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Review

A Review of Polysaccharide-Zinc Oxide Nanocomposites as Safe Coating for Fruits Preservation

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Abstract: Safe coating formulated from biopolymer can be an alternative for better packaging for fruits. Among biopolymers used for safe coating, polysaccharides attracted more attention due to its biocompatibility and edibility. However, polysaccharide-based materials have weaknesses such as low water barrier and mechanical properties which result in lower capability on preserving the coated fruits. Hence, the incorporation of nanoparticles (NPs) such as zinc oxide (ZnO) is expected to increase the ability of polysaccharide-based coating for the enhancement of fruit shelf life. In this review paper, the basic information and the latest updates on the incorporation of ZnO NPs into the polysaccharide-based safe coating for fruit are presented. Various research has investigated polysaccharide-ZnO nanocomposite safe coating to prolong the shelf life of fruits. The polysaccharides used include chitosan, alginate, carrageenan, cellulose, and pectin. Overall, polysaccharide-ZnO nanocomposites can improve the shelf life of fruits by reducing weight loss, maintaining firmness, reducing the ripening process, reducing respiration, reducing the oxidation process, and inhibiting microbial growth. Finally, the challenges and potential of ZnO NPs as an active agent in the safe coating application are also discussed.

Keywords: nanocomposites; polysaccharides; safe coatings; zinc oxide nanoparticles

1. Introduction

The main function of food packaging is to protect food products from physical, biological, and chemical impact. The ability of food packaging to preserve food is by delaying food deterioration, retaining and prolonging the food's beneficial effect that comes from processing, and conserving the quality and safety aspect of the food by improving its shelf life [1]. However, the standard materials used for manufacturing food packaging (synthetic petroleum-based polymer) have their drawbacks, such as non-renewable, non-biodegradable, carcinogenic, and non-eco-friendly [2,3]. Therefore, it is crucial to develop biopolymer-based packaging that is inexpensive, biodegradable, renewable, available in nature, and harmless to the environment [4].

Safe coating is a layer-forming material used to enrobe food products. Safe coating can fortify or replace natural layers on food that can be consumed or not afterward [5]. The uses of safe coating can overcome many problems in food marketing. Most of the issues are associated with food deterioration and changes that may happen during production, transportation, and storage periods. Food deterioration and changes include loss of moisture, gas, solute, and oil due to food migration. Safe coating can improve the structural integrity of food, prevent the loss of volatile compounds that are responsible for flavor, and conveying food additives. Aside from maintaining quality related to food deterioration and changes, safe coating also improves food quality associated with its aesthetic appearance by minimizing physical damage development in food, hiding scars, and enhancing the surface shine of the food [6,7].

Safe coating becomes one of the prime concerns because of its ability to slow down the ripening process and prolong the shelf life of fruits [8]. Even when it is detached from its parent plant, fruits have their catalytic machinery to maintain its own life. The ripening process of fruits still occurs even after harvesting. This process occurs due to ethylene production and respiration of the fruit itself. Ethylene is a fruit ripening phytohormone that triggers many kinds of cell metabolism, including the ripening and senescence process. Based on the ethylene production and respiration pattern, fruits are further classified as climacteric and non-climacteric. Climacteric fruits still undergo the ripening process after harvest. Climacteric fruit is harvested when it achieves full maturity, where respiration rate and ethylene production are still at the minimum. However, the respiration rate and ethylene production are then increased dramatically to a climacteric peak, at the onset of ripening [9]. Non-climacteric fruit, on the other hand, does not undergo the ripening process after being harvested. This fruit produces a very small quantity of ethylene and does not respond to external ethylene. It also has a lower respiration rate, which, along with lower ethylene production, gradually decreases throughout the ripening process [9]. Besides the ripening process, fruits also undergo microbiological deterioration, physical changes, and biochemical changes that are also responsible for its postharvest degradation [10]. The uses of safe coating on fruits can help reduce the rate of respiration, inhibit moisture and volatile compound loss, and inhibit the ripening process. Thus, safe coating can increase the shelf life of fruit [11,12].

