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Antimicrobial nanoparticles and biodegradable polymer composites for active food packaging applications

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Abstract

The food industry faces numerous challenges to assure provision of tasty and convenient food that possesses extended shelf life and shows long-term high-quality preservation. Research and development of antimicrobial materials for food applications have provided active antibacterial packaging technologies that are able to meet these challenges. Furthermore, consumers expect and demand sustainable packaging materials that would reduce environmental problems associated with plastic waste. In this review, we discuss antimicrobial composite materials for active food packaging applications that combine highly efficient antibacterial nanoparticles (i.e., metal, metal oxide, mesoporous silica and graphene-based nanomaterials) with biodegradable and environmentally friendly green polymers (i.e., gelatin, alginate, cellulose, and chitosan) obtained from plants, bacteria, and animals. In addition, innovative syntheses and processing techniques used to obtain active and safe packaging are showcased. Implementation of such green active packaging can significantly reduce the risk of foodborne pathogen outbreaks, improve food safety and quality, and minimize product losses, while reducing waste and maintaining sustainability.

KEYWORDS

food safety, foodborne pathogens, nanocomposites, nanofillers, shelf life

Abbreviations: BNP, biodegradable natural polymers; CEO, cinnamon essential oil; CNT, carbon nanotube; EFSA, European Food Safety Agency; FDA, Food and Drug Administration; GO, graphene oxide; GRAS, generally recognized as safe; MSN, mesoporous silica nanoparticles; MRSA, methicillin-resistant *Staphylococcus aureus*; MWCNTs, multiwalled carbon nanotubes; NP, nanoparticles; PBS, polybutylene succinate; PCL, poly(ϵ -caprolactone); PFAS, perfluoroalkyl substances; PGA, poly(glycolic acid); PHB, poly(hydroxybutyrate); PHBV, poly(3-hydroxybutyrate-co-3-hydroxyvalerate); PLA, poly(lactic acid); PLGA, poly(lactide-co-glycolide); PVA, polyvinyl alcohol; rGO, reduced graphene oxide; ROS, reactive oxygen species; UV, ultraviolet

1 | INTRODUCTION

Foodborne diseases are a global public health concern, with a burden of 600 million cases per year of illnesses caused by contaminated food and a yearly average of 420,000 fatalities (WHO, 2015). In the European region, the impact of foodborne diseases results each year in 23 million people falling ill from unsafe food, leading to 5000 deaths worldwide. Symptoms vary from mild discomfort due to vomiting, fever, and diarrhea, to life-threatening diseases. In addition, outbreaks of foodborne diseases also have serious economic implications as they often involve

product recalls, damage credibility, and reputation of food brands.

Food packaging is essential in maintaining the safety and quality of products, from processing and manufacturing to handling and storage, until the products reach the consumers. For a long time, petroleum-based plastic materials (such as polyethylene terephthalate, polypropylene, and polystyrene) have been used for food packaging to protect the contents from contamination and spoilage and to facilitate transport and storage (Piergiovanni & Limbo, 2016). However, plastic materials cannot fully protect the food from the environment and, thus, cannot completely ensure product quality and safety. Importantly, synthetic non-biodegradable plastics have led to serious ecological problems due to environmental pollution by micro and nanoplastic debris, triggering the development of new biodegradable and less toxic biomaterials as alternatives. In addition, novel active nanomaterial-based agents are being incorporated into the packaging to further improve the ability of eco-friendly biopolymer films to protect food products from light, moisture, oxygen and other environmental factors that cause food degradation.

Active packaging ensures the quality and safety of the food product through the interaction between the packaging film, foodstuffs, and internal and external environments (Realini & Marcos, 2014). The technology refers to the incorporation of active compounds into packaging film. The antibacterial active packaging is a version of active packaging that contains antimicrobial agents that can interact with the headspace and packaged food with the aim to prevent bacterial contamination. The European Regulation (EC) No. 450/2009 defines active packaging as systems that “deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food” and intelligent packaging as “Intelligent food contact materials, which monitor the condition of packaged food or the surrounding environment, for instance, by providing information on the freshness of the food” (European Commission, 2009). In this way, antibacterial packaging functions by releasing the active agents onto the foodstuff or into the surrounding atmosphere and inhibits the growth phase of microorganisms present on the surface of the packaged food (Drago et al., 2020). Furthermore, the improved storage conditions may extend food shelf life and freshness and preserve food quality and safety.

The unique properties of nanostructures and nanomaterials, such as their small size, high surface-to-volume ratio, and quantum effects, make them suitable for diverse applications in the food industry. In particular, metal and metal oxide nanoparticles (NPs; such as Ag, ZnO, Cu/CuO, TiO₂, and MgO NPs), mesoporous particles, graphene, and carbon dots have attracted remarkable interest in the

food industry for their intrinsic antibacterial activity. The antimicrobial nanomaterials applied in active packaging keep the food safe from harmful and spoilage-causing bacteria (Figure 1), thus enabling longer-term freshness during storage and higher convenience for consumers (Hoseinnejad et al., 2018; R. Sharma et al., 2020). Furthermore, the incorporation of active antimicrobial NPs into the packaging material makes it lighter, stronger and less permeable to oxygen.

Active packaging based on hybrid nano-biocomposites protects the food, increases its shelf life, and reduces the use of traditional plastics. This review provides an overview of the most recent studies on the development of nanomaterial-enabled active packaging for food with a particular focus on nanofillers with antimicrobial activity and different types of biodegradable polymers. Additionally, the mode of antibacterial action, cytotoxicity, and legislation for the safety of nanomaterials for active packaging are discussed.

2 | BIODEGRADABLE POLYMERS

Nondegradable plastic persists in the environment and may interfere with the homeostasis of the ecosystems and exert toxic effects on human and animal health. Plastics are estimated to make up about 10% of global waste with about 70% of marine litter objects being single-use plastic products like straws or food and drink containers (Barnes, Galgani, Thompson, & Barlaz, 2009), and are even predicted to dramatically increase over the next decade, if the current trends continue (Harrison et al., 2018). Due to the very slow degradation, the conventional petroleum-based polymers entering the environment undergo mechanical fragmentation and become micro- and nanoplastics of <5 mm and <100 nm size range, respectively. The microplastics-associated microbial biofilms (so-called “plastisphere”) are a particular concern because the plastisphere microorganisms can serve as transport systems for pathogens and can change the particle buoyancy as well as the degradation rate of the plastic polymers, affecting the general distribution and fate of the plastic pollutants (Oberbeckmann et al., 2015).

The genotoxicity and cytotoxicity of such small plastic particles have been documented in fish and mussels (Oliveira et al., 2019) and concerns about their detrimental effects on human health have been raised (da Costa et al., 2016; Fadare et al., 2020; Revel et al., 2018), urging the scientific and policy making communities to harness all the available resources in order to further investigate and ultimately diminish the negative effects of plastics on human health and the environment (Stojkovic et al., 2020). Moreover, some additives, such as bisphenol A (Cooper, Kendig,

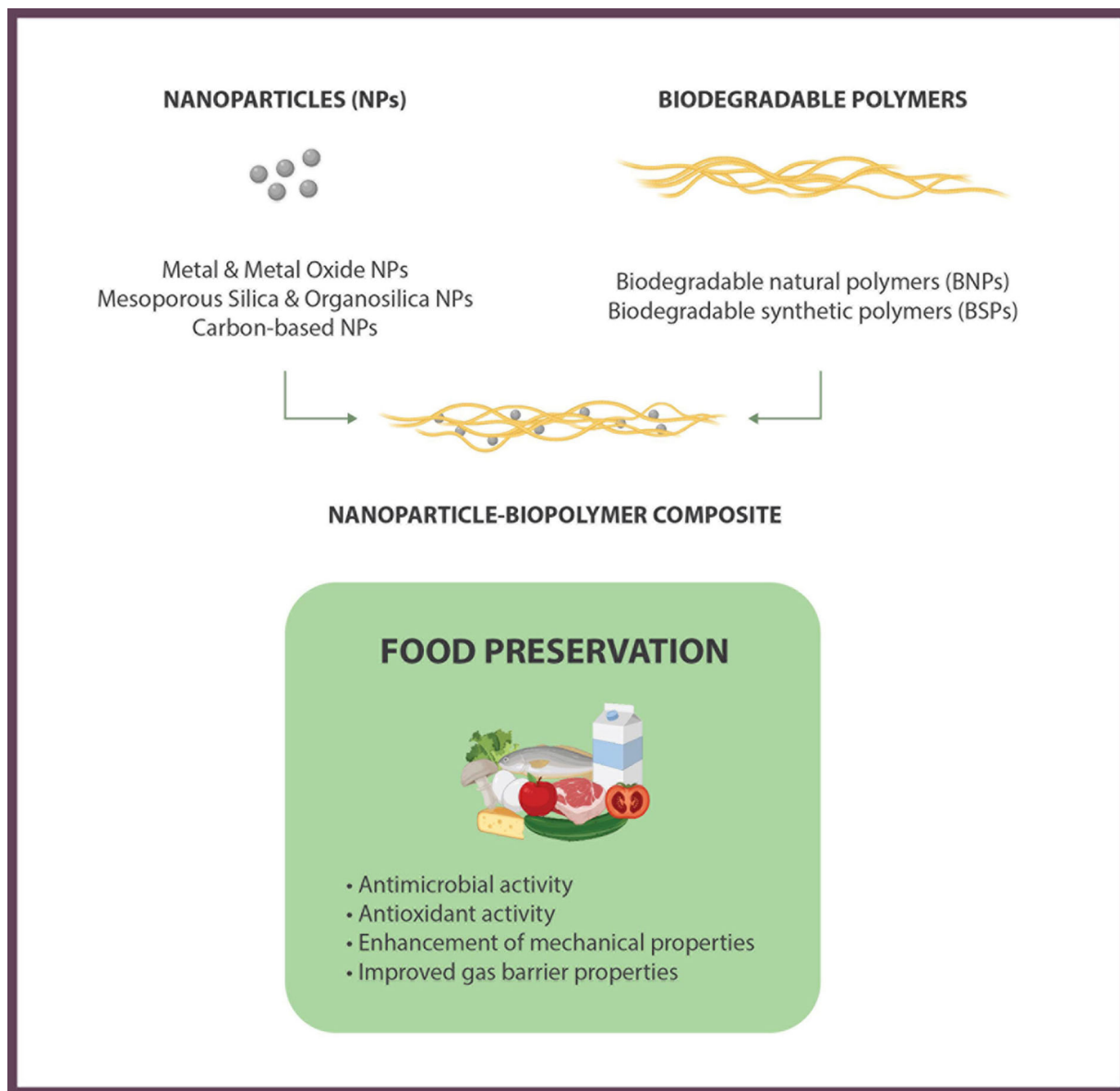


FIGURE 1 Active packaging fundamentals: composition of nanoparticle–biopolymer composites and their beneficial effects in food preservation

& Belcher, 2011), polybrominated diphenyl ethers, phthalates, or perfluoroalkyl substances (PFAS), which have been used for a long time in the food industry as moisture and oil penetration barriers can be classified as carcinogenic or endocrine disrupting (Llorca, Schirinzi, Martínez, Barceló, & Farré, 2018; Thurber & Curtzwiler, 2020). There was particular attention on the sulfonate and carboxylate PFAS forms such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA; Kucharzyk et al., 2017). Both PFOA and PFOS have high chemical and thermal stability rendering them very difficult to remove from the environment (Rahman et al., 2014). Many conventional methods of remediation, such as air stripping, thermal treatment, soil vapor extraction, and hydroxyl-based chem-

ical oxidation are not effective for PFOA and PFOS removal (Vecitis et al., 2010).

