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Nanotechnology in Food Packaging and Storage: A Review

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Abstract

Moving towards globalization, food packaging requires longer storage time, quality control, and hygienic measures based on international standards. Nanotechnology can meet all these needs and implement the essential factors for packaging, storage, protection, marketing, distribution, and communications. Polymer nanotechnology can provide new food packaging materials with suitable mechanical, inhibitory, and antimicrobial properties along with nanosensors to track and control food conditions during transportation and storage. In this survey, the latest innovations and applications of nanomaterials in food packaging using improved, active, and smart nanotechnology are reviewed. Moreover, the current business situation, understanding of the health concept in these technologies, as well as the limitations of recently advanced polymer nanomaterials that can effectively change the food packaging industry are discussed.

KEYWORDS: Active packaging, Antimicrobial properties, Food packaging, Nanotechnology

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Introduction

Nanotechnology is the recognition, production, and application of materials smaller than 100 nanometers on atomic, molecular, and macromolecular scales. Research in nanotechnology has grown dramatically over the past decade and many companies have specialized in making new forms of nanoscale materials with anticipated applications, including medical treatment and diagnosis, energy production, molecular calculations, and structural materials (Hulla *et al.*, 2015 Barani *et al.*, 2018). In 1808, nanotechnology allocated more than $16 billion in global research and development budgets employing more than 4,000,000 researchers all over the world (Hulla *et al.*, 2015). Nanotechnology will cover more than $3 trillion of the global markets by 2020. In addition, nanotechnology industries around the world will need about six million workers by the end of the decade to support those industries (Hulla *et al.*, 2015; Bhushan, 2016). Although nanotechnology is pervasive and attracts abundant financial resources, the food industry is one of the industries that are slowly developing nanotechnology usage. This is not surprising as the general preference for "natural" food products has historically prevented the use of emerging food technologies and nanotechnology is not an exception. However, the public opinion about the broad applications of nanotechnology vary from neutral to slightly positive (Ju *et al.*, 2017; Shahbazzadeh *et al.*, 2009; Ydollahi *et al.*, 2016; Moadab *et al.*, 2011; Aminzadeh *et al.*, 2011). Some studies showed that consumers are still concerned about nano foods (Nasiri *et al.*, 2019; Ahari *et al.*, 2018). The most active areas in nanotechnology research are packaging and nanotechnology-prepared foods. The beverage packaging market in 2008 was equivalent to $4.13 billion representing an annual growth of 11.65% (Ahari *et al.*, 2017). This is entirely consistent with some investigations that indicated people are more receptive to nanotechnology in fields other than food. The use of nanotechnology can expand and take over all the main functions of packaging, including maintenance, protection, marketing, distribution, and communication. This is why many of the largest food packaging companies in the world are actively seeking the potential of polymer nanotechnology to achieve new packaging materials with improved mechanical, inhibitory, and antimicrobial properties that can track and control food conditions during transportation and storage (Ahari *et al.*, 2017). The vital role of nanotechnology in the food packaging process is considered as the most extensive commercial application in the food sector. In recent years, research and innovation in food packaging have increased and various film-based materials, carbon nanotubes (CNTs), and wax nanocoatings have been developed for different foods. Maodab *et al.* (2011) reported that nanoparticles may be beneficial in producing new materials for food packaging with improved mechanical, impermeability, and antimicrobial properties, as well as higher durability. In addition to their antimicrobial properties, nanoparticles can be used as carriers to deliver antioxidants, enzymes, seasnings, anti-browning agents, and other materials for augmenting durability even after opening the package.

Being basically utilized in food packaging, the polymer nanomaterials for food packaging (PNFP) are discussed as follow:

- ***Improved PNFP****:* Using nanomaterials in the polymer network enhances the characteristics of that material related to the flexibility of polymer packaging, gas barrier properties, and temperature/humidity stability.
- ***Active PNFP****:* Applying nanoparticles allows interaction between the package, food, and environment that contribute to food protection.
- ***Smart PNFP****:* Using nanomaterials in the polymer network can indicate the packaged food and/or the conditions of its surrounding environment. Moreover, it can be used as a barrier against counterfeiting.

Improved Packaging

Despite diverse advances in nutrition, there is still a risk of infection with microorganisms, such as mold, bacteria, and viruses that threaten human health. Direct consumption of antimicrobial substances in food can be harmful to the health of consumers. As a result, antimicrobial packaging has become very important. One of these packaging methods is improved packaging that entails polymer chains with a 5% weight of nanoparticles and nanocomposites used in products, such as carbonated beverage bottles, films, and edible oils. One of the most critical features of this type of packaging is its higher capacity to prevent the entry of gases, regulate the temperature, and improve resistance to food moisture. The Food and Drug Administration (FDA) has approved using nanocomposite materials in the food industry science. Polymers containing clay nanoparticles are among the first polymer nanomaterials that emerged on the market as enhanced materials for food packaging (Soltani *et al.*, 2009; Mosadab *et al.*, 2011).

Active Packaging

Active packaging is particularly designed for releasing or absorbing substances in the packaged food or its surroundings. Currently, active PNFP has essentially been developed for packaging applications with antimicrobial properties. Some other promising and functional applications include oxygen scavengers, ethylene removers, and carbon dioxide absorbers or emitters. The most common nanoparticles used to promote antimicrobial PNFP are CNTs, metal nanoparticles, and metal oxide nanomaterials. These particles act in direct contact, but can also move steadily and react favorably with food organics. Nanoparticles of silver, gold, and zinc are metal nanoparticles with an antimicrobial function that have been studied extensively. Several commercial applications have been noted for silver nanoparticles (AgNPs). Silver, which has high-temperature stability and low volatility, is a significant antifungal and antimicrobial agent at the nanoscale. Moreover, silver is claimed to be effective against 150 different bacteria (Shahbazzadeh *et al.*, 2011).

Titanium dioxide (TiO_2), zinc oxide (ZnO), silicon oxide (SiO_2), and magnesium oxide (MgO) are the most studied metal oxide nanoparticles due to their ability for blocking UV and are ineffective photocatalyst agents. The use of TiO_2 as an ineffective photocatalyst for surface coatings in packaging is being investigated (Kakoolaki *et al.*, 2019). The antibacterial properties of ZnO and MgO nanoparticles have been recently found. The ZnO and MgO particles are expected to be more feasible and healthier for food packaging than nanosilver. Nanomaterials, namely ZnO nano-based optical catalysts for sterilization in indoor exposure, have been recently introduced. The ZnO has been reported to have an antimicrobial activity, which has an indirect rela-tionship with particle size. Although UV light is not required for antimicrobial activity (as opposed to TiO_2), visible light stimulates it and its exact mechanism has not yet been determined. The ZnO nanoparticles merge in several polymers, such as polypropylene (PP), in which UV light is absorbed without heat re-radiation and improves the stability of polymer compounds. The CNTs have appropriate antibacterial properties and promote polymer network properties. Direct contact with CNT masses was shown to be lethal for *Escherichia coli*. The latter impact may be due to the breakdown of microbial cells and irreversible damage by long narrow CNTs (Gokularaman *et al.*, 2017; Roy *et al.*, 2018). The usage of CNT is currently discontinued because of the claims of some studies suggesting that CNTs are dangerous due to their toxicity to human cells, especially when being in direct contact with the skin (Shahbazzadeh *et al.*, 2011; Ahari *et al.*, 2017).

Nanotechnology can help active packaging to reduce the spoilage of different kinds of food by direct and indirect oxidation through combining O_2 nanoabductors. For example, direct oxidation reactions cause fruits to turn brown and vegetable oils to turn sour. Food spoilage with indirect O_2 action involves the spoilage of food by aerobic microorganisms. The presence of O_2 in a package can initiate or accelerate oxidation reactions, which can cause food spoilage and facilitate the growth of microbes and aerobic molds. Both direct and indirect oxidative reactions reduce quality in diverse aspects such as odor and taste and cause unpleasant color changes. Oxygen abductors remove O_2 (waste or input) and thereby slow down oxidative reactions. Several nanoparticles, including TiO_2 nanoparticles, are used to produce O_2 abductor films. Some silver-based nanoparticles that have antimicrobial activity are also able to absorb and decompose ethylene. Ethylene is a natural plant hormone produced by ripening. Removing ethylene from the packaging environment helps to elevate conservation time for fresh products, such as fruits and vegetables (Ahari *et al.*, 2017).

Smart Packaging

Materials in contact with food are mainly used to indicate the condition of food and its packaging. This technology can alert the producer or consumer with a visible indicator of food freshness, perforated packaging, storage at the right temperature in the supply chain, and being spoiled (Cushen and Cummins, 2017). Key factors in their application are cost, strength, and compatibility with different packaging materials. The first improvements were based on devices that were used with the product in a typical standard package to perfectly control the package, time-temperature history of the product, and effective expiration date. Industries assess food expiration dates by considering the conditions of distribution and storage, especially temperature. However, we know that such conditions are not always expressed correctly and that food is constantly exposed to inappropriate temperatures. This is especially worrying about products that require a cold chain. Time-temperature indicators, which began to appear in some food products in the late twentieth century, allow producers to verify food storage at the right temperatures. These indicators fall into two categories, one of which is based on the transfer of a dye between a porous material that depends on temperature and time. The other group uses a chemical reaction, which starts when the label is applied to the package and causes color change (Omanovic-Miklicanin *et al.*, 2016). This indicator reassures the consumers of what they are buying and enables producers to track their food on the production line. In addition, controlling food while moving into the production chain helps companies to identify weaknesses. Furthermore, micro-penetration and sealing defects in packaging systems may lead to unwanted exposure to a lot of oxygen in food products, which results in unpleasant changes. Nanoparticles can be applied as reactive particles in packaging materials to represent the package state. Nanosensors can respond to environmental changes, such as room temperature and humidity, and oxygen exposure, as well as spoiled products, or microbial contamination (Ahari *et al.*, 2008).

