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Bio-nanocomposite films- production, applications and safety issues: A comprehensive review

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ABSTRACT

Biopolymers have been widely utilized for their unique peculiarities of eco-friendliness, easy availability, safety and biocompatibility over their synthetic alternatives. In order to enhance the packaging characteristics as well as to widen their areas of utilization, mixing biopolymers and/or adding nano-sized fillers is found to be an effective method. Packaging is one of the major industries responsible for the nation's GDP and economy. Hence, recent innovative techniques such as bio-nanocomposite film packaging are obtained wide popular acceptance among producers and consumers. Improvement in the film's physical, chemical and functional properties contributes higher shelf life of food products. Bio-nanocomposite films open an excellent opportunity for sustainable growth and development for the packaging industry. Bio-nanocomposite reduces pollution and in the future, it will be the best replacer for many plastics. At the same time, proper care must be given to the safety issues of nanomaterials and alternatives for toxic and harmful materials are to be developed. The migration of nano-compounds from bio-nanocomposite films is to be addressed and needs a permanent solution. The compatibility of bio-nanocomposite films provides enormous applications for it. As compared to conventional films the feasibility and overall performance of bio-nanocomposite films are superior. The low cost and weight of bio-nanocomposite film provide more significant demand for it in the market. The polymeric matrix incorporated with properly-dispersed nanofillers may enhance the physicochemical qualities of the obtained bio-nanocomposites, mainly mechanical, barrier, thermal and optical properties.

1. Introduction

Biopolymers have been widely utilized for their unique peculiarities of eco-friendliness, easy availability, safety and biocompatibility over their synthetic alternatives (Basumatary et al. 2022). Biological macromolecules such as proteins and carbohydrates are common biopolymers in the areas of bio-materials and packaging purposes, especially for conventional and novel applications (Rhim et al. 2013; Rhim et al. 2014). Some of the properties of biopolymers, such as mechanical and water vapor transmission, should be made better to switch them as potent alternatives for fossil derivatives (Rhim et al. 2013; Rhim et al. 2014). Hence to enhance the packaging characteristics as well as to widen their areas of utilization, mixing biopolymers and/or adding nano-sized fillers is found to be an effective method (Rhim et al. 2013; Reddy et al. 2014). Blending of biopolymer is one of the effective methods to reformulate biopolymers with improved properties. When compared to individual component-derived films, biopolymer-incorporated films possess improved properties (Ghanbarzadeh et al. 2010; Sionkowska 2011).

There is an increasing trend towards using reformed biopolymers films or biopolymer-related blends for packaging since they offer added benefits over artificial or non-biodecomposible films for the protection of foodstuffs (Ghanbarzadeh et al. 2010; Benbettaïeb et al. 2015). To create novel biomaterials with desirable properties and superior performance, low nanofillers loaded (5 % by wt) bio-nanocomposite technology has been proven effective (Rhim et al. 2013; Reddy et al. 2014). The polymeric matrix

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incorporated with properly-dispersed nanofillers may enhance the physicochemical qualities of the obtained bio-nanocomposites, mainly mechanical, barrier, thermal and optical properties.

Films made from carboxy methyl cellulose (CMC) or starch (ST) lack the required mechanical and water vapor permeability properties, apart from their excellent film-forming properties. These problems of films can be solved by blending them to create bio-decomposable blended films with altered characteristics or by incorporating nanofillers to make bio-nanocomposite films derived from CMC or ST with enhanced qualities (Ghanbarzadeh et al. 2010; Peressini et al. 2003; Chen et al. 2009).

Due to the hydrogen bond formation, the CMC biopolymer is entirely miscible with ST biopolymer (Ghanbarzadeh et al. 2010; Tongdeesoontorn et al. 2011). All these properties together help formulate a CMC/CST blend that can obtain a homogeneous matrix blend for bionanocomposite development, a new biocompatible packaging (Almasi et al. 2010). Ghanbarzadeh and his co-workers (2010) evaluated the influence of CMC content on the physical and chemical characteristics of CMC/ST blend films and was revealed that the films exhibited superior mechanical and barrier properties compared to pure ST film.