To diminish the use of PFAS and other compounds that prove to be difficult to remediate, a number of new technologies have been explored aiming to develop novel more sustainable food packaging materials that are, in the same time, able to meet company's and consumer's expectations. To endorse a common circular economy vision in which by 2025 all plastic packaging will be reusable, recyclable, or compostable, more than 500 businesses, governments, and other organizations have signed the New Plastics Economy Global Commitment (Global Commitment, 2020). Already more than 170 countries have banned six single-use plastic items including food containers, straws,

plastic grocery bags, and six-pack rings that are hard to recycle and often found in the environment. Naturally sourced biopolymers are the first choice when attempting to achieve a less toxic and more sustainable food packaging because of their biocompatibility, biodegradability, and nontoxicity (Popović et al., 2018; Spasojević et al., 2019). Biodegradable natural polymers (BNP) are biopolymers derived from biomass and other agro-resources. They are a chemically diverse category, comprising polysaccharides, proteins of animal or plant origin, lipids, and polyesters from microbial sources. Examples of different BNP categories used as packaging materials are presented in Table 1.

The most commonly used BNP for producing packaging are proteins and polysaccharides and their derivatives because they are widely available and can polymerize into brittle and rigid materials. Casting method is the most frequently used method for the preparation of films based on polysaccharides and proteins. It includes two main steps: dissolution or dispersion of biopolymers in suitable solvents, and evaporation of solution under ambient conditions or in a controlled relative humidity environment (L.-F. Wang et al., 2016). Some other conventional methods that include extrusion, compression, and injection molding of biopolymers are performed as dry processes. Although these methods require precisely defined parameters (pressure, temperature, shear impact) and more equipment compared to the casting method, they can be easily adapted for industrial implementation (Park et al., 2014). Polysaccharide- and protein-based films show good barrier properties to gases, low- to medium-relative humidity, moderate mechanical properties, but are poor barriers against water vapor due to their hydrophilic nature. The major limiting factor for direct application of BNP-based films in food packaging systems is their high sensitivity to moisture.

Biodegradable synthetic polymers exert an array of physicochemical and mechanical characteristics that render them suitable for packaging applications. Biodegradation of these synthetic materials is typically achieved through their slow chemical hydrolysis in an aqueous environment that can be improved by enzymatic catalysis (Zhong et al., 2020). Poly(α -hydroxyester)s, such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and their co-polymers poly(lactide-co-glycolide) (PLGA), are being extensively reported for antimicrobial packaging applications (Biswal et al., 2020; Holman et al., 2018; Lyu et al., 2019; Radusin et al., 2019; Rao et al., 2016). Polymeric nanocapsules based on poly(ϵ -caprolactone) (PCL) were also reported for encapsulation of *Thymus capitatus* and *Origanum vulgare* essential oils, showing higher activity against foodborne pathogens than that of the corresponding pure essential oils (Granata et al., 2018). In

addition, the incorporation of quaternary ammonium salts into the backbone of PCL led toward efficient contact-killing activity against *Escherichia coli* and *Bacillus subtilis*, with enhanced degradability (Bakhshi & Agarwal, 2017). Polyvinyl alcohol (PVA) film shows an advantage compared to other synthetic polymers because it can be reusable. Another example is kraft paper that is an extensively used material for food packaging due to its biodegradability and safety. However, it can lose its properties upon absorbing water when it becomes a good support for the growth of microorganisms.

To improve the flexibility and extensibility and even moisture-sensitivity of the packaging film, plasticizers are mixed with degradable polymers, such as glycerol, polyethylene glycol, sorbitol, propylene glycol, ethylene glycol, lipids (Haq et al., 2014). Lipids can improve the film barrier properties to the water vapor. In such packaging films, lipids may form a protective emulsion layer or may be in a separate layer within a multilayered composite film (Chiumarelli & Hubinger, 2014). However, the plasticizers can exert certain negative effects such as modification of the food aroma or increased oxygen permeability of the films.

3 | NANOFILLERS

Rapid developments in the nanotechnology field in the past several years are expected to bring significant benefits to the food and food-related industries through the whole food chain, including packaging, transportation, storage, and delivery. The European Commission's definition for identifying nanomaterial states that a material is to be considered a nanomaterial if half or more of the total number of particles in a material have one or more of their external dimensions between 1 and 100 nm (Lövestam et al., 2010). Biopolymer–nanocomposites display decreased gas and moisture permeability and increased mechanical strength in comparison to the pure polymers or microscopic composites. Nanofillers can be classified into: nanoplatelets, nanofibers, and NPs, having one, two, or three nanoscale dimensions, respectively. Nanofillers may also provide antibacterial activity. Figure 2 illustrates some antibacterial mechanisms of nanomaterials used in the development of active packaging. Some recent works employing different nanofillers are summarized in Table 2. Nanofillers may be used in two different concepts of conceiving food packaging. When integrated into active packaging, nanofillers act on the headspace or directly on food to increase the shelf-life. However, within the intelligent packaging nanofillers do not have any action on food, but monitor the condition of the packaged product and share the information. The packaging that combines intelligent

TABLE 1 Natural and synthetic biopolymers used to produce film for food packaging

Biopolymer	Source for production	Properties	References
Natural biopolymers—Carbohydrates			
Starch	Corn, potato, wheat, cassava, rice	Widely available and inexpensive polysaccharide, compatible with antioxidants and antimicrobial agents, good film-forming and gas barrier properties, hydrophilic behavior, low water vapor barrier, poor mechanical properties	(Chakravartula et al., 2020)
Cellulose	Agricultural waste (corn stalks, rice straw, sugarcane bagasse), woody plants	The most abundant natural polymer; tasteless and odorless, highly crystalline, fibrous and insoluble, thermally and chemically stable, inherent antimicrobial properties	(Hassan et al., 2018; C. Wu et al., 2019)
Chitosan	Alkaline deacetylation of chitin; exoskeletons of crustaceans (crab, shrimp, crawfish)	Good film-forming ability with moderate mechanical strength, inherent antimicrobial activity, compatible with many other biopolymers, high water sensitivity (solubility issue), low barrier properties	(Akhter et al., 2019; Chakravartula et al., 2020; Fernandez-Salz, 2011)
Alginate	Brown seaweeds of <i>Phaeophyceae</i> class	Ability to react with di-valent and trivalent cations, gel and film-forming ability	(de Oliveira Filho et al., 2019; Hassan et al., 2018; Munteanu & Vasile, 2020)
Agar	Red seaweeds of <i>Rhodophyceae</i> class (<i>Gelidium</i> sp. and <i>Gracilaria</i> sp.)	Ability to form strong gels and gums, films are transparent, heat-sealable, biologically inert, brittle, thermally unstable, water sensitive (high water vapor permeability)	(Mostafavi & Zaeim, 2020)
Carrageenan	<i>Chondrus crispus</i> and other species of red seaweeds	Water-soluble, forms pseudoplastic gels, κ -carrageenan has the best film-forming ability	(Roy et al., 2019)
Natural biopolymers—Proteins			
Soy protein	Soybeans, soy flakes	Insoluble in water, soluble in acid or alkaline medium, flexible films with remarkable gas barrier properties, acidic films display better antimicrobial activity but weaker mechanical properties than films prepared in alkaline medium	(Emiroglu et al., 2010; Z. Yu, Dhital, et al., 2019)
Zein	Corn protein—major coproduct of oil and bioethanol industries	Good film-forming properties, good barrier properties, high compatibility with biopreservatives, ability to form blends with lipids and fatty acids, insolubility in water, brittleness, problems with mass production	(Spasojević et al., 2019; Yemenicioğlu, 2016)

(Continues)

TABLE 1 (Continued)

Biopolymer	Source for production	Properties	References
Wheat gluten	Wheat flour	Hydrophobic nature, insoluble in water, can be dissolved in aqueous solutions of high or low pH at low ionic strength, Films are clear with shiny surface and show good resistance to water	(Kurek et al., 2014)
Gelatin	Partial hydrolysis of collagen; pig skin, bovine hide, pork and cattle bones, fish skin	Good gas barrier property, light absorption, high moisture sensitivity, low mechanical strength, low process ability	(Kadam et al., 2015; S. Shankar et al., 2016)
Collagen	Pork and cattle bone, pig skin, fish skin	The most exploited raw material for biopolymer packaging, flexible polymer with complex structure, films display excellent oxygen barrier at 0% RH, barrier decreases with increase of RH	(Popović et al., 2018)
Whey protein	Milk—coproduct of cheese production (remain after the coagulation of caseinate)	Transparent, odorless, and tasteless films with high moisture sensitivity (water solubility) and good barrier properties to oxygen and lipids	(del Carmen Beristain-Bauza et al., 2017)
Casein	Skim milk	Films obtained from aqueous solutions, opaque films with excellent sensory properties, high water vapor permeability	(Ponce et al., 2016)
Chemically synthesized biopolymers			
PLA		Large availability, high transparency, processability and rate of composability, brittleness and low thermal and gas barrier properties	(Radusin et al., 2019; Requena et al., 2018)
PCL		Hydrophobic and semicrystalline polyester, films display low tensile strength and high elongation at break	(Ahmed et al., 2019)
PBS		High processability, low mechanical strength, and microbial corrosion	(Petchwattana et al., 2016)
PVA		Excellent physical and optical properties, excellent film-forming, emulsifying, and adhesive properties, low barrier performance, low biodegradation rate, high production costs	(de Oliveira et al., 2020; Jayakumar et al., 2019; Qin et al., 2020)

Note: Studies presented are from the past 10 years.

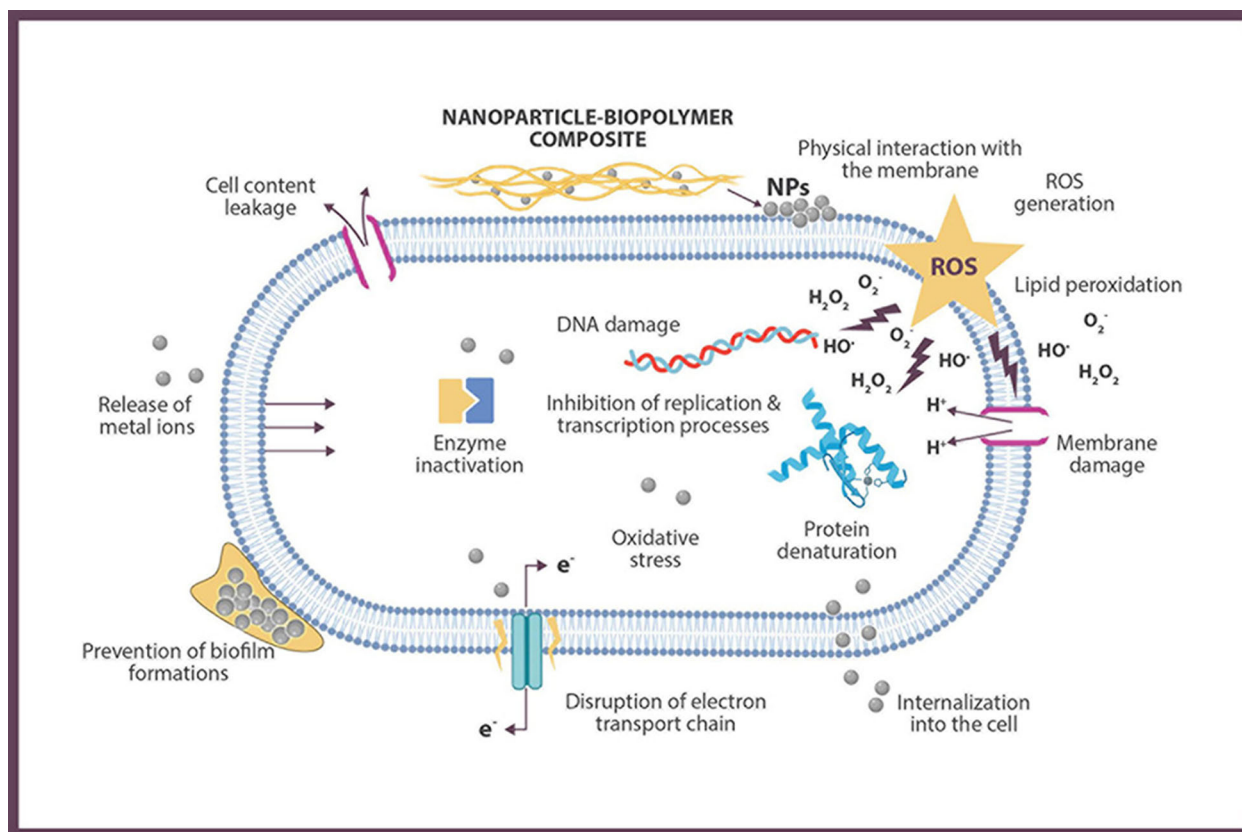


FIGURE 2 Various antibacterial mechanisms of nanoparticles used as inforcers in food packaging

and active components is smart packaging (Drago et al., 2020; Vanderroost et al., 2014).