Nanosensors in food packaging can detect certain chemical compounds, pathogens, and toxins in foods. Consequently, they are useful for eliminating the need for inaccurate expiration dates. These sensors provide real-time status of food freshness. Recent advances in smart PNFPs include oxygen markers, novelty, and pathogen sensors. There is a growing interest in the development of irreversible and non-toxic oxygen sensors to prevent the presence of nitrogen or oxygen in non-oxygen food packaging systems, namely spotted packaging (Ahari *et al.*, 2008). Lee *et al.* developed a UV-activated colorimetric oxygen marker using TiO_2 nanoparticles to sensitize light-reducing methylene blue with triethanolamine in an encapsulated polymer medium using UVA light (Mills and Hazafy, 2009). As soon as exposure to UV radiation the sensor turns white and remains colorless until it is exposed to oxygen and the original blue color is restored. The rate of color recovery is proportional to the amount of oxygen exposure. Mills and Hazafy used nano crystallized SnO_2 as an optical sensitizer in a colorimetric indicator with variable film color dependent on the presence of oxygen (Mills and Hazafy, 2009). Furthermore, pH markers based on organically modified organic silicate nano-particles have recently been introduced (Ahari *et al.*, 2008). Novel indicators show the quality of packaged food in response to changes in fresh food products as a result of microbiological growth. Smolander reported that a definite prerequisite for the successful development of novel indicators is an understanding of quality metabolites. The freshness sensor must be able to react sensitively to these metabolites. Freshness is judged based on a color change due to microbial metabolites generated during spoilage. It should be noted that the formation of different metabolites depends on the nature of the spoiled flora of the packaged product and the type of packaging. Sensors embedded in a packaging film should be able to detect food spoilage organisms and initiate color change to alert the consumer about the expiration date (Smolander, 2008; Smolander and Chaudhry, 2010). A list of freshness indicators that react to the presence of quality-reflecting metabolites has also been reported (Ahari *et al.*, 2019). Several types of gas sensors have been developed, which can be used to determine the amount and type of microorganisms by evaluating their gas emissions. One of the most popular types of sensors is a metal oxide gas sensor with high sensitivity and stability (Lotfi and Ahari, 2019). Sensors based on conductive nanoparticles embedded in a non-conductive polymer network are being studied for detecting and determining food pathogens by generating a specific response pattern for each microorganism. Currently, three types of bacteria, namely *Bacillus spp.*, *Salmonella*, and *Vibrio parahaemolyticus* must be detected using the response pattern created by these sensors. Another advance in this area is "electronic language" technology, which is composed of an array of sensors that signals food conditions. The device contains a variety of highly sensitive nanosensors detecting the gases released by spoilage microorganisms, which produce a color change that shows the freshness or spoilage of food. DNA-based biochips are also being developed and can detect the presence of dangerous detrimental bacteria or molds in food, such as meat, fish, and fruits. Other advances in this field are in the early stages of research, including devices that provide the basis for preserving packaging technology, which will begin to be protected if food begins to spoil (Lotfi and Ahari, 2019).

Maintenance Applications of Polymer Nanocomposites

When food is not consumed immediately after production, it should be placed in a package with several characteristics. A package type should have the potential to protect food from contamination, dust, oxygen, light, contaminating microorganisms, moisture, and oth-er harmful destroying agents. In addition, the packaging must meet desired conditions, such as being safe, suitable for static use, cheap, light, reusable, resistant to final conditions during processing or accumulation, unsusceptible to physical factors, and impermeable to an environmental reservoir or transfer conditions. These are the prominent features of any substance which is supposed to be included in this technology (Barani *et al.*, 2018).

A critical issue in food packaging is mobility and impermeability. No material is entirely impervious to atmospheric gases, water vapor, or natural substances that enter during food packaging, or even the packaging material itself. In some applications, strong retainers are not desirable for moving or releasing gas. For example, in packages designed for fresh fruits and vegetables, shelf life depends on access to a continuous source of oxygen for cellular respiration. Plastics are used for carbonated beverage containers and must have strong oxygen and carbon dioxide barriers to prevent the oxidation and decarbonization of beverages. In other products, the transfer of carbon dioxide is a smaller issue than both oxygen and water vapor. Regarding these complexities, a necessity is felt for quite different packaging characteristics for food products. The packaging industry only allows longer transportations between production units and consumers (Barani *et al.*, 2018).

Old materials for food packaging encompass metal, ceramics (glass), and paper (cardboard). Although these materials continue to be used, plastic is becoming a significant alternative for food packaging due to its light weight, low cost, ease of processing and ductility, as well as considerable diversity in the physical properties of organic polymeric materials. Polymers commonly used for food packaging include polyphenols, such as PP, low- and high-density polyethylene (LDPE and HDPE), polystyrene (PS), polyethylene terephthalate (PET), and polyvinyl chloride (PVC). Accordingly, polymers have made a revolution in the food industry and provided more advantages than traditional materials which were inherently permeable to gases and other small molecules (Bradley *et al.*, 2011; de Azeredo *et al.*, 2011). Some polymers are better in this case than others. For example, PET is a good barrier against oxygen (6-8 nmol m^{-1} s^{-1}Gpa=O_2 permeability), while HDPE does not protect efficiently (200-400 nmol m^{-1} s^{-1} Gpa=O_2 permeability). On the other hand, HDPE shows better resistance against water vapor than PET. No common pure polymer has all the protective and mechanical properties needed for any possible use in food packaging. Therefore, mixed polymer films or polymeric compositions are usually used. For instance, ethylene vinyl alcohol (EVOH), as a water-sensitive material, can be placed between the layers of hydrophobic polymers, such as polyethylene (PE), when powerful oxygen inhibitors over a wide range of moisture and strong oxygen protectors are required (Barani *et al.*, 2018). Moreover, directly combining polymers is effective for obtaining optimum gas barrier and mechanical properties that are not available by other polymer monomers. Cleverly integrated combinations can result in more controllable properties of the achieved film (Shankar and Rhim, 2016). Polymer compounds and multilayer films have led to the production of packaging materials with good gas barrier properties and without the inherent limitations of many single-layer films made of solid protective polymers. However, they cost higher, are more productive, require additional additives and adhesives, which complicate their adjustment by random factors, and have recovery problems (Ahari *et al.*, 2017). As a result, the polymer industry still tends to produce single-layer films with improved gas and mechanical properties, especially those made of biocompatible materials.

Polymeric nanocomposites (PNCs) are the newest materials for solving the problems mentioned above. The PNCs are obtained by dispersing a nanoscale neutral filler in a polymer network. Filling materials can include nano-clay or silicate plates (fra-in vide), silica nanoparticles (SiO_2), CNTs, starch nanobots, graphene, cellulose-based nanofibers, nano-whiskers, chitin or chitosan particles, and other non-organic materials. Therefore, improve-ing the polymer barrier properties is the most precise application of PNCs in the food industry. The PNCs are stronger, more flame resistant, and have better thermal properties, including dots melting, glass transition temperatures, and decomposition, compared to control polymers that do not contain any nanoscale fillers. Conversions in surface wetting and hydrophobicity have also been reported (Clifton *et al.*, 2020). Some of these improvements in physical properties can be extremely effective. For example, a layer-by-layer method is used to fabricate a PNC material composed of clay nanosheets dispersed between polyvinyl acetate (PVA) with a modulus of hardness (GPa 11±106), which is almost twice as large as pure PVA and is comparable in hardness to some Kevlar types (Ahari *et al.*, 2017). A similar construction method has been utilized for engineering clay/poly (ethyleneimine) PNCs that preserve the textural structure of linen products when used as a coating during long burning times. Finally, PNCs should provide better opportunities for the food industry in addition to reducing costs and wastage due to the smaller volume of polymers required in packaging materials with the same mechanical properties or even better. Furthermore, nanocomposites may have environmental advantages over traditional plastics: When a nanofiller is released into a biocompatible polymer (PLA), the PLA biocomposite biodegrades faster than PLA without such additives (Segura González *et al.*, 2018).

Preparation and Determination of Nanocomposite Properties

polymer nanotechnology for food packaging is still in a development stage. The envisaged direction is to look at the complete life cycle of the packaging (raw material selection, production, analysis of interaction with food, use and disposal) integrating and balancing cost, performance, health and environmental considerations (Figure 1). Researches on the production methods and characterization of PCNs used for food preservation began in the late 1990s. Most of the published researches from "Rus Mont Morillonite" (MMT) has been used as a nanoscale component. A wide range of synthetic polymers, such as PE, nylon, and PVC, as well as biopolymers, such as starch have been investigated. In most published studies on PCN, varying amounts of nanoclay (usually 1%-5% by weight) have been used (Kuswandi, 2016 & 2017). The silicates used in PCN synthesis have a layered structure with a layer thickness of about 1 nm. The side dimensions of these layers can be up to several micrometers. As a result, the ratio of dimensions (length to thickness ratio) of these fillers is high and includes values higher than 1000. The defined distance between these layers form clusters is known as "gallery". Inorganic cations between layers can replace other cations, such as lithium and sodium (Zare, 2017; Bee *et al.*, 2018).

There are usually three possible arrangements of these silicate clays that are obtained in a polymer matrix during dispersion:

1. Non-interlayers: If the polymer cannot be placed between the silicate layers, a non-interlayer nanocomposite is obtained.
2. Intercalated structure: Separation of the clay layers by increasing the space between layers.
3. Exfoliated structure: Complete separation of clay platelets and formation of random arrangement. Although this is the ideal arrangement of nanocomposites, it is more difficult to achieve during synthesis and processing. These structures are shown in Figure 2.