The superior films possessing water vapor permeability (WVP) as well as mechanical properties can be obtained by utilizing CMC/ST mixture as a bio-material matrix. Almasi and co-researchers integrated nanoclay in the blend of CMC/ST to develop nano clay-occupied carboxy methyl cellulose/starch films with enhanced performance (Almasi et al. 2010). Similarly, reinforced bio-nanocomposites were prepared using CMC/Starch along with graphene oxide and keratin-grafted graphene oxide (Rodríguez-González et al. 2012). The findings stated that the thermal and mechanical characteristics of the mixture were rapidly improved through the inclusion of graphene oxide in modified and unmodified forms. Hence the effort to convert bio-degradable polymers into compounds with higher functional properties could be a challenge when used with derivatives of fossils; however, related research works are popular nowadays.

In the sectors of nanotechnology, the trend of using elongated cellulose nanocrystals is getting popular and illustrated that the cellulose nanocrystals integration into the biological polymer can produce bio-nanocomposite polymers with special packaging requirements (Chen et al. 2009; Alves et al. 2015; El Miri et al. 2015).

The nanofillers like silicate, clay, and titanium dioxide (TiO2) included in the biological polymers act against microbes and scavenge oxygen which causes deteriorative reactions. It can also serve as a biosensor and enhance barrier qualities (Azeredo 2009; Azeredo et al. 2011). They can react with the produce by emitting useful substances such as antibacterial components, oxidation-preventing components, or by expelling undesirable components like oxygen and water vapor and thereby act as an active packaging ingredient of food. Since it can recognize the packed produce quality like bacterial degradation or life span and it utilizes methods to communicate data regarding food state and security, it is also considered to be a smart food packaging (Azeredo et al. 2011).

Studies report that bio-polymers integrated with nanocomposite materials, a potential component in enhancing packaging functions, can also play a significant part in mitigating various pollution problems and many research works on the scope of biopolymers and nano-sized fillers in forming bio-nanocomposites are successfully going on.

Biopolymers

Biopolymers can be defined as polymers consisting of covalently bonded monomeric units that form a molecular chain. The first part, “bio” represents the meaning of biodegradable. Thus, microbes degrade biopolymers by giving out environment-safe products such as carbon dioxide and water. Due to their biodegradability, renewability and abundance biopolymers can be exploited as a substitute for petroleum plastic (Liu et al. 2005; Muratore et al. 2005).

In the early days, the category of biopolymers commonly used were organic biofiber like carbohydrate agar, polysaccharide, chitosan and cellulose; collagen, gelatin, wheat protein, milk protein and alginate which are protein derivatives. New technology has recently created synthetic biopolymers, including polyglycolic acid, polylactic acid, polycaprolactone, polybutylene succinate, polyvinyl alcohol, etc. Man-made biological polymers possess superior attributes like life span, elasticity, high polish, transparency, and strength and thereby have the potential to form a sustainable industry.

Biological polymers can be classified into different groups based on the source, which covers biomass-derived polymers (e.g. agricultural raw materials), microbially fermented synthetic biological polymers, biomass-derived and petroleum-derived traditionally and biomass derived from artificially prepared biopolymers. The former categories originated from sustainable sources, whereas the latter category is petroleum derivatives.

Widely studied biopolymers for making bio-nanocomposite packaging materials are mainly starch and derivates (Heydari et al. 2013). They are generally eatable, and are safe ingredients. It has been reported that starches are entirely eco-friendly and can induce the biological decomposition of non-decomposable materials while blending. Moreover, poor physical characteristics of starch could be corrected using aids like plasticizers, nano-sized filler materials, etc (Sorrentino et al. 2007).

When artificial biological polymers are considered, PLA is gained much importance. It is prepared from lactic acid, which may easily be obtained from carbohydrate plant sources such as sugar and maize (Sorrentino et al. 2007). PLA is biodegradable and its durability could be custom-made (Wang et al. 2013).

Derogations like low physical properties and permeability attributes were reported when petroleum-derived non-biodegradable materials were studied. The main concerns of biopolymers generally include brittleness, lower thermal disruption point and poor prolonged process resistance. Nevertheless, lower thermal disruption points, poor ability with and temperature and relative humidity, and poor elasticity are the problems faced as far as the synthetic biopolymers, PLA is concerned (Sorrentino et al. 2007).