3.1 | Metal and metal oxide NPs as antibacterial nanofillers

Metal and metal-oxide NPs exhibit different antibacterial efficiencies toward Gram-positive and Gram-negative bacteria, and may even have different effects on the bacterial strains within the same Gram-positive or Gram-negative group (Auger et al., 2018; Cavassin et al., 2015; Zanet et al., 2019). This effect originates probably from the presence of various membrane components in different bacteria that are the first barrier and the first target for NPs. For instance, lipoproteins and phospholipids are found on the surface of Gram-negative bacteria, while the surface of Gram-positive bacteria contains abundant pores and is of a much higher negative surface charge (Sánchez-López et al., 2020; L. Wang et al., 2017). Reactive oxygen species (ROS)-induced oxidative stress is one of the most prominent mechanisms associated with the antimicrobial action of metal- and metal oxide NPs. Four types of ROS are mostly produced: superoxide radical, hydroxide radical, hydrogen peroxide, and singlet oxygen. The

hyperproduction of one or several ROS can be induced by different NPs. For instance, CaO and MgO NPs generate superoxide radicals, ZnO NPs mainly generate hydrogen peroxide, whereas CuO NPs can produce all those ROS. The formation of hydrogen peroxide and hydroxyl radicals occurs with Ag NPs (Dakal et al., 2016; Durán et al., 2016). Increased ROS production leads to oxidative stress, impairment of biomolecules and membrane integrity, enzyme inhibition, and nucleic acid damage. ROS can also form holes within the bacterial membrane, causing the contents of the cell to leak (Gold et al., 2018).

3.1.1 | Silver NP-biopolymers

Ag NPs are the most commonly used nanofiller to endow the composite films with antimicrobial properties (Shiv Shankar & Rhim, 2015; Suppakul et al., 2003). The US Food and Drug Administration (FDA) authorized direct addition of Ag salts up to 17 $\mu\text{g}/\text{kg}$ as a disinfectant in bottled water (Duncan, 2011). The antibacterial effect of Ag NPs is achieved through the adhesion of Ag NPs to the bacterial membrane and/or penetration inside bacterial cells. Ag NPs may interact with membrane lipids or with DNA and proteins inside the cells (Yun'an Qing et al., 2018). Ag

TABLE 2 Examples of active packaging containing nanofillers

Composites		Antimicrobial activity toward:	Application	References
Nanoparticle	Biopolymer			
Ag	Nanostructured starch	<i>S. aureus</i> , <i>E. coli</i> , and <i>C. albicans</i>	Active food packaging	(Abreu et al., 2015)
Ag	PVA	<i>S. aureus</i> and <i>E. coli</i>	Active food packaging	(Sarwar et al., 2018)
Ag	Chitosan	<i>E. coli</i> , <i>S. aureus</i> , <i>A. niger</i> , and <i>P. citrinum</i>	Active food packaging for litchi fruits	(Wu et al., 2018)
Ag	Cellulose nanofibrils	<i>E. coli</i> and <i>L. monocytogenes</i>	Active food packaging	(Z. Yu, Wang, et al., 2019)
Ag	Agar	<i>E. coli</i> and <i>L. monocytogenes</i>	UV-screening and active food packaging	(Shiv Shankar & Rhim, 2015)
Ag	PVA	<i>S. Typhimurium</i> and <i>S. aureus</i>	Active food packaging for chicken meat	(Mathew et al., 2019)
Ag	Carrageenan	<i>E. coli</i> and <i>L. monocytogenes</i>	Active food packaging	(Roy et al., 2019)
Ag	Chitosan	<i>E. coli</i> , <i>Salmonella</i> , <i>S. aureus</i> , and <i>L. monocytogenes</i>	Active and intelligent food packaging	(Qin et al., 2019)
Au	cellulose/keratin	<i>S. aureus</i> (MRSA) and <i>Enterococcus</i> (VRE)	Active food packaging	(Tran et al., 2018)
Au	PVA	<i>E. coli</i>	Active food packaging for banana	(Chowdhury et al., 2020)
Au–Ag	Cellulose	<i>E. coli</i>	Active food packaging	(Tsai et al., 2017)
Ag–Cu	Agar	<i>L. monocytogenes</i> and <i>S. Typhimurium</i>	UV-screening and active food packaging	(Arfat, Ahmed, & Jacob, 2017)
Ag–Cu	Fish skin gelatin	<i>L. monocytogenes</i> and <i>S. Typhimurium</i>	UV-screening and active food packaging	(Arfat et al., 2017)
Ag–Cu	PLA	<i>S. Typhimurium</i> , <i>L. monocytogenes</i> , and <i>C. jejuni</i>	UV-screening and active food packaging for chicken meat	(Ahmed et al., 2018)
Se–Ag	Gelatin/furcellaran	<i>E. coli</i> , <i>S. aureus</i> , and <i>S. aureus</i>	Active food packaging for kiwi fruits	(Jamróz et al., 2019)
CuS	Agar	<i>E. coli</i> and <i>L. monocytogenes</i>	Active food packaging	(Roy & Rhim, 2020)
S	Chitosan	<i>E. coli</i> and <i>L. monocytogenes</i>	Active food packaging	(Shiv Shankar & Rhim, 2018)
Nanocellulose	Chitosan	<i>S. aureus</i> , <i>E. coli</i> , and <i>S. enteritidis</i>	Active food packaging for ground meat	(Dehnad et al., 2014)
Nanocellulose	Bagasse paper	<i>B. cereus</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>S. Typhimurium</i> , and <i>S. aureus</i>	Paper-based active food packaging	(El-Samahy et al., 2017)
CuO	Agar, alginate, carrageenan, chitosan, and CMC	<i>L. monocytogenes</i> and <i>E. coli</i> (O157:H7)	UV-screening and active food packaging	(Shiv Shankar et al., 2017)
ZnO	Ethyl cellulose/gelatin nanofibers	<i>E. coli</i> and <i>S. aureus</i>	Active food packaging	(Yuyu Liu et al., 2018)
ZnO	Corn starch, Guanidine-based starch	<i>E. coli</i>	Green-based active food packaging	(Ni et al., 2018)
ZnO	Soy protein isolate	<i>A. niger</i>	Active food packaging	(J. Wu et al., 2019)

(Continues)

TABLE 2 (Continued)

Composites		Antimicrobial activity toward:	Application	References
Nanoparticle	Biopolymer			
TiO ₂	Wheat gluten and cellulose nanocrystals	<i>S. cerevisiae</i> , <i>E. coli</i> , and <i>S. aureus</i>	Active food packaging	(El-Wakil et al., 2015)
TiO ₂	Whey protein isolate /cellulose nanofiber	<i>Pseudomonas</i> spp., Enterobacteriaceae, lactic bacteria, <i>S. aureus</i> , <i>L. monocytogenes</i> , and <i>E. coli</i>	Active food packaging for meat preservation	(Sani et al., 2017)
TiO ₂	Zein/sodium alginate	<i>S. aureus</i> and <i>E. coli</i>	Active packaging for meat, cheese, nuts, cereal	(Amjadi et al., 2020)
ZnO	PBS	<i>E. coli</i> and <i>S. aureus</i>	Active food packaging	(Petchwattana et al., 2016)
ZnO	Poly lactide/polyethylene glycol/ polycaprolactone	<i>S. aureus</i> and <i>E. coli</i>	Active food packaging for eggs	(Ahmed et al., 2019)
MgO	PLA	<i>E. coli</i>	UV-screening and active food packaging	(Swaroop & Shukla, 2018)
Zn-MgO	Alginate	<i>L. monocytogenes</i>	Active food packaging for smoked salmon meat	(Vizzini et al., 2020)
TiO ₂	Chitosan/PVA	<i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> (O157:H7), and <i>Candida albicans</i>	Active food packaging for soft white cheese	(Youssef et al., 2015)
Carbon dots	Bacterial nanocellulose	<i>L. monocytogenes</i> and <i>E. coli</i>	UV-screening and forgery-proof packaging	(Kousheh et al., 2020)
Iron-oxide coated GO	Chitosan hydrogel	MRSA, <i>S. aureus</i> , <i>E. coli</i> , and <i>C. albicans</i>	Biomedical and active food packaging applications	(Konwar et al., 2016)
MWCNTs	Chitosan/PLA	<i>E. coli</i> , <i>S. aureus</i> , <i>B. cinerea</i> , and <i>Rhizopus</i>	Active food packaging for fruit and vegetable	(Yaowen Liu et al., 2019)
SiO ₂ (silica)	Chitosan	<i>E. coli</i> , <i>S. Typhimurium</i> , <i>S. aureus</i> , <i>L. monocytogenes</i>	Active food packaging	(Bi et al., 2020)
MSN (SBA-15)	Chitosan	<i>S. aureus</i> , <i>E. coli</i>	Active food packaging	(C. Wu et al., 2019)
MSN	Potato starch	<i>Mucor</i> sp., <i>Mucor circinelloides</i>	Active food packaging for mushrooms	(Zhang et al., 2019)
MSN and amino-MSN	Bitter vetch proteins	<i>Micrococcus luteus</i>	Active food packaging	(Fernandez-Bats et al., 2018)
SiO ₂ and SiO ₂ -HDTMS/SPH	Polyhydroxy butyrate/poly-ε-caprolactone	<i>E. coli</i> (O157:H7) and <i>S. aureus</i>	Active food packaging and biomedical materials	(Lin et al., 2018)
MSN (MCM-41)	PHBV	<i>S. aureus</i> and <i>E. coli</i>	Interlayers or coatings for active food packaging	(Melendez-Rodriguez et al., 2019)

Note: Studies presented are from the past 10 years.