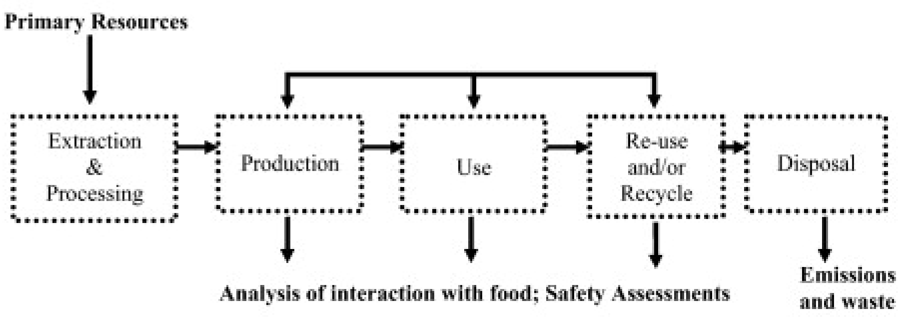

Figure 1. Full packing cycle

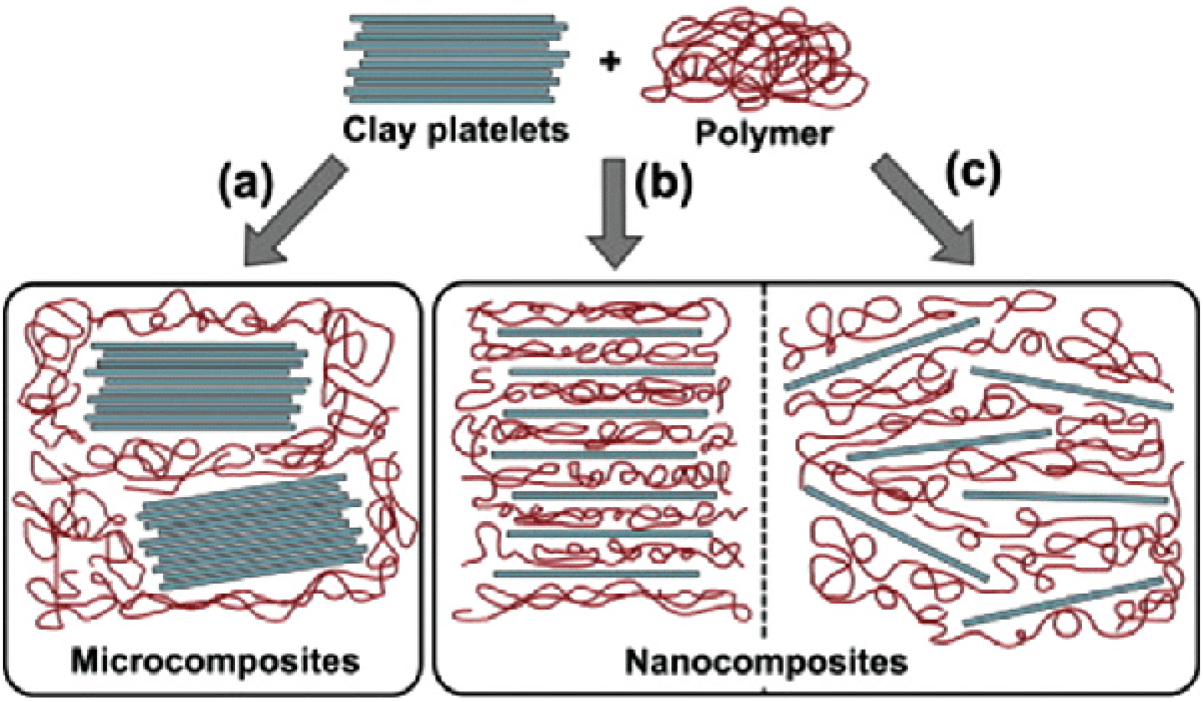

Figure 2. Morphology of clay / polymer nanocomposites (a) Tactoid (b) inserted (c) and exfoliated.

The structure of nanocomposites is usually determined using two complementary diagnostic methods, namely X-ray diffraction (XRD) and transmission electron microscopy (TEM). The XRD is used to identify an intercalated structure by determining the interlayer space (Mohammadi *et al.*, 2014). Placing polymer chains between the layers augments the interlayer spaces and according to Bragg's law causes the peak diffraction to move to a lower angle. However, if the interlayer space becomes too large, these diffraction peaks will disappear indicating a complete exfoliation of the layer silicates in the polymer matrix. In this case, TEM is utilized to identify the separated silicate layers (Kakoolaki *et al.*, 2019; Pooyamanesh *et al.*, 2019).

Nanocomposites are usually produced by soluble method, in-situ/interlamellar polymerization, and melt processing.

Current Business Situation, Health, and Prospects

Many companies have made PCNC packaging materials commercially available because they are not relatively expensive to manufacture. Some companies, such as Nanocore offer a wide variety of polymer nanocomposites in bullet form, in addition to several commercial product lines, including Aegis (Honeywell, USA), Durethan (LANXESS Deutschland GmbH, Germany), Imperm (ColorMatrix Co., USA), nano stuff (Nylon Co., USA), and Nanoseal (Nanopack Inc., USA). Pooyamanesh *et al.* presented a complete summary of one of the active companies in this field (Pooyamanesh *et al.*, 2019).

The most common PCNC available products are dedicated to particular applications, including the food and beverage industries. For example, Brewing Miller Company uses these products to make plastic bottles, which are resistant to the ingress of both oxygen and CO_2. Another exciting usage of PCNC technology is found in the US Army Natick Soldier Research, Development, and Engineering Center (NSRDEC), which saves signi-ficant time and money on the possible use of PNC plastics for meals ready-to-eat (MREs) packaging. In addition, MREs are surprisingly hard to store, and the strength of PCNC-based packaging may only be able to meet this (Pooyamanesh *et al.*, 2019).

Given the number of studies that consider food packaging as a possible endpoint for PCNC research, the number of researchers who examine these substances in storage or health tests using real food components is surprisingly small. An investigation observed that PET, PHBV, and PHB-based PCNCs at 5.5 wt% relative passages (Polymer/Pcomposite) of limonene d, an essential aroma compound in citrus fruits, were 3.2, 1.6, and 8.8 in size, respectively (Sasikala and Umapathy, 2018). This shows that PCNC packaging materials are not much similar to skin flavors, colors, or food odors. Another study was more concerned with the behavior of entire food systems demonstrating that the number of germs and mold on apple slices diminished dramatically over 10 days when packaged in iPP/PNCCaCo3 films. On the other hand, those stored in a pure isotactic PP experienced an elevation in overall mesophilic microflora during the same time (Avella *et al.*, 2007). In addition, this review showed that apples stored in PNC packaging had ripened more because of ethylene gas retention and had less oxidation than apples stored in common PP packages (Mohammadi *et al.*, 2016).

In terms of the safety of PNCs-made packaging materials, some concerns are raised regarding the permeability of packaging materials to foods. Investigations on the accessibility of PNC materials are controversial and limited. A survey in 2005 revealed that vegetables in contact with clay/starch nanocomposite films did not change in terms of iron and magnesium content. However, they had higher levels of silicon. The authors argued that the main elements of clay nanoparticles did not have a significant entry into food or the entry was within the limits of current EU regulations (Avella *et al.*, 2005). Another study showed that OB Uvitex, a common additive used in polyolefins with European approval for food contact use, has a very slow distribution in oily and aqueous foods, such as PNC-MMT/wheat-gluten film. It was found to be more than 60% in LLDPE films subjected to the same test (Mauricio-Iglesias *et al.*, 2010). Furthermore, the PNCMMT/wheat-gluten film allows aluminum and silicon to enter foods at levels well within the limits set out in European regulations. Finally, a paper showed that the distribution of triclosan and trans-, trans-1, 4-diphenyl-3, 1-butadiene (DPBD), as two other common additives in polyamide/clay PCNCs, is slower than pure polyamide (Türe *et al.*, 2012; Nataraj *et al.*, 2018). Although the mentioned studies suggest that PCNCs may moderate the entry of possibly harmful additives into foods, the health literature is fragmented at this moment. More detailed testing should be performed on PCNCs because various food and beverage companies use them to package their products. Importantly, the toxicological data of clay nanoparticles are still inaccessible as strategies for identifying and classifying clay nanoparticles and other nanoparticles in complex food networks are being developed. One study found that layered silicate nanoparticles had little cell and gene toxicity even when were part of a diet given to mice (measured acute oral poisoning, mean lethal dose, LD_{50}<5700 mg/kg body weight under excavated conditions) (Ansorena *et al.*, 2016; Nataraj *et al.*, 2018). However, the authors of this study have only tried one type of clay and morphology. Therefore, its application for a general proposition remains unclear. In addition, the PCNC films are found to increase the entry of nano clay composites into quasi-foods when films are under high pressures, which is an increasingly popular food sterilization/preservation method. Another study showed that MMT clays undergo new chemical and structural changes at pressures below 300 MPa. It was concluded that such changes should be considered when montmorillonite-polymer bond-ed composites are in contact with food. As a result of these assumptions, while PCNCs may represent the next revolution in food packaging technology, steps remain to be taken to ensure that consumers are safe from any potential risks posed by these substances (Mohammadi *et al.*, 2016).

Silver Nanoparticles and Nanocomposites as Antimicrobial Food Packaging Materials

The use of silver as an antimicrobial agent in food and beverage storage has a long history. The wide range of antimicrobial activity and relatively low cost of silver make it a suitable case for active water disinfectant in developing countries (Hadrup and Lam 2014, Akter *et al.*, 2018). In 2009, the FDA amended the Food Additives Regulations to allow the direct addition of silver nitrate, as a disinfectant, to commercially bottled water at a maximum concentration of 17 kg/g (Akter and Sikder, 2018).

Aside from applications in the food industry, silver has long been used as a disinfectant. Hippocrates, the ancient Greek physician, used silver powder on wounds to accelerate healing. However, perhaps the most significant antimicrobial advantage of silver is that silver can be easily incorporated into countless materials, such as fabric and plastic, making it particularly useful for applications where a broad and stable antimicrobial activity is desirable and traditional antimicrobials are useless. In the food industry, silver-containing plastics are incorporated in almost everything from refrigerated liners to cutting boards and food storage containers. In addition, silver has been a revolution in the medical device industry with an increase in silver-plated urinary catheters, cardiovascular implants, esophageal tubes, bandages, sutures, and other instruments on which the growth of bacteria endangers the patient's life. Up to now, the FDA has approved more than ten silver-containing zeo-lites or other materials for use as food contact materials for disinfection, as well as many silver-plated medical devices (Hadrup and Lam, 2014; Deshmukh *et al.*, 2019).

Despite the long history of silver as an antimicrobial agent, the mechanism of this activity remains a subject of active research. Some suggested explanations include interference with vital cellular processes by binding to sulfhydryl or disulfide functional groups on the surfaces of membrane proteins and other enzymes, disruption of DNA replication, and causing oxidative tension (oxidation) by catalyzing the formation of reactive oxygen species (ROS) (Cvjetko *et al.*, 2017; Deshmukh *et al.*, 2019). However, there is no consensus on which of these mechanisms is most important. For example, Dibrov *et al.* provided evidence that the binding of silver, especially to membrane proteins, disrupts ion and proton transport across membranes (Dibrov *et al.*, 2002). Another study found that silver ions penetrate the cell, where they interfere with ribosome activity and disrupt the production of several key enzymes responsible for energy production (Yamanaka *et al.*, 2005).