Moreover, research studies have always recommended the enhancement of biopolymer properties to offer sustainability as well as environmental safety. Biological polymers incorporated with nanofillers are reported as a guaranteed method for improving the physical and transmission attributes of biological polymers.

Nanofillers

A rapid filler surface area rise occurs when the filler dimensions (nanophase) are considerably lowered. This is advantageous since biological polymers incorporated with nanoparticles depend on the large circumference of the nano-sized fillers, which leads to a border area or partition among the biopolymer structure and nano-sized filler material. The broad surface promotes change in the movement of molecules, the stress relaxation characteristics apart from the thermo-physical, and the permeability qualities of the biological nanocomposite polymers (Azeredo et al. 2011). These are generally custom-made for acquiring the capability of withstanding physical and thermodynamic impacts in the stages of preparation, distribution and keeping of food, particularly in food packaging

Different variants of filler materials of nano dimensions less than 100 nm have been used to improve the biopolymer attributes. Generally, the kind of nanofiller on which research work have been done includes nanoparticles, nanotubes, nanorods, and nanofibrils. Regarding a comparative study on attributes of the bio-nanocomposite ingredients produced from various nanofillering materials, only a little research work has been conducted so far. Kanmani et al. (2014) conducted a study on the physical as well as chemical traits of antibacterial films obtained from gelatin and silver nanoscale particles, Rafieian et al. (2014) carried out a research to study the thermal, mechanical and structural attributes of these films derived from gluten and cellulose nanoscale particles. The physical characteristics of nano bio-composite films can be significantly improved by incorporating ZnO nanoparticles into fish gelatin (Rouhi et al. 2013).

The primary classification of nano-sized filler materials is organic or inorganic such as clay, organic biopolymers, organic antimicrobial agents, metal, and metal oxides. Amid the innumerable nanofillers, clay can be considered the most popular improved form of bio-nano composite material used for packaging. (Abdollahi et al. 2013; Di Maio et al. 2013; Shin et al. 2014). Since it is naturally occurring and abundant in the pure form, cost-effective and resulted in remarkable reinforcement and relative flexibility in the processing of bio-nanocomposite materials. The clay material that is most broadly examined was MMT clay and this is because of its larger surface area and length-to-diameter ratio (Bruna et al. 2014; Pinto et al. 2015). Clay possesses respective surface areas as well as a length-to-diameter ratio. Some of the comparative evaluations of the impact of clay form above the obtained nanoparticle incorporated polymers have been conducted. Lee et al. (2014) determined the influence of Cloisite Na^+ and Cloisite 10A on the rheological traits of bio-nanocomposite films prepared from meal protein of sesame seed. Rhim et al. (2011) compared the results of different kinds of clay such as Cloisite Na^+, Cloisite 30B, and Cloisite 20A on the attributes of agar and clay combined films. The impacts on the attributes of the films were distinct because of the changes in clay attributes.

The mostly studied metal for manufacturing these materials was reported to be metal mainly because of their antimicrobial characteristics, stability and lower volatility at elevated temperatures. Meanwhile Zno is the properly studied metal oxide due to its deodorizing ability and antibacterial quality (Rhim et al. 2013; Rouhi et al. 2013; De Moura et al. 2012; Youssef et al. 2014).

Generally, a small quantity of fillers (<5%) is sufficient to enhance biopolymer characteristics. Usually, nanofiller quantity has a considerable function on the physical characteristics of the composites. Many works have stated that with an increase in the quantity of nanofillers, bio-nanocomposite materials show an increment in the strength and young's modulus, while lengthening at rupture is found to be reduced (Tang et al. 2008). The reason for the increment in the physical attributes of the composite particles because of the high stiffness of the nanofillering materials and superior bonding between polymer and filler at the boundary. Reaction at the interface would result in stiff biological nanomaterials attributed addition of stiff nanofillers, thereby modifying the thermodynamic properties of composite materials desirably.

Moreover, bio-nanocomposite materials also show enhanced gases and water vapor barrier attributes. Earlier research works have illustrated that modification of transmission characteristics was influenced by the kinds and quantity of nanofillers added and its length to diameter ratio

(Rhim et al. 2013). Among these, length to diameter ratio was reported to show an essential influence on the transmission characteristics of bio-nanocomposite constituents. Nanofillers with large aspect ratios show higher affinities to the integration of filler thus desirably modifying the permeability attributes (Choudalakis et al. 2009). Because of the superior blocking attributes, these composite materials got significant recognition for packaging applications of food as this might help in achieving significant keeping quality of food produce.