NPs were also shown to downregulate the expression of antioxidant enzymes such as glutathione, superoxide dismutase, and catalase depriving the bacterial cell of defense against oxidative stress (Yuan et al., 2017). Another mode of action involves the release of highly toxic silver ions that bind protein sulfhydryl groups or intercalate within the DNA chain. Moreover, Ag⁺-ions released by Ag NPs can

generate a high concentration of ROS and interact with bacterial proteins from the respiratory chain causing cell death (Long et al., 2017). Unfortunately, Ag NPs may also kill human cells as shown in vitro (Carrola et al., 2016; Jiang et al., 2018; Kim & Shin, 2014; L. Li et al., 2018; Sabella et al., 2014). In active packaging, Ag NPs are immobilized into polymeric materials, which allows controlled release

of Ag⁺-ions suppressing in this way their cytotoxicity. The migration of silver from the packaging film to food may occur through the detachment of Ag NPs from the composites or the oxidative dissolution of silver ions. It was shown that the highest level of silver migrating from various nanocomposites into food simulants occurred with acidic food (Echegoyen & Nerín, 2013). Moreover, food heating, and especially microwave heating, induces migration of toxic Ag/Ag⁺ from the packaging film to the foodstuff. Cushen et al. (2014) developed a model of Ag NPs migration from food packaging. Using modeling to predict the level of migration of Ag NPs is highly beneficial to the industry because it could reduce time and costs usually associated with the migration studies.

The addition of Ag NPs may also improve the mechanical properties of packaging films. Abreu et al. (2015) applied a solution casting method to prepare nanostructured starch films containing organically modified nanoclay and Ag NPs. They demonstrated that Ag NPs improved the clay dispersion, contributing to higher homogeneity of films and improving their mechanical properties. The presence of Ag NPs reduced water vapor and oxygen permeability, providing excellent barrier properties. Moreover, the nanocomposite showed antimicrobial activity against *Staphylococcus aureus*, *E. coli*, and *Candida albicans*. A composite film prepared of cellulose nanofibrils impregnated with Ag NPs through casting and evaporating at room temperature showed strong inhibition effectiveness against *E. coli* and *Listeria monocytogenes* and no significant cytotoxicity to human epithelial cell or colon cell lines (Z. Yu et al., 2019). Similarly, PVA packaging film reinforced with nanocellulose and starch-capped Ag NPs showed no cytotoxic effect on the hepatocyte function (Sarwar et al., 2018). The authors suggested that starch served as a protective layer that prevented the undesired release of silver ions from capped NPs. Besides contributing to antimicrobial properties of films that efficiently inhibited the growth of *E. coli* and *S. aureus*, Ag NPs in a PVA film enhanced its thermal stability and mechanical properties, such as breaking stress and elongation at break. Moreover, the PVA/AgNPs film had low water vapor permeability and high moisture retention due to glycerol hydroxyl groups' affinity toward water molecules (Sarwar et al., 2018).

A green synthesis method is generally used for preparing Ag NPs for food packaging applications. Wu et al. (2018) synthesized laponite-immobilized silver NPs by reactive template grain growth method using quaternized chitosan as a green reducing agent and stabilizer. Prepared Ag NPs and laponite improved chitosan films for the wrapping of litchi fruits, by increasing the film density, improving its mechanical properties and making it more uniform and homogenous. The tripartite interactions between NPs, chitosan, and laponite were proposed as a crucial

factor in reducing the water vapor permeability, oxygen transmission rate, solubility, swelling of the films, and in increasing the film's antimicrobial activity against *E. coli*, *S. aureus*, *Aspergillus niger*, and *Penicillium citrinum*. The film enabled to extend the shelf life of litchi fruit from 4 to 7 days. Melanin, as a reducing agent, enables green synthesis and capping of Ag NPs within the carrageenan film (Roy et al., 2019). In addition, melanin, as a well-known ultraviolet (UV)-blocking agent, enhanced the UV-barrier property of the films. Finally, carrageenan/Ag NPs packaging film notably reduced the growth of *E. coli* and *L. monocytogenes*.

In the polymer industry, enormous efforts have been made to produce environmentally friendly materials for food packaging. Biodegradability of PVA-based nanocomposites can be improved through the green synthesis method using a natural extract as a plasticizer. Mathew et al. (2019) applied ginger extract having a reductant activity to improve the plasticity of PVA and to partake in the in situ synthesis of Ag NPs. This nanocomposite was degraded entirely under simulated environmental conditions after 100 days. In addition, the mechanical strength of the prepared films was greatly enhanced, suggesting high compatibility between the nanofillers and PVA. The prepared films show superior inhibition on *S. Typhimurium* and *S. aureus* growth. Qin et al. (2019) developed a chitosan bio-nanocomposite with potential for use in active food packaging. Multifunctionality was enabled via incorporation of Ag NPs, acting as antimicrobial agents, and anthocyanin-rich purple corn extract, functioning as an antioxidant and pH indicator (Figure 3). Silver NPs, produced through the green synthesis, and oxygen groups of chitosan interact through coordination providing a strong synergic effect that decreased UV-vis light transmission, water vapor permeability, and the moisture content of the film. Improvement of tensile strength was ascribed to the reduction of the mobility of polymer chains and flexibility of the matrix, caused by Ag NPs embedding into the chitosan films. Films showed color changes at pH 3 to 10 due to the structural transformations of anthocyanin, the phenomenon that could be utilized for food freshness monitoring (Qin et al., 2019).

Ag NPs are also used in combination with other NPs to further improve packaging film characteristics. The synergism was observed between selenium and silver NPs where selenium NPs enhance water resistance and antimicrobial activity, while Ag NPs improve the mechanical properties of furcellaran/gelatin films (Jamróz et al., 2018, 2019). Such films were tested for the preservation of kiwi fruit. The addition of Se-AgNPs into furcellaran/gelatin matrix resulted in denser and more compact films, which were less soluble in water. The kiwi fruits had shelf life prolongation of 2 days when protected with

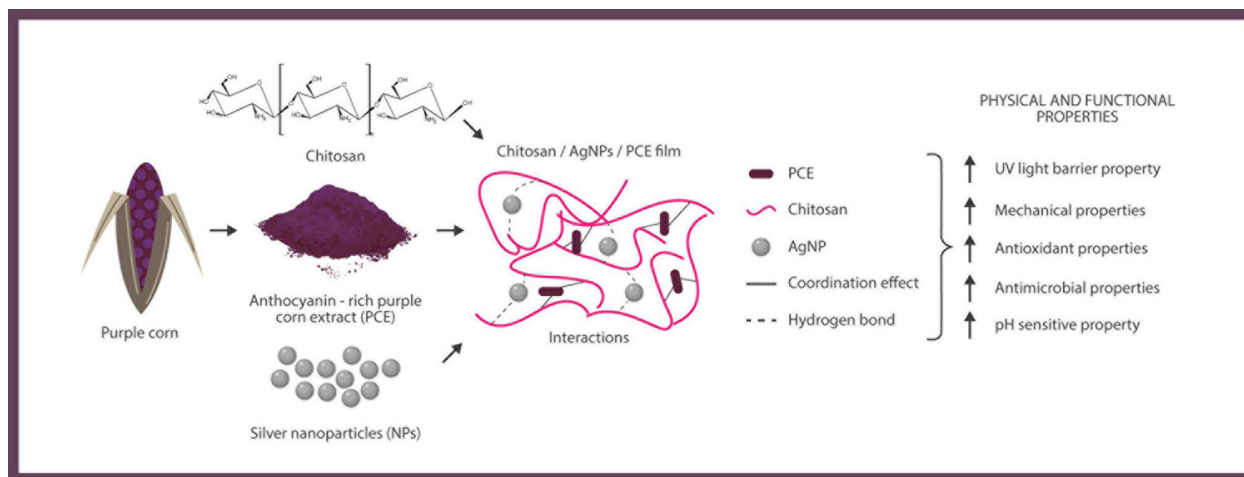


FIGURE 3 Active packaging obtained by incorporation of silver nanoparticles and purple corn extract (PCE) into chitosan films improved the physical properties of chitosan films and increased antioxidant and antimicrobial properties

Note: Illustration adapted from Qin et al. (2019).

furcellaran/gelatin/Se-AgNPs film, which was attributed to the antimicrobial effectiveness of incorporated NPs.

3.1.2 | Gold NP-biopolymers

An antibacterial packaging incorporating gold NPs was also elaborated. In contrast to most antibacterial nanomaterials that generate cytotoxic ROS, the gold NPs kill the bacterial cells via ROS-independent mechanisms. Furthermore, the antibacterial action of Au NPs was shown to largely depend on particle size, functionalization, and the bacterial strain to which they are applied (Ortiz-Benítez et al., 2019). Nano-gold particles either bind to the bacterial membrane and change its potential or internalize into the cells where they decrease adenosine triphosphate levels or inhibit tRNA binding to the ribosome (Cui et al., 2012). In contact with *E. coli* and *S. aureus*, Au NPs were shown to damage bacterial membrane (Badwaik et al., 2012; Rai et al., 2010).

When PVA films were cross-linked with glyoxal and reinforced with Au NPs as nanofillers, the composites had improved hydrophobicity, mechanical and barrier properties (Chowdhury et al., 2020). Intermolecular cross-linking contributed towards formation of a packed pores structure that decreased water vapor transmission rate and increased water and thermal stability of the film. The nanocomposite showed effective antimicrobial activity against *E. coli* because Au NPs created holes in the cell walls and inactivated bacterial enzymes through blocking of their thiol groups. The material demonstrated superior capability for the preservation of wrapped organic bananas compared to PVA film cross-linked with glutaraldehyde and graphene oxide (GO; Figure 4).

Au NP is a promising enhancer because of its safety and the simple method of post-deposition onto the cellulose paper. Au NPs may also enhance the silver coating of a cellulose film (Tsai et al., 2017). Excellent antibacterial activity of the silver coated cellulose paper was enhanced by Au NPs against *E. coli* (Tsai et al., 2017). An *in vitro* size-dependent cytotoxicity study suggested that Au NPs of a diameter ≥ 15 nm can be considered harmless, while smaller ones may cause mammalian cell necrosis (Yah, 2013).

3.1.3 | Copper and copper oxide NPs

Copper oxide NPs (CuO NPs) were demonstrated to reinforce mechanical (such as tensile strength and elastic modulus) and antibacterial properties of carbohydrate polymers. The effect of CuO NPs addition to carbohydrate biopolymers was tested using agar, alginate, carrageenan, chitosan, and carboxymethyl cellulose (Shiv Shankar et al., 2017). All obtained CuO NP-composite films showed homogeneous, uniform, compact, and smooth surface structure except the agar/CuO NP—nanocomposite film that exhibited agglomerates and clusters of CuO NPs. Only agar/CuO nanocomposite films did not show antimicrobial activity indicating that the available surface area of the NPs was low. Interestingly, incorporation of Cu NPs into the agar polymer, did not significantly improve the mechanical properties nor the thickness of the films.