Cell wall damage due to silver (Ag) binding to membrane proteins and DNA shrinkage has been observed in *E. coli* and *Staphylococcus aureus*. DNA shrinkage in response to the presence of Ag ions has been stated as a defense mechanism, which, while protecting DNA from damage, limits the ability of cells to proliferat (Batarseh, *et al.*, 2004). In contrast, some reported that silver complexes of glutamic and tartaric acids are actively involved in DNA cleavage, and suggested that the binding of Ag ions to membrane enzymes and proteins plays a relatively minor role in silver antimicrobial activity. Gram-negative bacteria, such as *E. coli* are generally more sensitive to silver treatment than gram-positive bacteria, namely *S. aureus* because the transfer of positively-charged silver ions across the membrane of gram-positive bacteria, which is thicker and rich in outer peptidoglycans, is slower than the thinner membranes of gram-negative species (Guo *et al.*, 2019; Rezvani *et al.*, 2019). Finally, there is evidence suggesting that the antibacterial activity of silver zeolites could be attributed to silver ability for catalyzing the production of ROS, which causes cell death as the result of oxidative stress (Bakhsheshi-Rad *et al.*, 2018).

Antimicrobial Activity of Silver Nanoparticles

Since the first published reports of the antimicrobial properties of silver colloids, AgNPs have been identified as potent agents against many species of bacteria, including *E. coli*, *Enterococcus faecalis*, *S. aureus*, *S. epidermidis*, *V. cholerae*, *Pseudomonas aeruginosa, P. putida, P. fluorescence*, *Shigella flexneri*, *B. anthracis, B. subtilis, B. cereus*, *Proteus mirabilis*, *Salmonella enterica, S. typhimurium*, *Micrococcus luteus*, *Listeria monocytogenes*, and *Klebsiella pneumoniae*. Moreover, AgNPs are effective against the strains of these species which are resistant to potent chemical antimicrobials, including methicillin-resistant S. aureus, methicillin-resistant S. epidermidis, vancomycin-resistant enterococcus, and extended-spectrum β-lactamase-producing Klebsiella (Bakhsheshi-Rad *et al.*, 2018; Tang and Zheng 2018). In addition, AgNPs are toxic for some fungi, such as *Candida albicans*, *Aspergillus niger*, *Trichophyton mentagrophytes*, and yeast isolated from bovine mastitis, as well *Chlamydomonas reinhardtii* algae and *Thalassiosira weissflogii* phytoplankton. Furthermore, they inhibit at least two viruses, namely the human immunodeficiency virus and smallpox (de Souza *et al.*, 2019).

There is a disagreement about the toxicity of AgNPs for bacterial cells. The most conservative view is that silver atoms separate from the surfaces of AgNPs and cause cell damage by precisely the same mechanisms observed for conventional silver antimicrobials. Some studies showing that AgNPs are more toxic than the equivalent of isolated silver ions refer to the "Trojan horse" model for the toxicity of engineered nanoparticles (Cho *et al.*, 2018), according to which AgNPs play an efficient role in transferring large amounts of silver ions into cells in a short time. A study confirmed the hypothesis that AgNPs are only Ag^+ carriers demonstrating that these particles are ineffective in slowing the growth of *E. coli* species resistant to Ag^+ (Cho *et al.*, 2018; de Souza *et al.*, 2019). In addition, *E. coli* cells exposed to 9% nm AgNPs presented the same abnormality in transmembrane potentials and decreased ATP levels similar to *E. coli* cells exposed to $AgNO_3$ with the lower absolute molar concentrations (μM vs. mM). It is important to note that the chemical nature of silver leads to antibacterial effect, and gold nanoparticles (AuNPs) with similar size have no efficient antimicrobial activity (Vazquez-Muñoz *et al.*, 2017; Cho *et al.*, 2018). While AgNPs probably act as a source of Ag^+ ions, they may also have additional antimicrobial mechanisms. For example, when the released Ag^+ concentration is uniformed, the AgNPs seem to be more toxic to algae than the equivalent doses of $AgNO_3$. In contrast to the above-mentioned study, another report indicated that AgNPs are very influential against silver-resistant strains of *Proteus mirabilis* and *E. coli*, and highlights the fact that particles with different sizes, shapes, or other properties may behave differently even in the same system. There is also evidence that AgNP levels effectively catalyze the formation of free radicals in bacterial cells, which can lead to cell death through oxidative stress (Vazquez-Muñoz *et al.*, 2017; Smith *et al.*, 2018).

However, perhaps the most astonishing evidence that AgNPs are toxic to microorganisms through mechanisms different from Ag ions is the result of researches by Elechiguerra *et al.* These authors revealed that the concentration of released Ag^+ ions from AgNP was under the tested conditions was not sufficient to fully justify the toxicity of AgNPs (Elechiguerr *et al.*, 2005). Above all, these authors could show that

AgNPs bind to membrane proteins, form cavities, and cause other morphological changes (Figure 3). The AgNPs were also observed to react with phosphorus DNA groups. Morphological changes (cavitation) were observed in the cell membrane as a result of exposure to bacterial AgNP independently by Sundi and Salopek-Sondi (Sondi and Salopek-Sondi, 2004), which were more likely to bind AgNP to membrane surfaces causing lipopolysaccharides to adhere and subsequently structural integrity and impenetrability to be lost. Therefore, perforated membranes become more porous resulting in disrupted molecular and ionic transport, as well as accelerated AgNPs entering into the cell that can cause more damage to DNA and other cellular components inside the cell.

In summary, AgNPs are powerful broad-spectrum antimicrobials as the minimum inhibitory concentration of 2 mL/kg-4 has been reported for AgNPs with a diameter of 45-50 nm against *E. coli, V. cholera, S. flexneri*, and at least one strain of *S. aureus* that compete with the antibacterial properties of penicillin against non-resistant strains. In addition, this power can be efficiently modified with the particular physical effects provided by nanomaterials. For example, Akhavan and Ghaderi (Akhavan and Ghaderi, 2009) showed that silver nanowires, when exposed to external electric fields, have about 18.5% to 63% better antimicrobial power due to the elevation in silver ion production at the wire terminals. Furthermore, Fuertes *et al.* (Fuertes *et al.*, 2011) demonstrated that the optical excitation of AgNPs coated with a thin layer (1-2 nm) of porous silica at visible light frequencies that resonate with AgNP surface plasmon bands significantly increased the antimicrobial activity against *E. coli* by ROS photosynthesis or photocatalyzed by the release of silver ions. It is also a reversible effect that provides a portal in photo switchable antimicrobial behavior. Such studies suggest that an electric field or external light source can be a controllable, non-invasive sterilization method if silver nanostructures are embedded into food storage containers.

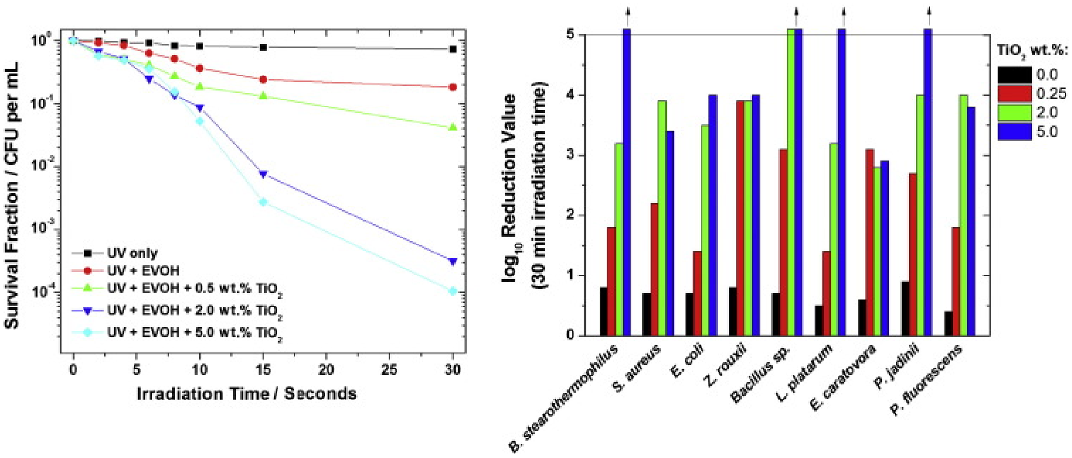

Figure 3. Mechanisms of Silver Nanoparticle Bacteriocidicity. (A and B) Silver nanoparticles (AgNPs) are lethal to bacteria in part because they damage cell membranes.

Polymeric Nanocomposites Containing Silver Nanoparticles

One of the greatest advantages of mineral or inorganic nanoparticles over molecular antimicrobials is their ease of use in polymers to form available antimicrobials (Pozdnyakov *et al.*, 2016). This is especially true regarding the controlled diffusion properties of AgNPs, which can be designed for the long-lasting durability of strong antimicrobial agents. There-fore, silver/polymer nanoparticles are attractive materials for usage in medical devices, as well as food packaging materials to maintain durability. While silver zeolites have been utilized for some time to make antibacterial polymer composites, AgNP nanocomposites augment stability and slow down the release of silver ions into stored foods, which is essential for stable antimicrobial activity. For example, when the antimicrobial activity of SiO_2/AgNP nanocomposite materials was compared with the antimicrobial activity of silver zeolite (Ag) and a SiO_2/$AgNO_3$ composite, the last two materials had about ten times more severe antimicrobial response. However, nanocomposi-tes made a longer period of activity possible (Pozdnyakov *et al.*, 2016; Prozorova *et al.*, 2019). Although a zeolite material may provide an excellent immediate effect, the stable antimicrobial activity of nanocomposite is more suitable for food packaging that requires transport distance or long storage life. Note that in the case of uncoated AgNPs, the SiO_2/AgNP composite material was found to be effective against a wide range of bacteria and fungi. It was more effective against gram negative bacteria than gram positive ones and can be incorporated into a PP polymer matrix to create antibacterial films for food-contact containers (Prozorova *et al.*, 2019).

Many PNCs have been reported in experiments. For example, Sanchez Valdes *et al.* coated a five-layer plastic film (Polyamide=PE/tie/PA-6/tie/PE; PA-6 and tie=PE bonded with maleic anhydride) with an AgNP/PE nanocomposite layer and found an antimicrobial effect against *Aspergillus niger*, a common food contaminant. In addition, they found that this effect and activity depends on the coating method. The methods that resulted in a harder surface, and therefore a higher level of silver ions release, were more active than the methods that led to a softer surface. Munster *et al.* published several studies on PNCs AgNP/PA-6 and AgNP/PP (AgNP particle size of about 800 nm) that have antimicrobial activity against *E. coli*, *S. aureus*, *Candida albicans*, multi-stranded worms (*Spirobis spirorbis*), sea squirt (*Ciona intestinalis*), and algae (*Ulva intestinalis*) (Sánchez-Valdes *et al.*, 2018; Grijalva-Chon *et al.*, 2017).