Integrating nano-sized fillering materials offers several desirable functions like an antibacterial agent, biological sensor, and absorption of oxygen that are vital for packaging applications but also enhance the biopolymers' physical, thermal, and permeability traits. The integration of antimicrobial nanofillers could ensure food security by checking the multiplication and attack, also destroying microbes and disease-causing microbes contained in the food. The broad interfacial area of nanofillering substances allows a large number of microbes for adsorbing on the fillers hence contributing to the antibacterial efficacy of these composite constituents (Azeredo 2009).

A few nanofillers such as MMT, chitosan, silver and ZnO possess an antibacterial characteristic that may be suitable for packing food materials. Very often, other antimicrobial ingredients like enzymes (e.g. peroxidase, lysozyme) and artificial microbicidal components (e.g. benzoic acid, propionic acid, sorbic acid) with bio-nanocomposite ingredients. Since silver has substantial toxicity towards a broad range of microorganisms, it is the most extensively opted antimicrobial nanofillers (Azeredo 2009). Kanmani et al. (2014) conducted research upon the antibacterial characteristics of gelatin-derived highly reactive films incorporated with Ag nanoparticles and nano clay. Rhim et al. (2013) conducted a work on bio-nanocomposite derived from agar added with Ag nanoparticles filler, whereas De Moura et al. (2012) designed nanocomposites whose base material is cellulose containing Ag nanomaterials which are antimicrobial in nature. All of the research works demonstrated assuring antimicrobial impact of these materials.

Highly reactive nanofillers integrated into biological polymers can perform as biological sensors. Bio-nanocomposite biosensor shows the capability to communicate variations in the environmental parameters like temp, RH, oxygen concentration and quality of food articles (Azeredo 2009; Bouwmeester et al. 2009). Food product storage is solely dependent on external factors, so measuring changes in each factor is an essential step for proper storage. Defects in the sealing of packaging are often going unidentified, thus, the sensor is essential to demonstrate unwanted variations in food produce and to warn customers in case quality has deteriorated or degraded. A carbon nanotube is a nano-type filler that possesses sensoring characteristics. Han et al. (2011) stated that Georgia Technology is an institution that exploits nanofillering materials as a sensor in packaging fabrication. Georgia Technology Institution designs biosensors using PA and nanotubes of multi-wall carbon to monitor and indicate microbes, toxic substances, and food and beverage spoilage.

Some nanofillers show the ability to scavenge oxygen and the inclusion of these nanofillers in the film control and retain the amount of O_2. This is relevant because an enormous amount of O_2 within packages results in product deterioration (Azeredo 2009). Oxidation can cause the browning reaction of fruits and vegetables, whereas fat oxidation leads to the formation of rancid odors and flavors. At the same time, pigment oxidation ends in color changes in the product are mainly due to pigment oxidation and this will disturb the aesthetic looks of the product, thereby less appealing. Oxygen scavenger packages promote the maintenance of food freshness. TiO2 is an example of a nanofillers that possess the ability to scavenge oxygen in the presence of ultraviolet. Xiao-e et al. (2004) designed oxygen-scavenging films by incorporating TiO2 within the package.

Kanmani et al. (2014) standardized the qualities of films produced from different types of biopolymers like with the addition of different quantities of ZnO nanoparticles to perform the function of nanofiller. They pointed out that ZnO addition as a nanofiller resulted in increased color, UV blocking, functional properties of the films, and decreased transmission rate of water vapor, tensile strength, and elastic modulus for all kinds of biopolymer. Nafchi et al. (2013) carried out a study on the characteristics of bio-nanocomposite films prepared from sago starch and bovine gelatin along with nanorod-rich zinc oxide nanofillers. They highlighted a decreased oxygen transmission and mechanical and heat seal properties of the films were found to be increased. As the amount of ZnO nanorod contents increases moisture content and water absorption capability of the bio-nanocomposite films are found to have decreased. The films also possessed the ability to absorb UV radiation.