CuNPs in active packaging were also used in combination with AgNPs to obtain the synergic effect of two metals. Ahmed et al. (2018) prepared a PLA nanocomposite loaded with bimetal Ag-CuNPs and cinnamon essential

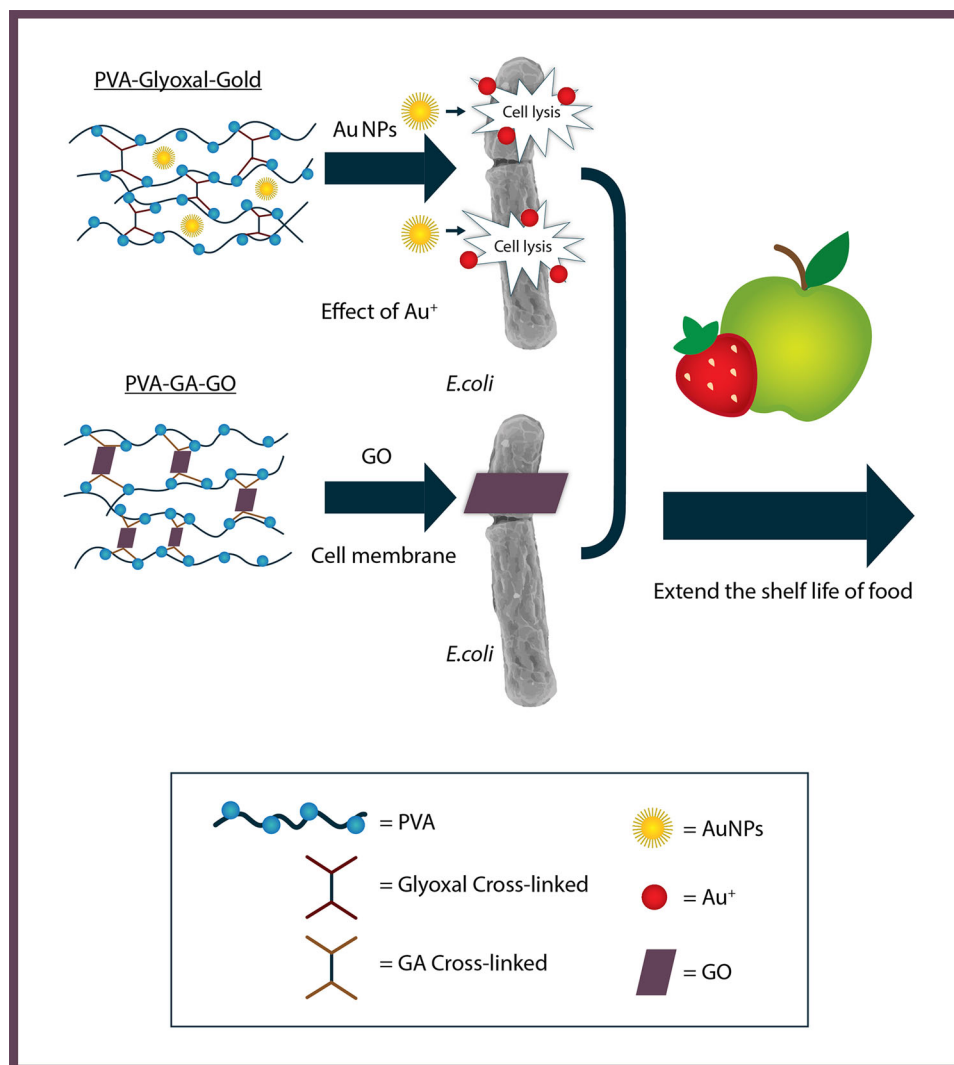


FIGURE 4 The structure of PVA-based composite films containing gold nanoparticles (AuNPs), glutaraldehyde (GA) as a cross linking agent and graphene oxide (GO), representation of their antimicrobial mechanisms and application for extending shelf life of fruits. Illustration adapted from (Chowdhury et al., 2020).

oil (CEO) through the compression molding process. The obtained Ag-CuNPs/PLA films had improved barrier properties, a decreased light transmittance, modified morphology, and thermal stability compared to the pure PLA films. Storage tests demonstrated that such films became more rigid over time due to the evaporation of the CEO. The antimicrobial effectiveness of films was confirmed against *Salmonella* Typhimurium, *L. monocytogenes*, and *Campylobacter jejuni* in chicken meat as a model food system, during 21 days of refrigeration. The antimicrobial activity decreases after 150 days of storage at 4 °C, because of the loss of the CEO component that restricts the release of NPs. The bimetallic Ag-Cu NPs incorporated into agar-based film via a solution casting method and plasticized with glycerol showed also a high antimicrobial activity (Arfat et al., 2017). The thickness of the film, its tensile strength, thermal stability, oxygen-barrier, and light transmittance

were all shown to be dependent on the concentration of NPs.

Cu/CuO NPs in active films inhibit bacterial growth by producing highly toxic ROS or releasing Cu²⁺-ions. Cu NPs accumulate on the bacterial membrane, decrease transmembrane electrochemical potential, and disrupt bacterial cell wall (Din et al., 2017). The ruptured bacterial membrane facilitates entry of the NPs into the cell, but also induces leakage of the cell content into the extracellular space. Released Cu²⁺-ions induce ROS-induced oxidative stress, which produces oxidative damage to DNA and proteins. In addition, Cu²⁺ can interact directly with the bacterial proteins and denature them. Cu²⁺ is estimated to interact with sulfhydryl groups that are necessary for the activity of many essential enzymes, like phosphatases and kinases (Makvandi, Wang, et al., 2020). Similar toxic mechanisms of Cu/CuO NPs toward human cells were

also shown (Katsumiti et al., 2018). Practical applications of Cu/CuO NP-biopolymer composites strongly depend on the stabilization of NPs within films that control the release of Cu²⁺-ions thus preventing cytotoxicity to human cells.

3.1.4 | Titanium dioxide NPs

TiO₂ particles are commonly used as a food additive to provide a whitening effect that makes the foodstuffs look brighter and more appealing (Ropers et al., 2017). In food packaging, TiO₂ NPs are used for their wide range of antimicrobial properties (Makvandi, Gu, et al., 2020; Stankic et al., 2016). Until recently, TiO₂ NPs were considered as safe. However, the European Food Safety Agency (EFSA) revealed some concerns due to the ability of TiO₂ NPs to alter the intestinal barrier, which pushed some EU countries to ban TiO₂ NPs in food (Boutillier et al., 2020). Numerous reports confirmed the toxic effects of TiO₂ NPs on human cells (Acar et al., 2015; Coccini et al., 2015; Radziwill-Bienkowska et al., 2018). In vivo tests revealed that upon inhalation or oral exposure, TiO₂ NPs accumulate in lungs, digestive tract, liver, heart, spleen, kidneys, and heart (Baranowska-Wójcik et al., 2020; Shukla et al., 2013; Song et al., 2016). However, nanosized TiO₂ is still among the few approved nanomaterials used by the food industry.

The main mechanism of antimicrobial activity of TiO₂ NPs involves ROS production under exposure to UV light (Makvandi, Wang, et al., 2020). This photocatalytic mechanism involves: (i) electron-hole pairs production and migration to the TiO₂ NP surface, (ii) interaction of photo-generated holes with adsorbed H₂O/OH⁻ leading to the generation of hydroxyl radicals, (iii) electron binding to empty oxygen portions and superoxide formation.

TiO₂ NPs incorporated into the wheat gluten-based biopolymer ensured antimicrobial properties of the food packaging (El-Wakil et al., 2015). Wheat gluten was chosen as a plant protein because it is inexpensive and shows good film-forming properties. Advanced nanocomposite film was fabricated by incorporation of glycerol, cellulose nanocrystals, and TiO₂ NPs to the wheat gluten by casting/evaporation. Obtained films showed enhanced mechanical properties in terms of increased tensile strength and decrease of elongation at break, and low water vapor permeability and water vapor uptake due to the increased surface hydrophobicity. The addition of TiO₂ NPs was shown to further prevent water absorption. Kraft paper coated with three layers of this nanocomposite excelled at antimicrobial activities against *S. cerevisiae*, *E. coli*, and *S. aureus* after UVA light irradiation. In another study, packaging films composed of whey protein

isolate/cellulose nanofiber nanocomposite with TiO₂ NPs and rosemary essential oil demonstrated antimicrobial activity against *Pseudomonas* spp., Enterobacteriaceae, *S. aureus*, *L. monocytogenes*, *E. coli*, and lactic bacteria, in inoculated lamb meat (Sani et al., 2017). The shelf-life of meat samples packed with this biodegradable film was extended up to 6 days compared to controls. Besides ensuring the microbial quality of food, the TiO₂-biopolymer composite preserved the sensory quality of the meat samples.

Recently, novel zein/sodium/TiO₂NPs-alginate nanobiocomposite fibers for food packaging applications were obtained using the electrospinning technique (Amjadi et al., 2020). Even though the pure zein polymer is flexible, tough, compressible, hydrophobic, and safe, it has weak mechanical properties. Carbohydrates were added to reinforce the mechanical properties of zein fibers while sodium alginate facilitated its electrospinning and the addition of TiO₂ NPs improved mechanical and thermal properties as well as gas and water barrier properties and provided high antibacterial activity. The antibacterial activity against *S. aureus* and *E. coli* was even further enhanced by incorporating natural pigments, such as betanin. The obtained nano-biocomposite complex showed good biocompatibility and homogeneous morphology despite its multicomponent structure. TiO₂ improved the properties of the fabricated nanofibers by forming hydrogen bonds with zein and producing highly regular and compact polymer chains.

Fresh white cheese prepared from buffalo milk is characterized by rapid spoilage under aerobic conditions. It deteriorates usually in less than 7 days when packed using traditional petrochemical plastics. To provide prolonged storage and shelf life of buffalo milk cheese a chitosan/PVA/TiO₂NPs nanocomposite was elaborated (Youssef et al., 2015). Mechanical properties, such as tensile strength and elongation at break, were significantly improved by the addition of TiO₂. This was associated with a uniform dispersion of NPs providing stable stress distribution and diminishing the formation of stress concentration centers. In addition, the TiO₂NPs incorporation increased the hydrophobicity of the film, improving its protective effect against moisture during the storage period. The chitosan/PVA/TiO₂ films exhibited good antibacterial activity against *S. aureus*, *Pseudomonas aeruginosa*, and *E. coli*, as well as good antifungal properties against *C. albicans*, thus enabled prolonged cheese shelf life.

3.1.5 | Zinc oxide NPs

ZnO is classified as an essential micronutrient for human and animal health and considered as GRAS (generally recognized as safe) by the FDA. ZnO is used by food industries

as a nutritional supplement and as a food additive due to its white appearance (Auger et al., 2019; Jeon et al., 2020; Z. L. Wang, 2004). In nanosized form, ZnO is used for food packaging because its presence in the polymeric matrix provides improved mechanical strength, barrier properties, and material stability together with high antibacterial and antifungal activities. The dynamic role of ZnO NPs in food preservation entails the production of ROS, especially hydrogen-peroxide in the presence of light (Auger et al., 2019; Vidic et al., 2013). Their antibacterial efficiency was shown for a broad range of microorganisms such as *B. subtilis*, *S. aureus*, *E. coli*, *Salmonella*, *P. aeruginosa*, *C. jejuni* (Auger et al., 2019; Stankic et al., 2016; Vidic et al., 2013; Zanet et al., 2019). Indeed, a greater sensitivity to H₂O₂ was observed in bacteria that were more sensitive to ZnO NPs (d'Água et al., 2018). In contrast, research conducted on *S. aureus* indicated the independence of antimicrobial activity of ZnO NPs from increased production of ROS and pointed to an alternative antibacterial mechanism affecting the energy metabolism of the cell and amino acid biosynthesis pathway (Kadiyala et al., 2018). ZnO NPs may also release cytotoxic Zn²⁺-ions upon their partial dissolving (Vidic et al., 2014). Namely, ZnO NPs can release Zn²⁺-ions intracellularly that disturb bacterial redox potential (Randazzo et al., 2020). Seray et al. (2020) described poly(butylene adipate-co-terephthalate)/ZnO NPs films. The NP embedding into the polymer assured the controlled release of Zn²⁺-ions, resulting in the safe application of the film.