To determine AgNP antimicrobial impact on food shelf life, researchers have tested AgNP/polymer nanocomposite materials using real food systems. For example, Fayaz *et al.* (2009) dipped and extracted sterilized carrots and pears into alginate solutions containing biosynthesized AgNPs to form "edible antibacterial films". They observed less water loss for treated carrots and pears in 10 days, which was more acceptable for the consumers judging by color, texture, and taste (Figure 8, bottom) (Huang *et al.*, 2019; Saedi *et al.*, 2020). In a similar study, fresh asparagus leaves and buds coated with AgNP/polyvinyl pyrrolidone nanocomposite films increased their storage life to 25 days at 2°C. In addition to lower weight loss, greener color, crisper and softer texture, covered asparagus had less microorganism growth (psychrophiles bacteria, yeast, and mold) during this period (Moradi *et al.*, 2015). An edible film containing AgNP dispersed in glycogen has also been reported. Chinese jujube, which was placed in harder and heavier food storage bags made of AgNP/TiO_2/nanoparticle PE films, presented less spoilage, browning, and slower ripening in 12 days, compared to fruits stored in control materials. Orange juice stored at 4°C in LDPE films containing P105 (a mixture of TiO_2 and 10 nm nanosilver) showed a statistically significant reduction in the growth of *Lactobacillus plantarum* over 112 days (Ydollahi *et al.*, 2016). In other applications, cellulosic pads containing AgNP produced from silver ions have been indicated to reduce the microbial level of exudates (secreted substances) from the beef stored in modified atmospheric conditions. Moreover, in a slice of fresh melon stored in cellulose pads containing AgNP, the number of microbes (mesophiles, psychrotrophic bacteria, and yeasts) was lower and the microbial growth retardation time was longer. Silver particles accelerate and catalyze the loss of ethylene gas. Consequently, fruits stored in the presence of AgNP have a shorter ripening time and a longer storage life (Ydollahi *et al.*, 2016). Although many advances have been made in silver nanostructures for food packaging applications, there is still a lack of comprehensive studies in different polymer systems, and extensive investigations are required to clarify the relationships affecting the antimicrobial resistance of AgNP PNCs.

Other Antimicrobial Nanoparticles

The antimicrobial properties of nanoparticles composed of other materials have been investigated. The TiO_2 particles are particularly promising. In contrast to AgNP, the antimicrobial activity of TiO_2 nanoparticles is photocatalyzed, and therefore, TiO_2-based antimicrobials are active only in the presence of ultraviolet (UV) light. For example, it has been identified that TiO_2 nanoparticles are effective against food due to pathogens under UV radiation but not in the dark (Ydollahi *et al.*, 2016). Mohammadi *et al.* dispersed TiO_2 nanoparticles throughout EVOH films via the ultrasonic method. They observed the lethal properties of their active light against nine microorganisms, including yeasts and bacteria mentioned in food poisoning and food spoilage (Figure 4) (2014). Another study on food packaging showed that PP films coated with TiO_2 nanoparticles inhibited the growth of *E. coli* in fresh lettuce slices (Peiris *et al.*, 2018). Numerous researchers have combined the antimicrobial properties of TiO_2 nanoparticles with silver or AgNPs to form films or particles with enhanced antimicrobial activity (Mohammadi *et al.*, 2014). Food packaging films containing TiO_2 particles may use another advantage of protecting food against the oxide-zing effects of UV radiation while maintaining good optical clarity because TiO_2 nanoparticles are effective light absorbers with short wavelengths and light stability. This technique is currently being used to protect against UV rays in sunscreens, textiles, and wood oil. However, one must be careful because some forms of titanium nanoparticles may cause photocatalysis of oxidation and decomposition of the polymer (Ahari *et al.*, 2017).

Figure 4. Antimicrobial effect of photosynthetic TiO2 / EVOH nanocomposite materials. Left: Healthy fraction of E. coli residue immersed in a suitable liquid medium in the presence of EVOH / TiO2 nanocomposite films (loading percentages of several nanoparticles) as a function of UV irradiation time. Right: Logarithmic reduction of the total number of food microorganisms after 30 minutes of irradiation time in the presence of TiO2 / EVOH. Columns with arrows pointing upwards show examples in which a logarithmic decrease of more than five has been reported.

AgNP in Food Packaging

Many studies have shown the effectiveness of AgNP-containing packaging materials against microbial growth in foods. Biosynthesized AgNPs using *Trichoderma viride* were placed in a sodium alginate film-forming solution. The films were formed by casting and were analyzed for antimicrobial activity against *E. coli* and *S. aureus* by disk diffusion method on Müller-Hinton agar plates. The culture media was diluted with NaCl and was attached to the leaves with an alginate film containing AgNP. After incubation for 24 h at 37°C, clear areas were observed around alginate film samples containing AgNP indicating the antimicrobial efficacy of alginate film containing AgNP against both gram-negative and positive bacteria. The AgNP coatings and AgNP-free coatings were attached to cucumbers and pears with the pre-sterilized surface, which improved storage life from 6 to 10 days for cucumbers and 8 to 10 days for pears (Hosseini *et al.*, 2017; Simbine *et al.*, 2019; Riahi *et al.*, 2020).

The AgNPs were prepared by the dropwise addition of a solution containing $NaBH_4$ and polyvinylpyrrolidone (PVP), which produced a pale brown color indicating the formation of AgNPs. AgNP-PVP coating was applied to a well-prepared asparagus spear-shaped plant. Asparagus samples homogenized in saline peptone solution were exposed to microbial analysis for 25 days at 5 days intervals. Afterwards, aerobic psychedelic bacteria were counted using the plate counting technique containing algae extract after incubation for 72 h at 30°C. Moreover, parasites and fungi were counted in Sabouraud medium after incubation for 120 h at 25°C. Coatings that had AgNP significantly inhibited the growth of aerobic psychedelic bacteria, parasites, and fungi. In addition, the visual changes in asparagus samples were reduced. The LDPE nano-composite films containing AgNPs were prepared by melt mixing in a twin extruder and were used to package fresh orange juice at 4°C. This juice is stored for 56 days and alternately has been incubated using plate counting technique with algae extract and after incubation for 3 days at 30°C and counting parasites and fungi through the plate counting method containing algae extract of potato glucose with 10% tartaric acid stored at 25°C for 5 days. Both of these values were drastically reduced by AgNP-containing films compared to those without LDPE films. The resulting percentage of Ag ions in orange juice was less than 10 ppm indicating low Ag migration from the packaging material (Farrokhi *et al.*, 2017).

Absorbent pads are often used in retail food packaging to absorb the water and fluid that leaked from meat products to keep them fresh and prevent them from coming into contact with unhealthy fruit juices. However, this juice can facilitate the growth of pathogenic bacteria and spoilage agents even if it is absorbed by the pads. Therefore, cellulose, which is ordinarily a part of pads, can be utilized as a nanoreacting and stabilizing agent for antimicrobial AgNPs. In freshly stored cantaloupe slices in modified atmosphere packaging, the delay phase of microorganisms was enhanced by adsorbent pads containing AgNPs. As a result, microbial loads were kept under control during storage at approximately 3 log CFU/g (cantaloupe slices packed without pads) (Lloret *et al.*, 2012; Adibelli *et al.*, 2020). Synthesized cellulose/AgNPs under UV/heat reduction of $AgNO_3$ are adsorbed on foamed cellulose fibers. Fruit and meat products that were minimally processed were packed in trays containing adsorbent pads with or without AgNP and were stored for 4 days at 4°C. Next, these pads were exposed to microbial analysis for 12 days at intervals of 2 days. The total count of viable microorganisms, fungi, and parasites was reduced to 99.9% in kiwi and cantaloupe juices in contact with AgNP sorbents. In meat juice, the total count of viable bacteria and lactic acid bacteria (90%), was kept under control. It has been reported that the antimicrobial effects of silver in food contact applications are more prominent in fruit juices than meat juice because proteins counteract spoilage microorganisms with the antimicrobial activity of Ag^+ ions. The LDPE Ag_2O film bags designed by Zhou, Lv, He, He, and Shi have diminished microbial spoilage in apple slices. The quality of apple slices stored at 5°C in the LDPE/Ag_2O bags was satisfactory after 12 days, while for samples packed in an ordinary LDPE bag, a noticeable quality loss was observed after 6 days. The highest percentage of Ag ions released from the nanostructured bag is following the guidelines of the World Health Organization (WHO), in which the maximum permitted limit for silver in drinking water is declared as 0.1 mg/L (Farrokhi *et al.*, 2017).

In addition to the antimicrobial activity, AgNP absorbs and decomposes ethylene and plays a more important role in enhancing the storage life of vegetables and fruits. Each apple was cut into wedges of eight pieces and was packed in LDPE bags with and without Ag_2O nanoparticles. All were stored at 5°C and 15°C.

The browning and weight loss of apple slices were significantly delayed during storage. Furthermore, Di Mora *et al.* reported that AgNPs of 41 nm and 100 nm enhanced the tensile and inhibitory properties of H films. They include AgNPs into films resulting in a significant augmentation in tensile stren-gth from 28.3 MPa for H films to 51 MPa for films containing AgNPs of 41 nm and 38.5 MPa for films that had AgNPs of 100 nm. These results indicate that smaller AgNPs exerted better effect. Similarly, the permeability of films against water vapor decreased from 800 gmmkPa^{-1} h^{-1} m^{-2} for films without these particles to 0.48 gmmkPa^{-1} h^{-1} m^{-2} (Farrokhi *et al.*, 2017).

Health Effect of Antimicrobials Based on Nanosilver

Researches on nanosilver have advanced our knowledge about the possible outcomes of commercially applying this technology. Nanoscale silver particles are being used much more than any other nanoparticle in known factory products (Roy *et al.*, 2020). By August 2018, some forms of nanosilver had been used in the manufacturing of about 400 products, such as textiles (e.g., linens and socks), cosmetics (e.g., toothpaste and makeup products), kitchen utensils (e.g., food storage containers and refractory containers), detergents and soaps, utensils (e.g., refrigerators and washing machines), building materials, and toys (e.g., paints, putties, and adhesives). The AgNPs may also appear in commercialized food pack-aging materials in the future. However, the influence of these products on human health and the environment is not yet clear (Ahari *et al.*, 2018).