Bio-nanocomposite films are a mixture of natural and synthetic biopolymers with nano-size materials. These are known as a new class of unconventional materials for packaging. Bio-polymers are originated and produced from biological molecules, while nano-size materials are used for value addition. In order to improve the physico-chemical and mechanical properties of the packaging materials, composite materials are used. The major difference between nanocomposite material from conventional composite materials is mainly due to the high surface-to-volume ratio. Nanocomposites are integrated with different systems of organic and/or inorganic materials. It is an innovative way to reduce environmental problems through green technology. Biodegradability and biocompatibility are essential features of bio-nanocomposite films. Hence, current academic and industrial research focuses on its numerous applications in various fields.

Preparation

There are different types of bio-nano composite films produced by various methods. Some of the films produced are explained below.

Cassava Starch Based Bio-nanocomposite Film

According to Llanos and Tadini (2018) bio-nanocomposite films can be produced by casting technique using chitosan and cassava starch as polymers along with montmorillonite (MMTNPs) or bamboo nanofibers (BNFs). Glycerol was used as a plasticizer for the reduction of the viscosity of the film (Llanos and Tadini 2018).

Flow chart of the production

Dissolve chitosan (1g/100 mL) in glacial acetic acid solution

↓

Magnetic stirring (190 rpm for 8 h)

↓

Vacuum filtration

↓

Addition of glycerol (30g/100g)

↓

Addition of nanoscale particles such as clay and nanofibers at 0.5 and 1 g, respectively

↓

Magnetic stirring at 400 rpm for 20 min at ambient temperature

↓

Ultrasound bath for 15 min

↓

Pour into acrylic plates of square shape

↓

Drying at 32^{o}C in an air circulation oven for 24 h

Similarly, bio-nano composite films based on cassava starch can be produced. Heating of the solution at 90^{o}C for 30 min is applied for the gelatinization of starch.

Soy Protein Isolate Based Bio-nanocomposite Film

The melt extrusion technique can be used to produce soy protein isolated-based bio-nanocomposite film (Kumar et al. 2010). Montmorillonite was used as the nanofiller material. The components (soy protein isolates, montmorillonite and glycerol) were mixed and kept at ambient temperature for 2 h. In order to obtain the extrudate, extrusion of the mixture was done using two screw extruders. The obtained product was dried at 50^{o}C for 48 h. The extrudate was ground using a grinder. The acquired powder was dissolved in deionized water and the pH of the solution was adjusted using HCl or NaOH solution. The solution was heated upto 95^{o}C and held for 20 min. Then the solution was cooled to 65^{o}C and transferred into a petri dish. The solution was cast in a Petri dish by keeping it at room temperature for 48 h. The bio-nanocomposite film was obtained by peeling the petri dish. It was reported that this technique improved the film's mechanical, thermal and dynamic properties.

Coconut Shell Based Bio-Nanocomposite Film

Hahary et al. (2016) reported the production of bio-nanocomposite film from coconut shells. Pre-treatment of coconut shell was done using a 4% alkali solution of NaOH at 70^{o}C for 3 h. Subsequently, a mechanical stirring of the sample was carried out followed by bleaching of the sample using acetate buffer. The dispersion was subjected to acid hydrolysis using 65% sulphuric acid at 45^{o}C for 60 min. The coconut shell was washed and filtered. The obtained shell is dried in a hot air oven at 80^{o}C for 24 h. The coconut shell was transferred into butyl methacrylate dissolved in ethanol. The suspension was stirred for 1 h and kept overnight. Then the solution was filtered and followed by drying in a hot air oven at 80^{o}C for 24 h. The sample was immersed in distilled water, acetone and DMAc each for a period of 1 h. The mixture of cellulose and LiCl/DMAc solution was prepared by stirring for 1 h. The obtained transparent solution was cast onto a glass plate and kept overnight. Washing of regenerated film was done using distilled water and dried at room temperature. The modulus of elasticity, tensile strength, crystallinity index and thermal stability of the obtained film was improved. Proper interaction between the coconut shell and regenerated cellulose improved the functional properties of the film.