Several novel nano-biocomposites combining ZnO NPs and essential oils have been developed for food packaging in the last few years. C. Wu et al. (2019) used protein isolate-based biocomposite films, synthesized through a liquid precipitation method that incorporated both plant-sourced cinnamaldehyde and hexagonal wurtzite ZnO NPs. The aim was to obtain an antibacterial and antifungal material harnessing the synergistic effect of ZnO NPs and cinnamaldehyde. ZnO NPs improved mechanical characteristics and oxygen and water vapor barrier properties of the biopolymer. The composite films exhibited homogeneous, uniform, compact, and nonporous surfaces. Interestingly, both cinnamaldehyde and ZnO NPs prevent light transmission, providing a beneficial feature of these films because the oxidative deterioration of food was diminished along with an extension of the food shelf life. The antifungal activity of the film against *Aspergillus niger* was increased in comparison to the pristine film. In another study, a polylactide/polyethylene glycol/polycaprolactone blend with incorporated ZnO NPs and clove essential oil in the form of a film was developed using the solution casting technique (Ahmed et al., 2019). Addition of ZnO NPs significantly improved tensile strength, but decreased elongation at the break of the nano-biopolymer film compared to

the pure form. ZnO NPs also decreased water vapor permeability due to the formation of tortuous paths that hinder the diffusion of water across the composite films. ZnO NPs, together with the clove essential oil, made the biopolymer chains more flexible, yielding the film that contains small pits. These micropores significantly increased the surface of the film. The authors suggested that such increased surface facilitated antimicrobial activity. Indeed, the high antimicrobial efficacy of the film was found against *S. aureus* and *E. coli* inoculated in scrambled eggs during 21 days of storage at 4 °C.

Antimicrobial composites were also synthesized based on ZnO NPs imbedded into biodegradable polymers like polybutylene succinate (PBS) resin (Petchwattana et al., 2016), or corn starch-based carboxymethyl cellulose (Ni et al., 2018). Physicochemical tests of the composite confirmed the intermolecular interactions between ZnO and starch and their excellent compatibility. Grain boundaries of ZnO form an electrostatic field that triggers water-repulsion, ensuring a good water vapor barrier and resistance to solvents upon soaking. The nano-biocomposite films were homogeneous and continuous and showed good thermal stability. Antibacterial activity of the constructed films was dependent on the migration of ZnO NPs that was the highest in the acidic environment.

Electrospinning method has been used for the encapsulation of ZnO NPs into biopolymer fibers. For instance, it was applied for addition of ZnO NPs into hydrophobic ethyl cellulose/gelatin nanofibers (Yuyu Liu et al., 2018). The tensile properties (average elastic modulus, tensile strength, and elongation at break) were enhanced in the fibers containing ZnO NPs. The hydrophobic surface property was achieved by decreasing the number of gelatin chains and the reduced N atom content on the nanofiber surface by ZnO NPs. Antibacterial properties observed against *E. coli* and *S. aureus* were dependent on the ZnO concentration and the UV irradiation (Yuyu Liu et al., 2018).

As shown in these examples, ZnO NPs improved mechanical, thermal, antibacterial, and antifungal parameters when incorporated into the packaging material. However, some concerns exist regarding the ZnO NP cytotoxicity and it was observed in in vitro and in vivo studies in animal and human cells (Jeon et al., 2020; McClements & Xiao, 2017). Moreover, the generation of ROS can induce oxidative stress, lipid peroxidation, increase in Ca²⁺-concentration inside the cell, alteration of mitochondrial membrane potential, lysosomal destabilization, inflammatory cytokine release, DNA damage, and cell death. All these mechanisms underlying ZnO NPs cytotoxicity are dose-dependent. In contrast, the biopolymer composites containing ZnO NPs did not exert a toxic effect on the human cells to such extent. Cierech et al. (2019) tested

release and cytotoxicity of ZnO NPs and of poly(methyl methacrylate)-ZnO nanocomposites on HeLa cells. They found out that the ZnO release level was more than three times lower than the concentration of ZnO NPs in the nanocomposite, which suggests the possibility of nanocomposites' safe application in food packaging.

3.1.6 | Magnesium oxide NPs

MgO is a natural mineral produced on large scale and recognized by the FDA as GRAS for food applications. MgO NPs integrated into a biocomposite usually enable fabrication of a highly transparent film. Still, MgO NPs are less used in food packaging applications than TiO₂ or ZnO NPs because of their less pronounced antibacterial activity (Vidic et al., 2013), although MgO NPs generates ROS (Auger et al., 2018, 2019; He et al., 2016; Vidic et al., 2013). An ROS-independent mechanism was also proposed for MgO NPs antibacterial effect on *E. coli* because it was shown that MgO NPs damaged cell membrane in the absence of oxidative stress (Leung et al., 2014). MgO NPs are highly biocompatible as shown in various in vivo and in vitro studies (Mazaheri et al., 2019; Mittag et al., 2019; Vidic et al., 2013).

Using the solvent casting method MgO NPs were incorporated in PLA polymer to optimize the tensile strength, oxygen barrier, and antibacterial properties because neat PLA has certain limitations, such as high moisture affinity, low thermal stability, poor barrier properties (Swaroop & Shukla, 2018). Interestingly, MgO NPs made no chemical bond with PLA, but only interacted physically. Packaging films exhibited no physical defects, while white dots formed of aggregated NPs throughout the samples contributed to surface roughness. Tensile strength of the films increased, while elongation at break and thermal stability significantly decreased, most probably due to the agglomeration of NPs. Moreover, MgONPs/PLA film showed only a moderate antibacterial effect against *E. coli*, but had improved oxygen barrier and increased water vapor permeability. The opposite effect of MgO NPs incorporation in PLA on various gas permeability can be attributed to different polarities of water vapor and oxygen gas.

3.2 | Mesoporous silica NPs as antibacterial nanofillers

High specific surface area (approximately 1000 m²/g) of mesoporous silica NPs (MSN) and their large pore volumes (approximately 1 cm³/g) are of substantial benefit for the construction of efficient antimicrobial composites. A large number of different antimicrobials can be filled inside the

mesopores of MSN while their release rate can be controlled, and hence their antimicrobial activity can be governed through different means. Previous research showcased that MSNs can be employed for loading and delivery of different types of drugs and even large biomolecules, and that the release of cargo molecules from MSNs can be governed by different surface modifications of MSNs (Knežević & Durand, 2015; Knežević et al., 2012). Capabilities for stimuli-responsive drug delivery were also demonstrated upon exposure to light, magnetic field, enzymes, change of environmental conditions (pH value, temperature), which may also be of interest for antimicrobial applications (Knežević & Kaluđerović, 2017; Q. Yu et al., 2020). These attributes highlight MSNs as great candidates for constructing active packaging composites, considering the plethora of possible antimicrobial cargo molecules for loading MSNs, possibilities for versatile functionalization of MSNs and the use of various biopolymers for constructing composite materials with desired attributes.

The aminopropyltriethoxysilane-functionalized surface of MSN (SBA-15 type) was recently utilized for attaching antimicrobial molecule vanillin through a reversible imine linker and its slow release from the composite with PCL was demonstrated, in an effort to develop active responsive packaging materials (Stanzione et al., 2017). A bio-nanocomposite film with pH-controlled release function and antimicrobial activity was developed using composites containing chitosan and curcumin-loaded MSNs (C. Wu et al., 2019). For this, curcumin was first loaded inside the pores of MSNs by gradual evaporation of the solvent and the films were subsequently prepared by the solution casting method. Antimicrobial activity of the films determined by measuring the zone of growth inhibition of *S. aureus* and *E. coli* O157:H7 indicated that the addition of curcumin into the chitosan films improved their antimicrobial activity compared to the pure chitosan films. The chitosan/curcumin blend films showed slightly higher antimicrobial activity than the films with curcumin incorporated into MSN NPs. The release of curcumin was evidently slower in the case of the composites containing MSN, which would be beneficial for achieving prolonged antimicrobial activity of the packaging material. The FTIR analysis of the films revealed the formation of additional hydrogen bonds between SBA-15-Cur and chitosan, which lead to the improved mechanical properties of the films.

Zhang et al. (2019) synthesized packaging films by the casting method, using potato starch and CEO loaded into MSNs. To determine the functionality of the prepared films, their antimicrobial activity against *Mucor* spp. molds was tested. These mold strains are commonly found in white mushrooms during storage. In contrast to pure potato starch and MSNs/potato starch films, MSNs-CEO/potato starch films showed antimicrobial activity

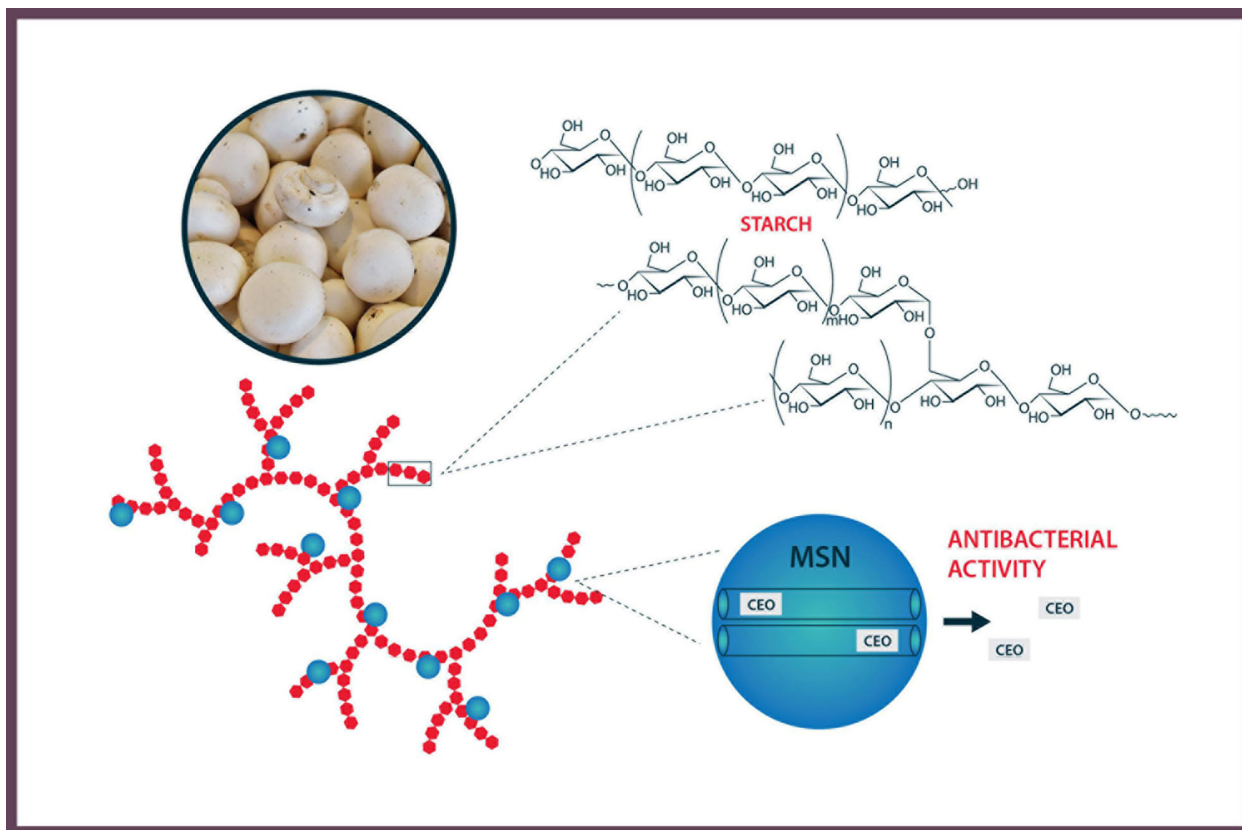


FIGURE 5 Structure of the starch-MSN composite material containing cinnamon essential oil loaded inside the mesopores of MSN for antimicrobial activity against bacteria specific for white mushrooms. Note: Illustration adapted from Zhang et al. (2019).

due to the release of the CEO from MSNs (Figure 5). In addition, the incorporation of MSNs-CEO into potato starch enhanced the tensile strength and thermal stability of the films, due to the intermolecular hydrogen bonding between surface silanols of MSNs-CEO and the biopolymer.