Toxicological examinations of AgNP in the body remained surprisingly limited. Therefore, generalized results on the impacts of AgNP exposure versus food exposure methods are not extensive. For example, it is still unclear to what extent biochemical pathways are applied to AgNPs that facilitate the processing of Ag ions or how much AgNPs pass intact through the lining of the intestine or are undissolved in the highly acidic gastric environment. Moreover, we are not yet sure how much AgNPs can cross natural biological barriers, such as the membrane separating brain tissue from flowing blood, fetus, or into breast milk. It should be noted that there is almost no investigation on the effects of AgNP accumulation due to chronic exposure or the relationships between the characteristics of particles (i.e., size, shape, and surface charge) and poisoning. We know very little about how the toxicity of Ag or AgNPs changes when these samples are dispersed in plastic coatings. A review of toxicological studies on living organisms in artificial environments has provided a complete analysis of this issue (Ahari *et al.*, 2018).

Toxicological studies in the artificial environment have shown that AgNPs may be dangerous for mammalian cells. Pulmonary fibroblasts and glioblastoma cells exposed to AgNPs decreased ATP levels, increased ROS production, damaged mitochondria and DNA, and caused chromosomal aberrations in a dose-dependent pattern, compared to controls. These findings suggest that AgNPs can be toxic to cells and genes, rapidly reproducible, and potentially carcinogenic. At low concentrations in the artificial environment, AgNPs change the cell cycle of human hepatoma cells. Furthermore, unusual cell morphology, cell contraction, and chromosomal damage might result from higher concentrations of AgNPs, which is much greater than what could be induced by the same concentrations of Ag^+. The latter result shows that the toxicity of AgNPs is not only due to the release of Ag^+ ions. Placing spermatogonia mouse stem cells in AgNPs of 15 nm at a low level (mL/10 µg) in the artificial environment leads to morphological changes and mitochondrial damage. Therefore, AgNPs may be a threat to male reproductive health under certain conditions. The AgNPs also cause cellular toxicity to mouse hepatic cells exposed to oxidative loads (e.g., the potential of damaged membranes and ROS formation), which is much lower in concentration than particles composed of other metal oxides. The ability of size-dependent cell poisoning by oxidative stress and ROS formation has also been demonstrated in mouse perforation macrophages. While at least one AgNP-based ointment is toxic to human fibroblasts and toxic skin cancer cells, it causes concentration-dependent morphology, signs of oxidative load, lipid oxidation, DNA fragmentation, and programmed cell death. The very high concentrations of these particles may lead to tissue death. However, it has been concluded that AgNPs are safe for skin contact at concentrations up to 25.6 μg/mL (Ahari *et al.*, 2018). However, this dose can be expected to be highly dependent on AgNP features. Consequently, the efficiency of this mass-based dose criterion is discussable, especially because the properties of AgNP have not been elucidated in this study. Contrary to these reports on the cell toxicity of AgNP, some other studies presented different results. A group of researchers observed that human cells were not affected by the proportionate concentrations of antimicrobial AgNPs and several groups found that AgNPs formed in the middle of layered PNCs or bone cement caused no observable toxic effects on human osteoblasts in test conditions [84]. While a growing number of studies in the artificial environment show that AgNPs are toxic to a variety of mammalian cell types, studies on living organisms have examined the systematic effects of AgNP delivery by the lingual pathway that is more ambiguous. Although AgNPs may be distributed in all the organs of a mouse fed by a stable nanoparticle diet, only a few toxic impacts are observed, except in high concentrations. An oral suction study on pigs that were weaned showed AgNP accumulation in the liver. However, the effects were not exactly toxic. On the other hand, inflammation and lymphocyte purification have been observed in the liver of mice fed by nanoparticles and microns. This influence can be aggravated at nanoscale particles (Ahari *et al.*, 2018; Yusuf and Casey, 2020).

Evaluation of the health status of a new agent, which is in contact with food, should be accompanied by determining how easily it is removed from packaging materials and enters various foods. Unfortunately, limited studies have assessed the ability of nanoparticles as a whole, and AgNPs in particular, for entering hard polymer environments and crossing the joint packaging/food surface. Simon *et al.* used a physicochemical method to theorize that AgNP embedded in food packaging may be dispersed in food in visible sizes only when the particle radius is very small (about 1 nm). In addition, the package should be composed of a polymer with relatively low dynamic fluidity (polyolefins, such as HDPE, LDPE, and PP) and there should be no major interaction between the particle and polymer. Although large-scale experiments have identified this prediction, while it had not been published yet, at least one study has revealed evidence of entry into food. In this case, it was orange juice from LDPE packaging materials containing antimicrobial nanoparticles Ag or ZnO. However, food-contact materials, on which AgNPs are placed, might be a more urgent concern as they are in direct contact with the food network. In a previous report on AgNPs incurporated in cellulose pads for use in the packaging of modified fresh meat, the authors found that visible amounts of Ag ions adhered to meat secretions and not to the meat itself. Therefore, even attempts to examine the relationships between particle characteristics, polymer type, food pH/polarity, and especially environmental conditions related to food production, storage, and packaging (e.g., tempera-ture, pressure, humidity, high exposure, and storage time) are limited. Consequently, it is difficult to have a widespread recognition of this essential aspect of AgNP-based food contact health (Ahari *et al.*, 2013).

Although the results of studies on AgNP toxicology may seem unpleasant, they should be kept in prospect. Some studies have poorly characterized the particles or not characterized them at all. Relationships were observed between the impacts of exposure to isolated cells in the artificial environment. However, they are not always clear in all organisms, especially when questions remain about nanoparticle toxicology. In addition, some studies have controversial or exaggerated findings. For example, a published text with some headings indicated that AgNPs interfere with DNA replication during PCR cycles, as they do during reproduction in *E. coli* cells (Yang *et al.*, 2009; Hannon *et al.*, 2016). Although the study title "silver nanoparticles of food preservative interferes with correct DNA replication and binds to DNA" requires the article to report a toxic effect on a consumer product, it has not dealt with food packaging at all. As a result, scientists must make an extra effort to stick to these facts and avoid exaggerated titles, results, or predictions to minimize the possibility of misleading or public newsagents about the health of consuming products that contain nanoparticles.

Nanosensors and Nanotechnology-based Assays for Food-related Analytes

Fresh products or meats that are rotten or taste bad show odors, colors, or other sensory characteristics that can be easily detected by consumers. When packaging materials prevent the presence of a strong sensor, consumers should trust the presented dates, which may not be usable if the milk is stored at an above-average temperature for one hour in a truck or a warm car (Ahari *et al.*, 2013).

Solutions to this problem may be offered by the chemical and electro-optical properties of single nanoscale particles. During low-to-high engineering, nanomaterials can be fabricated to detect the presence of gases, odors, chemical contaminants, and pathogens, or to react to changes in environmental conditions. This is not only useful for quality control to ensure that consumers can buy products at their best freshness and taste but also has the potential to improve food health and reduce the recurrence of illnesses due to food. Consequently, such technology benefits consumers, industry stakeholders, and food regulators. Furthermore, some companies launched nanotechno-logy products for sale that help customers determine which particular foods are perfectly palatable. This section shows some of the newest and most interesting works conducted in this field (Ahari *et al.*, 2014; Razavilar *et al.*, 2019).

Detection of Small Organic Molecules

Beyond the benefits for supermarkets and food manufacturers, nanotechnology-based sensors have the potential to revolutionize speed and accuracy so that industries and regulators can detect the presence of molecular contaminants or impurities in complex food networks. Many of these assays are based on the observed color change that occurs in metal nanoparticle solutions in the presence of analytes. For example, AuNPs functionalized with cyanide acid groups, selectively bind to melamine (Figure 4), an impurity used to artificially inflate the measured protein content of pet foods and infant formulas. Melamine-induced accumulation causes AuNPs to undergo a concentration-dependent discoloration of the reproducible analyte from red to blue, which can be used to accurately measure the amount of melamine in cold milk and infant formula at concentrations as low as 2.5 ppb and could be seen by the naked eye (Razavilar *et al.*, 2019). A similar method tested the presence of melamine in samples by adding freshly separated solutions of gold ions and a chemical reducing agent. In this system, when melamine is present in a sample, it joins the reducing agent and prevents the formation of AuNP. Therefore, test samples without melamine turn red during the assay because AuNP formation occurs as the result of the reaction between Au ions and the reducing agent. Detection based on melamine color in cold milk using AuNPs and thiols with modified coronal ether with a detection limit of 6 ppb has also been reported (Ahari *et al.*, 2014).

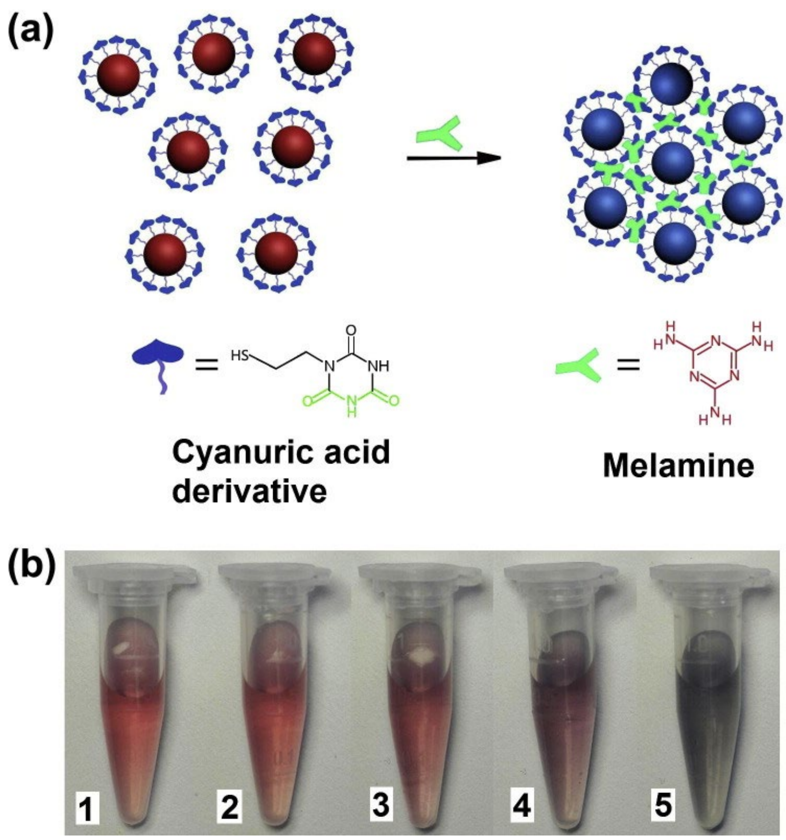

Figure 4. (a) Shows a diagram of Melamine colorimetric detection in solution with the use of modified gold nanoparticles (AuNPs). AuNPs are fused to a cyanuric acid derivative, which is selectively bound to Melamine by hydrogen bonding interactions. When attached to Melamine, accumulated AuNPs show red (blue) different absorption properties than free AuNPs (red). (b) Spectacular color changes of Melamine -AuNP sensor in real milk samples: 1) AuNP solution without any additives, 2) By adding what is extracted from the control milk, 3), 4), 5) by adding the extracted material containing 1ppm (final concentration: 8ppb) Melamine, ppm 2.5 (final concentration: 20ppb) Melamine, and five ppm (final concentration: 40ppb) Melamine, respectively.