Whey Protein Isolate Based Bio-nanocomposite Film

Qazanfarzadeh and Kadivar (2016) studied the properties of whey protein-based bio-nanocomposite film prepared by casting with oat husk nanocellulose. Impurities of the oat husk were removed by washing with warm water followed by air drying. Impurities removed from oat husk were ground to powder using an analytical mill. Subsequently, the obtained product was sieved to obtain oat husk powder. Next, bleaching of the oat husk powder was performed using acidified sodium chlorite solution. The pH of the solution was adjusted to 3-4 using glacial acetic acid. Lignin was removed by mechanical stirring at 70^{o}C for 5 h. The lignin-removed powder was treated with 5% KOH solution for 24 h at ambient temperature. The powder was further subjected to mechanical stirring at 90^{o}C for 2 h to obtain hemicellulose leached powder. After that, fiber was centrifuged and washed repeatedly with distilled water to make a neutral pH followed by air drying using the oven at 60^{o}C for 8 h. Oat nanocellulose was prepared by acid hydrolysis using a sulphuric acid solution. The sulphuric acid was removed by continuous centrifugation of the sample. The resulting product was sonicated for 30 min and freeze-dried. WPI-based nanocomposite film solution was prepared by dissolving oat husk nanofiber in 100 mL deionized water followed by the addition of 10 g WPI. Glycerol (50% wt of WPI) was used as the plasticizer. The pH of the solution was adjusted to 9 using sodium hydroxide. The solution was stirred mechanically at 80^{o}C for 20 min and degassed. The degassed solution was cast onto a Petri dish and kept at room temperature for 24 h. The Petri dish was peeled off to obtain a bio-nanocomposite film of high functional quality.

Alginate Based Bio-nanocomposite Film

Abdollahi et al. (2012) isolated nano-cellulose from microcrystalline cellulose and incorporated it in alginate to produce the bio-nanocomposite film. Sulphuric acid (64% w/w) was used to hydrolyze microcrystalline cellulose at 45^{o}C for 2 h. Repeated washing of the sample was done using centrifugation at 120000 rpm for 10 min. In order to adjust the pH, the suspension was dialyzed against deionized water. Subsequently, the dispersion was sonicated for 15-20 minutes. Sodium alginate was dissolved in distilled water at 70^{o}C for 30 min. Exactly, 5% of the nano-cellulose was dissolved in 50 mL of deionized water and subjected to sonication. Alginate solution was added into the nano-cellulose solution and stirred for 1 h. The obtained mixture was homogenized at 10000 rpm for 5 min. After that, the sample was subjected to sonication for further size reduction. The solution was degassed using a vacuum technique and cast onto Petri plates. Incubation of the solution was carried out at 40^{o}C for 24 h. The dried bio-nanocomposite film was removed from the Petri plates.

Bio-nanocomposite: food packaging applications

The major function of the packaging material is to protect the product from the external environment. In order to protect a food item, barrier properties of the bio-nanocomposite film such as water vapor barrier and gas barrier properties have to be kept in the proper range. Moreover, the product's shelf life directly influences the barrier properties of the bio-nanocomposite film. Bionanocomposite is gaining acceptance in the market due to its improved functional and mechanical properties. Bio-nanocomposites can be used for the packaging of processed cheese, meat and boil-in-bag products. It can also be incorporated with the extrusion and coextrusion process of different polymers for carbonated beverage bottles. Moreover, bio-nanocomposite packaging materials are helpful in reducing the environmental pollution. The impact of the packaging material in the soil and landfill can be minimized by using bio-nanocomposite packaging materials.

Bio-nanocomposite packaging is an innovative approach, which has several applications in the food industry. The incorporation of bio-composites improves bio-polymers' properties and makes it possible for several applications. The nano-fillers have a particular ability to increase the path length for gases and vapors. Furthermore, it contributes to the crystallinity of the packaging materials. The development of the antimicrobial system has emerged as the major application of new-generation bio-nanocomposite packaging. These types of packaging materials are used to protect meat and poultry from the pathogenic organism (Fortunati et al. 2012). Due to the different chemical nature and structure of metal and metal oxides, it is widely used as an antimicrobial agent. Zinc oxide has an antibacterial effect so it can be used in packages with modified nano-engineering techniques.

Pantani et al. (2013) studied the effect of zinc oxide PLA bio-nanocomposite on barrier properties against Gram +ve and Gram –ve bacteria. Silver nanoparticles incorporated in PLA materials decrease bacterial growth and provide an antibacterial effect (Pantani et al. 2013). Nanotechnology provides flexible films with a lower cost of design and fabrication.