Recently, novel bioactive films were obtained by a solution casting method from the protein concentrate extracted from bitter vetch (*Vicia ervilia*) seeds. The used concentrate was either in the native form, or enzymatically cross-linked by the microbial transglutaminase, (Fernandez-Bats et al., 2018). MSNs and their amino-functionalized derivative were used to reinforce mechanical and barrier properties of protein-based films. Also, bioactive oligopeptide nisin (bacteriocin effective against many Gram positive bacteria) was added to the film-forming solution. Antimicrobial activity of the films was tested by the agar diffusion method using an inoculum of *Micrococcus luteus*. The nanoreinforced bitter vetch proteins themselves exhibited antimicrobial activity, due to their phenolic compounds. The inhibition zone was significantly wider for the films with added nisin. Incorporation of both MSNs and APTES-MSNs improved the film mechanical properties, due to the ionic interaction of their positively

charged amino groups with the negative moieties of bitter vetch proteins. The addition of both types of NPs positively affected the gas and water vapor barrier properties of the films.

The antimicrobial packaging system based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV, and MSN (MCM-41 type) containing eugenol as an antibacterial molecule showed antimicrobial performance against *S. aureus* and *E. coli* for 15 days (Melendez-Rodriguez et al., 2019). After loading eugenol into the NPs, they were incorporated into PHBV by electrospinning. The obtained fibers were annealed below the polymer melting point to produce continuous films. The PHBV film and PHBV/MSN films without eugenol showed no growth inhibition, whereas films with eugenol-containing MSN exhibited remarkably increased antibacterial activity. The results from the tested closed system showed higher antimicrobial activity than that observed in the open system. This was ascribed to the volatile nature of eugenol that accumulated in the system and sustained the release capacity of the films. The results of the mechanical analysis showed that the incorporation of eugenol-loaded MSNs increased the mechanical strength of the PHBV films while the ductility was slightly reduced.

3.3 | Carbonbased NPs as antibacterial nanofillers

Graphene is one-atom thick, two-dimensional nanosheet of graphite having exceptional thermal conductivity, superior mechanical properties, large specific surface area, good electron transfer ability, and good biocompatibility (Pinto et al., 2013). In addition, GO or reduced GO (rGO) can be obtained from graphite upon chemical oxidation or electrochemical exfoliation. In the past few years, graphene-based materials attracted much attention and were used in the food industry to ensure food quality and safety (Sundramoorthy & Gunasekaran, 2014). Graphene-based NPs exert antimicrobial activity by damaging bacterial membrane and causing the leakage of the cell content, by inducing ROS hyper-production or by trapping microorganisms in its aggregated sheets (Shi et al., 2016; Zhu et al., 2017).

Exceptional tensile strength makes graphene a very attractive candidate for development of food packaging materials because its addition to films significantly increases endurance of the material whilst reducing the weight. For instance, multiwalled carbon nanotubes (MWCNTs) incorporated in chitosan/PLA composite via electrospinning gave fibers uniform size, smooth surface, and good nanotube distribution (Yaowen Liu et al., 2019). This material was tested for antimicrobial activity against *E. coli*, *S. aureus*, *B. cinerea*, and *Rhizopus*. Antimicrobial activity was manifested through the decrease of the membrane and cell wall permeability, which caused changes in the internal osmotic pressure. PLA/carbon nanotubes (CNTs)/chitosan composite fibers exhibit beneficial fruit and vegetable packaging properties confirmed by the conducted preservation experiments. Antimicrobial nanopackaging films were also developed by incorporating GO nanosheets and clove essential oil into PLA via solution casting (Arfat et al., 2018). The addition of GO into PLA matrix improved the flexibility of the composite films by lowering glass transition temperature, complex viscosity, oxygen permeability, and porosity. In addition, the developed composite film showed excellent antibacterial activity against *S. aureus* and *E. coli*. All the mentioned features indicate a high potential of graphene and GO for use as active packaging material for food safety and preservation.

Konwar et al. (2016) combined GO and iron oxide in a form of iron oxide coated GO with a chitosan hydrogel structure to develop a novel GO nanocomposite hydrogel for applications in the food industry. The nanocomposite was fabricated using the co-precipitation method, whereas the nanocomposite films were successfully synthesized by gel casting technique. For this, GO was first derived by oxidation and exfoliation of a graphite nanopowder,

followed by an in situ co-precipitation of $\text{Fe}^{3+}/\text{Fe}^{2+}$ ions onto the surface of GO nanosheets. Hydrogel system formation of chitosan–GO was done in the presence of glycerol as a chain extender. Glycerol assists the joining of the chitosan polymeric chains by electrostatic interactions and H-bonding. Obtained chitosan–iron oxide coated nanocomposite enabled fabrication of a compact packing film with a significantly improved thermostability, tensile strength and Young's modulus. Substantial antimicrobial activity of the composite hydrogel films was observed against *S. aureus*, *E. coli*, and *C. albicans*. The possibility of using these novel composite films in food industry applications is strengthened by the in vitro hemolysis potentiality test and the MTT assay, in which their noncytotoxicity was observed.

S. Sharma et al. (2018) used GO as a carrier of salicylaldehyde preservative to develop a packaging that released salicylaldehyde from GO only in the presence of over-ripe fruits. The composite was vacuum-filtered to obtain a robust wrapper for the fruit storage. The overripe fruits produce acid that cleaves the labile hydrazone and triggers the release of salicylaldehyde from GO (Figure 6). The biocomposite improved the fruit shelf life due to the efficient preservative release. In addition, it shows less toxicity as compared to the free preservative.

Carbon dots, as representatives of quantum dots, are another type of carbon nanomaterial used to develop active packaging. Carbon dots possess several superior characteristics, such as simple mass production, low toxicity, antioxidant activity, optical features, photo-stability, and antibacterial properties mostly due to their ultra-small sizes and a surface rich in functional groups. The mechanisms of their antimicrobial activity comprise adhesion of carbon dots to the surface of bacterial cells, followed by photo-induced ROS production and modification of the membrane permeability as well as cell internalization accompanied by intracellular oxidative damage (Al Awak et al., 2017; H. Li et al., 2018; Li et al., 2016; Shi et al., 2016).

Bacterial nanocellulose used by Kousheh et al. (2020) served as a carrier biopolymer for the incorporation of carbon dots. Carbon dots were obtained by a one-step hydrothermal method using the cell-free supernatant of a probiotic bacterium *Lactobacillus acidophilus*. The supernatant was rich in succinamic acid, cyclopentane, 1-cyclododecanone, undecanoic acid, cycloheptane, phenol, and benzoic acid, all of which are suitable for the formation of carbon dots. This protocol uses bacterial biomass that is abundant and represents a green resource for easy development. The as-synthesized carbon dots were incorporated into the bacterial nanocellulose membrane made from *Komagataeibacter xylinus* and proposed as a novel nanopaper with antimicrobial and antioxidant activity that potentially improves the food shelf life. Concerning

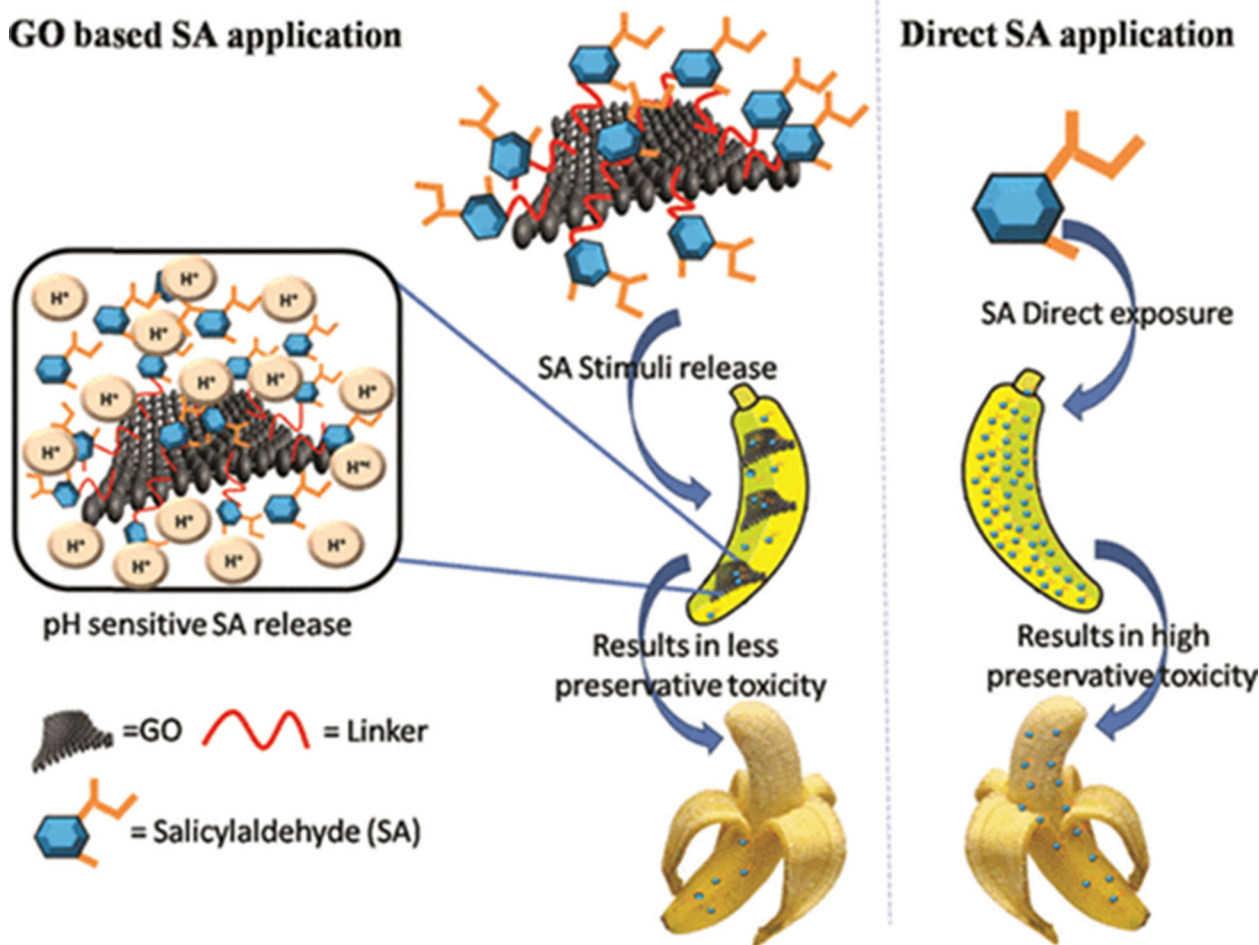


FIGURE 6 Active packaging based on graphene oxide (GO) and salicylaldehyde (SA) developed for preservation of fruits. With permission from (Sharma et al., 2018).

the mechanical properties, the developed nanopaper was flexible and stretchable and had decreased ultimate tensile strength. The presence of blackish carbon dots colored the nanopaper to dark brown color. Under UV light, the nanopaper was fluorescent due to carbon dots, extending this film's possible use to forgery-proof packaging.

