mg/kg in food and 0.05 mg/L in water. It is necessary for food packaging industries which are using antimicrobial potential of nanoparticles to imply these recommendations for the safety of consumers. Manufacturers of packaging contain nano particles as an antimicrobial agent requires *in vitro* genotoxicity, absorption, distribution, metabolism and excretion tests (Committee, 2011).

Likewise, in the United States of America the USFDA recommend that every industry should have to prepare a report for the toxicological effects of nanoparticles before its utilization in food packaging (USFDA, 2014). In March 2014 Environmental Protection Agency (EPA) abandons the plastic product of company, which is using nanoparticle without USFDA legislations (Martin, 2014). Since know Canada and many other countries did not have any proper food regulation regarding nanomaterials. Many countries have know introduced incomplete food safety regulations regarding application of nanoparticles in food sector (Berekaa, 2015).

Since 2012 Zinc Oxide (ZnO) is listed as a generally recognized as safe (GRAS) and it is used as food additive(Judith& Espitia, 2012).

Conclusion

ZnO NPs and Ag NPs have promising antibacterial properties against food borne pathogens. These nanoparticles have maintained their antimicrobial activity even when incorporated in polymeric matrices, which indicates their potential for food preservation through their use as antimicrobial food packaging. Various physical factors affect their antibacterial activity. Decrease in particle size and increase in concentration, increases their antibacterial activity. Higher antibacterial activity was observed at acidic pH. The strain specific antibacterial activity found to vary depending on the microbial species.

In case of Ag NPs regarding morphology, small spherical nano particles show higher antibacterial activity than triangular and large spherical nano particles. Nano plates shows higher antibacterial activity then nano rods. Regarding surface charge, the positively charged AgNPs were the most effective antimicrobial materials against all tested bacteria. The negatively charged silver nanoparticles had the least and the neutral nanoparticles had intermediate antibacterial activity. Studies show that aerobic bacteria are more susceptible toward antibacterial activity of Ag NPs as compared to anaerobic bacteria. In case of Ag NPs Gram negative bacteria are more sensitive than gram positive bacteria. Antibacterial activity of Ag NPs is higher in solid media as compared to liquid one.

The antibacterial study of ZnO NPs against various bacteria show that nano particle having flower and needle like morphology show higher activity than spherical nanoparticle. Gram positive bacteria are more sensitive toward anti bacterial activity of ZnO as compared to gram negative. The surface charge study shows that ZnO NPs with positive charge show higher anti bacterial activity than negatively charged nano particles. Both Ag and ZnO NPs have good antibacterial in edible and inedible packaging. Upper limits of Ag migration from packaging should not to exceed 0.05 mg/L in water and 0.05 mg/kg in food. ZnO NPs are generally recognized as safe to use in edible packaging.

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