Other sensing systems for small molecules are more dependent on fluorescence rather than absorption color change. For example, a sensor-based detection method, called improved fluorescence, is incorporated in enzyme-linked immunosorbent assay (ELISA) and can be used to detect the presence of gliadin. This system uses metal fluorescence with the addition of rhodamine-labeled anti-gliadin antibodies near nano-structured silver island films. Moreover, this method is applied to determine the gluten content of gluten-free foods and to selectively detect other protein analytes. Another fluorescence-based assay efficiently detects cyanide in drinking water at concentrations as low as 2 nM using fluorescence quenching of gold nanoclusters. A nanoscale liposome-based detector has also been developed for drinking water contamination with pesticides (Ahari *et al.*, 2014). Several bacterial protein toxins, including A serotype toxin botulinum, have been identified in picomole sizes (pM) using antibody-labeled luminous quantum dots, which can be beneficial in food safety and bioterrorism applications. Easy-to-read metal nanoparticle detectors have also been developed for many other small molecules, proteins, and metal ions, which suggest similar strategies that can be developed to properly diagnose a variety of food impurities, allergens, and contaminants (Ahari *et al.*, 2014).

Another popular method is electrochemical detection by nanomaterial-based sensors in the food industry. Electrochemical methods are more efficient for food networks, compared to optical techniques (colorimetry or fluorometry) because we can avoid the problem of light scattering and absorption by various food components. Many electrochemical sensors act on selective antibodies that bind to an induced nanomaterial, such as CNTs, and show changes in the conductivity of the material when binding to antibodies. For instance, conduction changes that occur during the ligation of LR-Microcystin (MCLR), a cyanobacteria-produced toxin, to the surface of a single-walled CNT coated with anti-MCLR can be easily detected at concentrations as low as 6 nM, which simply meets the strategies set by the WHO for this substance in drinking water. This method reduces sampling time to once, compared to the older MCLR measurement methods, such as ELISA. A similar strategy that utilizes AuNPs and glucose-sensitive enzymes can be used to measure glucose concentrations in commercial beverages, and a reusable piezoelectric AuNP security sensor that detects the presence of B1-aflatoxin in contaminated milk samples up to concentrations as low as 0.01 mL/ng. Other nanomaterial-based electrochemical systems include a security sensor based on chitosan nanocomposites and a cerium oxide nanoparticle that detect A-ochratoxin, a fungal food contaminant (Ahari *et al.*, 2014). Note that analytes are not limited to harmful substances. A study showed that CNT-based electrochemical detection in microfluidic devices can be utilized to assess antioxidants, flavor composition, and vitamin content in vanilla and apple berries. Numerous other examples of electrochemical detection of various biomolecules using nanomaterials are provided in a new review of the topic (Zhang *et al.*, 2017; Wang *et al.*, 2019).

Gas Detection

Extra moisture and oxygen lead to food waste, and many measurements of the amount of water vapor or gas inside the package are still required to damage the package. As a result, packaged foods are randomly inspected during production to facilitate the process and typically one package is tested out of 300-400 packs, which takes time and money. Researchers are still unsure if unsampled packages have standard quality and health. Consistently and easily displaying the amount of gas in a package should allow the detection of the health and quality of stored food long after production suggesting that non-invasive leak detec-tion and gas measurement methods might not be of value (Majid *et al.*, 2018).

To complete the sensor methods, many non-invasive gases are provided based on nanotechnology. For example, Mills *et al.* developed a nano-sized indicator ink activated by promising light to detect oxygen in packages based on TiO_2 and SnO_2 particles and an oxidation-reduction dye (methylene blue). The color of the detector alters gradually in response to even small amounts of oxygen as shown in Figure 5. It may be difficult to evaluate the amount of oxygen in packages with this technology. However, it does provide a simple and intuitive method for consumers and retailers to detect packets with modified atmospheres (MAPs) with the possibility of not manipulating the agreed seal. An example of a humidity sensor, shown in Figure 6, is based on carbon nanoparticles coated with carbon (Matindoust *et al.*, 2016, Majid *et al.*, 2018). In humid environments, the swelling of the polymer lattice leads to greater degrees of separation of the inner nanoparticles. These changes prevent the sensor from reflecting or absorbing various colors of light, which are demonstrated to quickly and accurately detect closed moisture sizes without aggressive sampling. A non-invasive method of measuring the amount of CO_2 in MAPs has also been developed based on the lifetime analysis of bright colors standardized by fluorophore encapsulated polymer nanoparticles. This CO_2 sensor has a detection range of 8%-100%, a resolution of 1%, and only 6% molecular oxygen permeability (Ahari *et al.*, 2014; Moradi *et al.*, 2015).

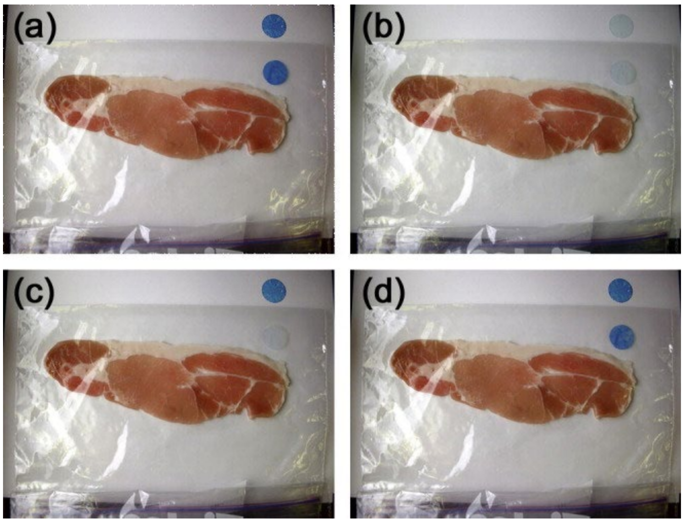

Figure 5. Pictures of O_2 sensors using UV-activated TiO_2 nanoparticles Methylene blue color indicator, one has been located inside a CO_2-balanced food package and one outside. In (a), the package is recently sealed, and both indicators are blue. Picture (b) shows the indicators immediately after activation with UVA light. After a few minutes, the indicator outside the package turns blue again, while the remaining indicator remains white in an oxygen-free atmosphere (c) until the package is opened, in which case the oxygen flux causes it to change back to blue. This system can be used for easy and non-invasive detection of leaks in any package immediately after production and in retail stores.

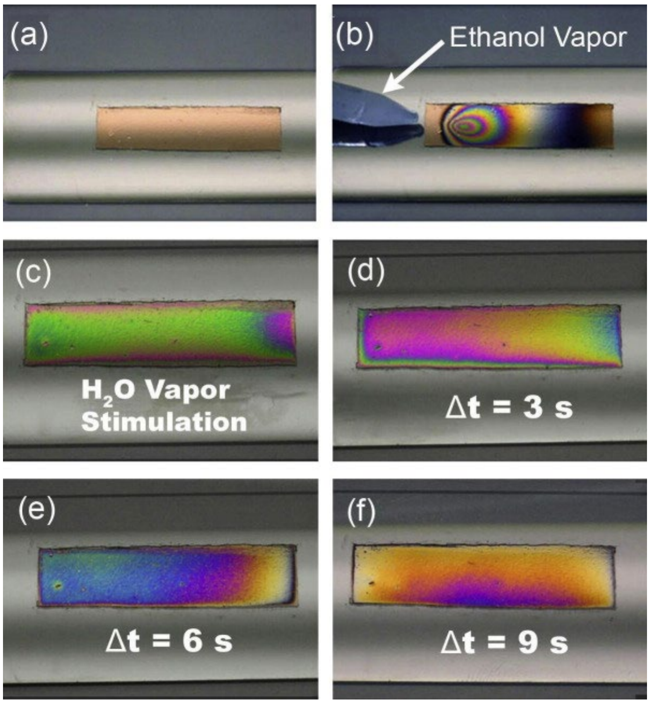

Figure 6. Moisture sensor using carbon-coated copper nanoparticles dispersed in a polymer network. a) When exposing to ethanol vapor results in rapid and reversible rainbow color. b) When exposed to water vapor, it swells the polymer, which causes the nanoparticles to show larger interparticle separation distances and therefore has different observable optical behavior. c) By dissipating moisture (d-f), the sensor returns to its original state and appearance.

Detection of gaseous amines, as indicators of meat and fish spoilage, is related to health or food quality in terms of fractional size per trillion (theoretical ppt) or ppm size affected by the fluorescence desorption of perylene-based fluorophore nanofibrils. It is completed using conductivity changes in SnO_2 nanoparticle composites and TiO_2 macromolecules, a set of electronic sensors that use $ZnOTiO_2$ nanocomposites or SnO nanofibers to detect the presence of volatile organics, including acetone, ethanol, and carbon monoxide. In addition, SnO_2-WO_3 nanocomposites are used to detect ethylene gas sensitive to fruit ripening (Moradi 2015 *et al.*, 2016).

Current Industrial Applications

Summarizing iRAP and BBC researches, Plastemart reported that the market for nano beverage and food packaging was $4.13 billion in 2008, and it was expected to grow up to $ 3.7 billion. Active technology owned the largest market share and is expected to reach 4.35 billion by 2015, and the smart technology sector may grow to $ 2.47 billion (Duncan, 2011).

In food products, nano-packaging was most applied for meat and bread products, as well as carbonated beverages and bottled water dominant in beverages. These systems were not much developed and few were then operational. In 2008, the Asia-Pacific region, especially Japan, led the market in nano-active packaging with about 45% of the market value of US $1.86 billion. The market is estimated to worth US $ 15 billion by the end of 2020. In the USA, Australia, and Japan, active and advanced packaging has been used almost successfully to enhance maintenance time while maintaining food quality and ensuring microbial health (Duncan 2011; Moradi *et al.*, 2015).