Antimicrobial Applications

Magnesia (MgO) could be utilized as a nanofiller to enhance the antibacterial attributes of the material. In a study conducted by Sanuja et al (2014), they revealed that clove oil incorporated MgO-reinforced chitosan bio-nanocomposite have inhibitory property on S. aureus.

To mitigate biological threats in food packaging, work has also been conducted using oxides of photocatalytic semiconductors like Titanium dioxide which shows inhibitory effects on a wide range of microorganisms. It possesses good physical and chemical stability, property of self-disinfection, safety and indestructible characteristics (Bodaghi et al. 2015).

In a study conducted by Radusin et al (2016), organically altered nanoclays were found to be incorporated to attain antimicrobial properties. However, ions of metals such as silver, copper, gold and platinum and oxides of metals such as Titanium, Zinc, and Magnesium are commonly used for nanocomposite preparation.

In a study, Carbone et al (2016) revealed that, along with antimicrobial attributes, gas barrier and mechanical characteristics could be improved by a starch-based nanostructured film with nanoparticles of silver and clay. This is mainly contributed by the involvement of silver nanoparticles with a number of hydroxyl groups. In the case of silver nanoparticles, adsorbents made of cellulose can act as carriers and found that it has got a noticeable effect on total aerobic bacterial and Enterobacteriaceae counts in samples.

Polyurethane films incorporated with nanoparticles of ZnO are found to have an inhibitory effect not only on *E. coli* but also *B. subtilis*. Since these nanoparticles are safer for humans than other metal oxides, they are also integrated with gelatine, low-density polyethylene (LDPE), chitosan, etc. Studies show that, after 24 hours incubation period, ZnO nanoparticles integrated with chitosan biopolymer inactivated *E. coli* at a rate of 99.92 percent (Al-Naamani et al. 2016). Studies were conducted on the antibacterial effect of ZnO nanoparticles integrated materials on bacteria such as *S. aureus, Pseudomonas aeruginosa, B. subtilis, S. Typhimurium and Enterococcus feacalis*. It was found that the formation of reactive oxygen species leads to oxidative stress by which the cell membranes of bacteria are disrupted (Al-Naamani et al. 2016; Espitia et al. 2012; Sivakumar et al. 2014).

Shankar et al. (2015, 2017) studied the antibacterial effect of copper oxide nanoparticles incorporated into carbohydrate-based matrices on *E. coli and L. monocytogenes*. Natural as well as synthetic polymers like chitosan, cellulose, starch, etc could be used to prepare. Though copper accelerates the biochemical oxidative degradation of foods, they exhibit good antifungal characteristics.

Antioxidant Property

Walls of the packaging film can be incorporated with antioxidants and either released into the package or deteriorating substances are absorbed from the headspace. Various techniques are used for integrating antioxidant agents with the packaging, thereby antioxidant packaging material is synthesized. Some of them are polymer melting and extrusion; immobilization; dissolving and coating (Feng et al. 2017a; Feng et al. 2017b; Gomez-Estaca et al. 2014).

The health consciousness of consumers towards synthetic substances encouraged studies on the use of safe natural compounds and antioxidant-nanocomposite materials in packaging, which mitigates the oxidative degradation of foods (Biji et al. 2015; Vital et al. 2016; Tsai et al. 2018).

Composite films of Nanoparticles of lignin/ Polyvinyl alcohol were prepared and their antioxidant property was analyzed using DPHH radical scavenging method. With the addition of 4 wt% lignin nanoparticles, the activity was found to be 129–157 mm Trolox per gram (Tian et al. 2017)

Composite packaging films of silymarin-zein nanoparticle/bacterial cellulose nanofiber were prepared and their antioxidant properties were studied. EC50 values of these nanoparticles were found to be, 214.7±6.9 µg/mL against superoxide anion and 38.5 ±1.1 µg/mL against DPPH. Change in values of Total Volatile Basic Nitrogen (TVBN) during 12 days of refrigerated storage were also studied by packing salmon fillets in the composite films and it was found to be 30 mg N/100 g salmon. In case of composite hydrogels made of PVA/chitosan/lignin nanoparticles, 74.3% DPPH scavenging activity was shown, when the hydrogel is integrated with lignin nanoparticles at a rate of 1wt%. A rise in antioxidant activity up to 78.6% was observed when 3 wt% l lignin nanoparticles were added (Tsai et al. 2018; Yang et al. 2018).