For use in food packaging, carbon-based NPs possess other numerous useful properties such as alleviating food waste with improved packaging, forming a selective barrier for moisture and air thereby enhancing food preservation. It can greatly help in reducing the use of plastic and increasing biodegradability and recyclability of packaging.

Nowadays, many novel nanostructured materials are under investigation for application in active food packaging. Most of the research on nano-biocomposites for active food packaging has focused on oxygen scavengers, water absorbers, carbon dioxide or ethanol generators, and antimicrobials (López de Dicastillo et al., 2020; Nikolic et al., 2019; Realini & Marcos, 2014; Suppakul et al., 2003;

Vasiljevic et al., 2020). To be accepted by the food industry and consumers, new packaging materials containing nanofillers should not only exert high efficiency in food preservation, but also should be inert concerning food sensory properties, as well as stable during the storage condition. The major concern on nanofillers comes from their potential toxicity because of the high surface-to-volume ratio that provides a high reactivity. There is a limited number of studies done on the cytotoxicology and migration of nanofillers into food from the packaging film. Thus, the utilization of nanofillers in active packaging films requires deeper consideration of their safety.

4 | REGULATORY AND MARKET STATUS

Food packaging is the third largest industry in the world, amounting to approximately 2% of the gross national product of the developed countries (Robertson, 2016).

Novel approaches, frequently based on nanotechnology, are being extensively developed for use in food packaging in order to achieve sustainable and cost-effective protection of foodstuffs. Such novel technologies require appropriate regulatory and legislative frameworks, which is why governing agencies all over the world intensively work on defining new regulations to encompass the emerging nanotechnology aspects. This global process has been in motion ever since Kraft foods (now Mondelez International) in 2000 started their first nanotech laboratory and the Nanotek consortium made up of 15 universities and national labs, to fund research into personalized or “smart” foods, such as nanotechnology-enabled products that could respond to a person’s nutritional needs (Suran, 2014). Nowadays, the European Union (EU) together with Switzerland has been the only region that included nanospecific definitions in the existing legislation (Amenta et al., 2015), while in the other regions, nanotechnology-based innovations are regulated in a more indirect manner, as shown below by the example of the US regulatory initiatives.

In the EU, regulatory bodies provide joint legislation for materials and articles for active and intelligent packaging. The Regulation (EC) No. 450/2009 establishes a union list of authorized components that strictly interact in a direct manner with the food or with its environment (European Commission, 2009). There is a derogation to this regulation concerning the substances that are separated from the food by a functional barrier (the definition of which is provided in another Regulation (EC) No. 10/2011) and are not transferred to food in a detectable quantity, with a detection limit of 10 mg/kg of food (European Commission, 2011a). This appears to specifically refer to the intelligent packaging materials, which are usually used outside of the primary packaging and are not very likely to be transferred to the foodstuffs. Furthermore, it is important to emphasize that the substances in nanosize, as well as the substances classified as carcinogens, mutagens, or toxic to reproduction, should always be listed in the product description regardless of whether they are used in direct or indirect contact with food, or of their migration behavior (Dainelli, 2015). However, many experts object to this derogation, pointing out that it may cause omission of the necessary listing in the product description for any nonmigrating components, not only those related to packaging. In other words, there is concern that such a relatively vague description of the regulation scope, that is, the components it refers to, may lead to insufficiently detailed product descriptions (Dainelli, 2015).

The procedure for authorization of substances constituting the components of active and intelligent materials and articles and their inclusion in the Community list of authorized substances starts with the application to the

EFSA. The application should be prepared according to the “Guidelines on submission of a dossier for safety evaluation by the EFSA of active or intelligent substances present in active and intelligent materials and articles intended to come into contact with food,” adopted by EFSA on July 21, 2009 (European Food Safety, 2009). After EFSA delivers its opinion, the Commission may adopt it and authorize the use of the component in question, by adding it to the union list. Furthermore, the Regulation (EC) No. 450c2009 (European Commission, 2009) sets guidance about the labeling of products that contain active and intelligent materials, stating that such products are to be labeled appropriately to allow the consumer to identify nonedible parts, for example, by adding the words “DO NOT EAT” to the label. Additionally, the products must clearly show on the label that they are active and/or intelligent.

It is necessary to discuss the regulation concerning antimicrobial packaging as well; taking this into consideration, we present in this review a number of components to be used as such. The regulation for the use of antimicrobial substances in food has always been quite strict in the EU, with a relatively small number of preservatives currently allowed in foodstuff (European Commission, 2011b). These listed substances can be used in antimicrobial packaging, provided there is no other relevant limitation in food legislation. A novel substance with potential antimicrobial effects on packaged food needs to be approved as a food additive. Obtaining approval for a new food additive in the EU is a costly and lengthy procedure, which is why manufacturers usually require very strong market justifications in order to start this process (Dainelli, 2015). This is, in principle, difficult to achieve for a new component, especially for the relatively small EU market.

In addition, the EU has enforced a new REACH regulation as of January 1, 2020, which requires Euro-based companies to provide more information on used nanomaterials (ECHA, 2020). At the same time, EFSA’s inventory of nanomaterials and their applications in the market shows that nanomaterials in contact with food as well as nanoscale food additives represent the most common application of nanomaterials (Peters et al., 2014). These two directions, one being a very strict and lengthy compliance procedure and the other of increased research in nanomaterials for use in food packaging, are currently not well synchronized in the EU. Thus, such a strict compliance process in the EU is not favorable to the manufacturers and distributors who opt instead to market their new active and intelligent nanoscale food packaging products outside the EU.

Other jurisdictions, such as the United States Food and Drugs Administration (FDA), do not specifically address

active and intelligent materials but treat them within conventional legislation for food contact materials (FDA, 2020). The material in the food packaging that is not intended to be added to the food, nor to have a technical effect on the food (so-called “indirect additives”), is regulated like all other food-contact substances (Restuccia et al., 2010). Moreover, the FDA has rather flexible guidance concerning the application of nanotechnology in FDA-approved products (FDA, 2020). These guidelines and regulatory approaches are being determined by the FDA Nanotechnology Task Force, formed in August 2006. This task force encourages regulatory science research in nanotechnology via internal research grants and publishes annual reports (FDA, 2020). Such science-based, product-focused regulatory policy for nanotechnology is also being implemented by another US regulatory body—the United States Department of Agriculture (USDA). The National Institute of Food and Agriculture (NIFA) within USDA manages four grant programs that fund nanotechnology research projects. NIFA also participates on the Nanoscale Science, Engineering, and Technology Subcommittee of the White House National Science and Technology Council and the National Nanotechnology Initiative defined by the 21st Century Nanotechnology Research and Development Act (USDA, 2015).

Nevertheless, all the guidance documents stemming from described initiatives in the United States still only provide recommendations that are not binding. Such flexible regulations are seen as favorable for the industry and manufacturers. The Canadian food packaging regulatory system is similar to the one in the US but it includes concerns of migrating food packaging components, which are not considered to be food additives (Rijk & Veraart, 2010). Other countries, especially Japan and Australia, follow this trend of flexible regulations due to the increasing demand for packaging solutions (for an extensive regulatory status of nanomaterials, see Bumbudsanpharoke & Ko, 2015; Lugani et al. 2021). Iran, Taiwan, and Thailand have introduced systems for tracking and labeling consumer products containing nanomaterials (e.g., NanoMark system), which are, however, substantially different from the labeling requirement in the EU (Amenta et al., 2015). In Japan, the safety of active packaging is regulated by the Japanese Food Sanitation Law of 1947, and the Food Safety Base Law of 2003. These regulations are combined with risk assessment criteria that are in compliance with those of the EU and the United States. (Dainelli, 2015). With a high interest in nanotechnology research on a global level (even among the developing countries) and a rise in acceptance among consumers, a further refinement of legislation is expected, followed by a higher rate of adoption among manufacturers.

5 | CONCLUSION AND FUTURE TRENDS

The public is aware of the need to reduce general waste stemming from the traditional plastic food packaging made from petroleum that is non-biodegradable and non-renewable. At the same time, the Food and Agriculture Organization has estimated that the post-harvest agricultural loss accounts for 20%–50% of the food production annually. Consequently, more efficient and sustainable food packaging is continuously under development in order to reduce waste, diminish post-harvest losses and increase safety. This review highlights the great potential of biodegradable films with nanomaterials for active packaging that provide extended shelf life and maintain or improve the condition of the packaged food. Bio-based polymers are eco-friendly materials with high performance that can replace traditional petroleum-based packaging. Nanofillers are used to improve food packaging mechanical properties; enhance barriers to water, oxygen, and other environmental factors; absorb moisture; protect against UV radiation; and release antimicrobials, while retaining their biodegradable properties. Antibacterial NPs such as TiO₂, ZnO, MgO, Ag, graphene, and carbon dots in bio-nanocomposite films could enhance food quality and safety over a longer period that would enhance the marketability of the new active packaging materials.

The main future prospect in active packaging is the safety improvement of antimicrobial nanofillers because there are limited studies on the cytotoxic impact of the nanomaterials in packaging films. The currently available bibliographic data reveal that the toxicity of nanofillers depends on their shape, size, surface-to-volume ratio, and doping. Nanofiller toxicity is also concentration- and time-dependent. Moreover, to produce efficient and durable antibacterial packaging, the controlled release of antibacterial agents must be achieved. Research is directed to specific incorporation of nanofillers to the packaging materials that will enable gradual release and reduce excessive particle concentration in contact with food. Further development of active packaging will also depend on comprehensive, systematic, and well-defined standards for antimicrobial activity and safety evaluation of nanofillers.

The commercialization of active packaging technologies is challenging because of the implementation costs and the complex legislative and regulatory issues. Despite currently existing complexities in achieving regulatory compliance, particularly in the EU, the high benefits that can be achieved by the use of active packaging stimulate the dynamic pace of commercialization of various packaging systems and accelerate their adoption by food

producers and consumers. Furthermore, the gap between commercial applications and research must be filled by close collaboration between academic and industrial groups.

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AUTHOR CONTRIBUTIONS

Nikola Ž. Knežević, Ivana Gadjanski, and Jasmina Vidić developed the concept of manuscript, and were involved in the organization of topics and distribution of sub-chapters, interim, and final version. Ivana Gadjanski and Nikola Ž. Knežević coordinated authors' contributions. Nejra Omerović and Nikola Ž. Knežević were involved in drafting the sections related to biodegradable polymers and MPS NPs-biopolymer composites. Kristina Živković was involved in drafting sections related to metal-oxide NPs-biopolymer composites and carbon NP-biopolymer composite. Minja Mladenović was involved in drafting sections on metal NPs-biopolymer composites. Jasmina Vidić drafted abstract, introduction, nanofillers, and conclusion. Jovana Vunduk, Ivana Gadjanski, and Ivanka Milenković were involved in drafting section on regulatory and market status. Mila Djisalov was involved in drafting inputs on antibacterial effects of various NPs. Critical input and corrections were made by Ivana Gadjanski and Jasmina Vidić who were responsible for editing the manuscript. All authors read and approved the final version.


CONFLICTS OF INTEREST


The authors declare no conflicts of interest.

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