In Europe, industrial applications were slowly advancing. It was mainly due to legal limitations and unawareness about the admissibility of European consumers regarding the efficiency of these systems, as well as the environmental and economic effects of these systems (Duncan, 2011).

However, the production and usage of such nanomaterials are costly today, compared to the benefits of the final commercial package. As a result, more nanoparticles involved in packaging are now more relevant in the research phase, in comparison with commercial applications. Despite the excellent performance of PNFPs, industrial applications are relatively slow with a small number of companies currently operating as leaders, such as Honeywell, Mitsubishi Gas and Chemical, Bayer, Triton Systems, and Nanocor. In general, there seems to be a hesitation to accept such technology because of its high costs and ability to change the quality of some products and the disadvantages of producing PNFPs. There are various problems in preparing nanocomposites with pre-polymerization and post-polymerization methods. Pre-polymerization may interrupt the polymerization process which is often critical, more time-consuming, and more costly to get better results and controllability. In turns, post-polymerization often takes time for nanoparticles to get fully dispersed in the composition (Sharma *et al.*, 2017, Cerqueira *et al.*, 2018). Optimization of processing conditions is important in production and can be costly and pay less attention to competitive initiatives. Transforming plastic tablets to a swollen bottle is a complicated process, which requires heating and blowing and forms the bottle with costly specific equipment designed for special materials. Nano-scale clay particles are currently the most common application of nanoparticles and cover about 70% of the market volume. Industrial applications of nano-clay in multilayer packaging films entail beer bottles, carbonated beverages, and heat-formed containers. Nanoclays are embedded in plastic bottles and hard-packed nylon food films, reduce gas permeability, keep oxygen-sensitive foods fresher, and augment storage life. Bayer polymers create a low-cost nano clay compound for the inner lining of boxes to keep juices fresher. Nano-clay used in PET beer bottles is produced by Nanocor Inc. and distributed by the Dye Network. The shelf life of beer in regular PET bottles is approximately 11 weeks and when nano-clay is used as a barrier, it is increased to about 30 weeks.

Impact on Human Health

Nanoparticles can enter organisms in three different methods, namely inhalation, penetration through the skin pores, and ingestion. Growing scientific evidence has reported that free nanoparticles can cross cell barriers and that exposure to some nanoparticles may lead to oxidative damage and destructive reactions. In the case of nanomaterials for food packaging, many people are concerned about the potential risk of nanoparticles transfer out of the package (Fen *et al.*, 2018).

Inhalation and entry through skin pores are almost exclusively related to workers in nanomaterials factories. For these workers, personal protection using gloves, goggles, and masks with particularly efficient filters is recommended. For end-users of foods packaged with nanomaterials, the first concern is to confirm the spread of nanoparticles from the package into food. In case this transfer occurs, the effect of swallowing these nanoparticles on the body from the mouth to the digestive tract is definite. It is vital to know how these particles will function when they enter the body, and if nanoparticles are absorbed by different organs, how they are metabolized, and how the body repels them (Fen *et al.*, 2018).

Few studies investigated the transfer of nanoparticles from package to food. Two studies analyzed the clay transfer from PET bottles and potato starch films and the polyester composition of potato starch. In both cases, non-significant visible translocations of nanoclay were observed. Another study reported the transfer of AgNPs from food containers made of PP nanosilver compounds. In this case, the amount of Ag transfer was lower than the set limits. Although these findings might assure the health of using these products, further research is needed in this regard (Fen *et al.*, 2018; Youssef 2018).

Moreover, the presence of nanoparticles in packaging films has positive influences on the transition from food packaging to chemical food, which may be accompanied by the potential for adverse health effects. De Abreu showed the transfer of caprolactam, 5-chloro-2-(2,4-dichlorophenoxy) phenol (triclosan) and trans, trans- 1 , 4 - diphenyl - 1, 3 - butadiene (DPBD) and dichlorophenoxy phenol (triclosan) from nano-clay-polyamide to different types of similar foods. Polymer nanoparticles were observed to reduce the transfer rate of these materials from the polymer network in food (Hosseini *et al.*, 2017).

We know little about what happens if these nanomaterials enter the body. The risk assessment of nanomaterials after ingestion has been taken into consideration for only a small number of nanomaterials used in food packaging. Some results about TiO_2, AgNPs, and CNTs indicate that nanoparticles can enter the blood through the digestive tract. These processes are highly dependent on the physicochemical properties of the nanoparticles, such as size, as well as the physiological condition of the organ into which they enter. Displacement ratios seem lower; however, this is the topic of many current pieces of research. After the nanomaterials reach the blood, the liver and spleen are the two major organs of distribution. Spin time elevates dramatically when hydrophilic nanoparticles have a positive surface charge. With certain nanoparticles, all organs may be at risk, and the chemical composition of the nanoparticles or the nanoparticles themselves can be detected showing the distribution of nanoparticles in these organs. These organs include the brain and testicles/reproductive system. Distribution in the fetus in the uterus has also been reported.

Research in these areas should be advanced to refute or confirm the hypothesis that nanoparticles are associated with various brain diseases. The impact of other particles used in food packaging on hea-lth is under investigation, such as ZnO nanoparticles and fluorides (Hosseini *et al.*, 2017).

Consumer Perception and Feeling

People's feelings are an important factor in their understanding and reactions to new technologies. Recent articles have demonstrated that in Europe and the USA, consumers have different perceptions of food nanotechnology. A report suggested that the public perception of the European consumers of nanotechnology is gradually increasing and they are skeptical about using nanoparticles in food, while positive about nanotechnology opportunities in several applications. In a variable and emotionally different state, 80% of American consumers in a new nanotechnology study have not heard much or even anything about nanotechnology, and they consider it effective for healthier and better food (Ahari *et al.*, 2020).

A study by the National Science Foundation in the USA on the general perception of nanotechnology products found that American consumers want to use special products containing nanoparticles even if there is a risk to health and safety. These investigations indicate that there is an urgent need to inform the public about nanotechnology and food (Inshakova and Inshakov, 2017). Nanotechnology can be used in all aspects of the food chain to improve both food health and quality control. Furthermore, we can utilize nanoparticles as new elements or food additives that even have a positive impact on the environment. However, they can lead to unforeseen health risks (Ahari *et al.*, 2020). There are also concerns about the implementation of risk assessment strategies and methods. There is a lack of public awareness of nanotechnology in general and nanotechnology applications, especially in the food industry. This should be reflected in the short period of public debate.

Results

Polymer nanotechnology can provide new packaging materials with improved capabilities and market analysis. Billions of dollars are predicted in markets for food products with nanotechnology in the next five years. These innovative nanotechnology-based packaging solutions must fully meet the requirements of food safety, including microbial growth control, oxidation delay, and visibility improvement, as well as product quality aspects, such as manageable fugitive flavors and odors. Moreover, these should provide comfort and stability. There are currently a lot of nanotechnology food and beverage products on the market or are being researched. However, without a general discussion of food nanotechnology, consumer acceptance of products, implementation of strategies, and methods for estimating the risks to the environment and health, it is difficult to determine how many of these products can find appropriate applications.

Some important issues should be considered for using nanotechnology in food packaging, including health concerns regarding the transfer of nanoparticles to food from the packaging material and their possible toxic effects. Although there are limited scientific data on the transport of the main types of nanoparticles, it is logical to presume that transfer may occur. Therefore, there is a need to diminish this transfer to zero, and to provide exact information on the effects of nanoparticles on human health following long-term exposure. It is essential for manufacturers not only to ensure the quality of products to enforce regulations but also to engage the consumer by providing clear information about the potential benefit/risk balance (Abbasi *et al.*, 2020).

Finally, it should be noted that biodegradable plastics, which are typically made from plant materials and were widely reviewed in this study, have become a primary field of research in nanotechnology (in 2008, the world's biodegradable plastics hit about 300,000 tons). Consequently, it needs a full guide as a green food packaging (Abbasi *et al.*, 2020). Major players, including Natureworks with a potential PLA capacity of 140,000 tons per year, are regarded among the world's biggest producers of biodegradable plastics, namely Cereplast, Metabolix, Novamont, and BASF. However, their barriers, problems, and mechanical properties are still destitute inadequate, compared to polymers derived from fossil fuels, which now limit their use. Therefore, more and more research will be needed for improvement. Certain biopolymers have specific antimicrobial properties making bio nanocomposites even more attractive to multiply their added value. There are several papers on this topic and interested readers can find newer items in the reference list.

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Conflict of Interest

The authors declared no conflict of interest.

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کاربردهای اندازه نانومتری در بسته‌بندی و ذخیره مواد غذایی: یک مطالعه مروری

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با حرکت به سمت جهانی شدن، بسته‌بندی مواد غذایی نیز نیاز به زمان نگهداری طولانی‌تر، کنترل کیفیت و بهداشت بر اساس استانداردهای بین‌المللی دارد. با پیشرفت مطالعات انجام‌شده در زمینهٔ استفاده از فناوری نانو در علوم غذایی، کاربردهای مواد نانو در زمینه‌های مختلف صنایع غذایی افزایش یافته است. فناوری نانو می‌تواند تمام این نیازها را برآورده کند و عملکردهای اساسی بسته‌بندی – ذخیره‌سازی، محافظت، بازاریابی و توزیع و ارتباطات را گسترش و اجرا نماید. کاربردهای فناوری نانو پلیمر در واقع می‌تواند مواد جدید بسته‌بندی مواد غذایی را با خواص مکانیکی، مهاری و ضد میکروبی همراه با حسگرهای نانو فراهم کند تا شرایط غذایی را در حین حمل و نگهداری ردیابی و کنترل کند. در این مقاله مروری، آخرین نوآوری‌ها و کاربردهای نانومواد در بسته‌بندی مواد غذایی با استفاده از فناوری نانو پیشرفته، فعال و هوشمند بررسی شد. وضعیت فعلی تجارت و درک مفهوم سلامت این فناوری‌ها و همچنین محدودیت‌های نانومواد پلیمری جدید که امکان تغییر کامل صنعت بسته‌بندی مواد غذایی را دارند نیز بحث شده است.

واژه‌های کلیدی: بسته‌بندی فعال، بسته‌بندی مواد غذایی، خواص ضد میکروبی، فناوری نانو

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