Biodegradation Property

Biodegradation of polymer is mainly influenced by polymer length, the extent of crystallinity and complexity of polymer matrices (Eubeler et al. 2010; Massardier-Nageotte et al. 2006; Pantani et al. 2013). The property of crystallinity is a principal attribute that influences the barrier and mechanical properties of the polymer (Pantani et al. 2013; Azizi et al. 2014; Sermsantiwanit et al. 2012).

Mesquita et al., 2016 evaluated the biodegradation property of bionanocomposites of Polyhydroxybutyrate/ Polypropylene-graft-maleic anhydride (2.5%)/clay (3%) using Fourier-transform infrared (FTIR) spectroscopy, weight loss and optical microscopy. FTIR analysis indicated that ester group bond cleavage is affected more by microbial activity. During mass loss analysis, a loss of 22.5% was observed, for 86 days of exposure in simulated soil. In micrographs, it was found that degradation was more prominent after 86 days than 14 and 28 days of exposure.

When the effect of thymol and nanoparticles of silver were studied on nanocomposite based on Polylactic acid, it was found that nanocomposites degrade at a faster rate compared to the polymer in the pure form and this is due to the high rate of hydrolysis caused by larger water diffusion via nanopolymer matrix (Ramos et al. 2014).

A disintegration study was conducted at 50 percent humidity in PLA-based limonene film formulations reinforced with Cellulose nanocrystals and it was found that nanocrystals and limonene altered the rate of disintegration. Nanomaterials-reinforced films are observed with a low rate of degradation (Fortunati et al. 2014).

Risk assessment and safety issues

Due to the significant variations in the physico-chemical properties of nanomaterials, the potential applications of nanocomposites in packaging are found to be a bit challenging. Therefore, an elaborate study of their physico-chemical properties and migration characteristics is essential for their utilization in the field of packaging (Honarvar et al. 2016).

The release of toxic substances from packaging material to food is of grave concern and on exposure, they may result in a dangerous toxicological impact on human health. However, the unavailability of sufficient data on the toxicological impact, accumulation and availability, makes adopting global standards or regulations on the use of nanomaterials difficult (Huang et al. 2015; Rovera et al. 2020).

The **European Food Safety Authority** (EFSA) is entitled to carry out the analysis studies and submit proposals to European Commission. Moreover, the applicability of the limits of migration fixed for macro-substances on nanomaterials is not advisable, which opens the need to determine new limits for this specific material category. When nanomaterials are used in active packaging, especially antimicrobial packaging, the materials purposefully released to the food matrix is not considered to be an ingredient of the package but an additive (Huang et al. 2018).

The nanomaterials can penetrate the human body via tissues of skin or inhalation. It has also been revealed that exposing humans to nanomaterials for a long duration could cause potential oxidative stress in cells, damage to the liver and kidney, and even damage to DNA. Inhalation and penetration through the dermal tissue into individuals working in nanotechnology plants is considered to be extremely common (Youssef et al. 2013; Naseer et al. 2018). The use of novel packaging materials integrated with nanomaterials shall be backed with sufficient toxicological data and migration studies at a global level for potential applications in food packaging to ensure consumer safety (Souza et al. 2016; Agriopoulou et al. 2020).

Conclusions

In conclusion, bio-nanocomposite film packaging has gained widespread acceptance among producers and consumers as a sustainable alternative to traditional packaging methods. These films improve the physical, chemical, and functional properties of packaging, leading to a higher shelf life of food products. They also reduce pollution and have the potential to replace many plastics in the future. However, proper care must be taken to address safety issues with nanomaterials, and alternatives for toxic and harmful materials must be developed. The migration of nano-compounds from bio-nanocomposite films must also be addressed, and a permanent solution found. The compatibility of bio-nanocomposite films provides enormous applications and has potential for wider use in other industries as well. The use of nano-fillers in bio-nanocomposite films also improves the shelf-life of the packaging material. The potential of bio-nanocomposite films in intelligent and active packaging is significant for the recent innovative packaging industry. Overall, bio-nanocomposite films are an emerging green technology for the sustainable development of the packaging industry and further research should be conducted to explore its opportunities.

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