Coating can be formulated from a variety of materials, such as polysaccharides, protein, lipid, and resin [7,13,14]. Because of its edibility and excellent biocompatibility, polysaccharides gained more attention in safe coating production. However, due to its hydrophilic nature, polysaccharides generally have weaknesses such as low water vapor resistance [15,16]. Additionally, a coating made from polysaccharides usually has poor mechanical properties [3,17].

Zinc is among the necessary nutrition in human bodies. It has diverse physiological functions in human bodies. Zinc interacts with many enzymes and protein to perform important structural, functional, and regulatory roles. Hence, it is important to consume zinc as deficiency will cause many physiological issues [18]. Zinc can be consumed naturally, especially from animal-based food such as beef, chicken, and pork. Plant-based foods are not a good source of zinc, as it is generally poorly absorbed in the human body [19]. Aside consumed from natural sources, zinc intake can also be improved by food fortification by zinc or by zinc supplementation. One material that can be used for this purpose is zinc oxide (ZnO) [15].

Due to its biocompatibility and safety to public health, ZnO NPs have been incorporated into the food coating material [8]. The incorporation of ZnO NPs into coating can provide good mechanical, structural, and barrier properties for the coating [20]. The purpose of this review is to discuss the various novel methods developed to show the possibilities to integrate zinc oxide nanoparticles (ZnO NPs) into polysaccharides as sustainable safe coating materials, especially for fruits. This review also addresses the basic concept regarding safe coating and safety issues concerning ZnO NPs migration.

2. Basic Concepts of Polysaccharide-Based Safe Coating

Safe coating and film are produced from layer-forming material such as gelling agents. While both terms have those similarities, they have differences in respect to their application to food products. The edible film is wrapping materials that are applied to food products separately. Meanwhile, the safe coating is a thin material that is used directly on the food products. Hence, even though both safe coating and film may be produced from the same gelling agent, their characteristics may differ significantly [21].

There are at least three ways to apply safe coating on food, including dipping, spraying, and vacuum impregnation. The dipping technique is one of the most used methods in coating food. Food is immersed in the coating solution and withdraw from to drain excessive solution. In some cases, such as alginate coating, second immersion into the crosslinking solution can be performed to form film on the food [22]. The dipping technique usually requires a short duration, ranging between 0.5–5 min. This technique's main advantage is its ability to coat the food surface thoroughly, even if the food has a rough and complex surface [23] (Figure 1). However, it is essential to note that this simple dipping technique also comes with limitations. Excellent adhesion of the coating solution to the food surfaces may be hard to achieve if the surface of the food products is hydrophilic. These cases usually occur on cut fruit. It usually requires a layer-by-layer technique, in which food is dipped in polyelectrolyte solution with opposite charges to increase adhesion with the actual coating [24–27]. The dipping method also has weaknesses such as thick coating and diminished efficacy due to dilution and dissolving effect [28]. Residual accumulation of coating materials and microbiological contamination can also occur [29]. Some process parameters that need to be controlled in the dipping method are the type of solvent, temperature and viscosity of the coating solution, immersion and withdrawal speed, and dipping repetition and immersion time [30,31].

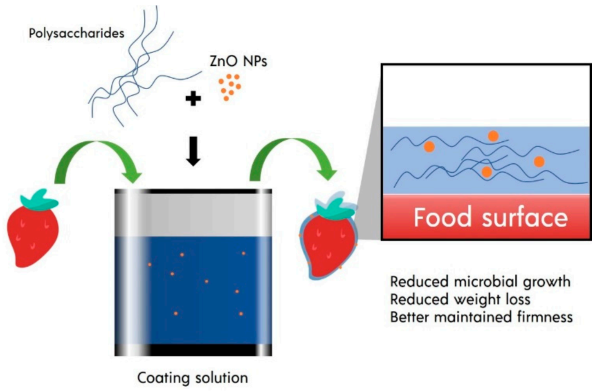

Figure 1. ZnO NPs in the polysaccharide-based safe coating.

The spraying technique is also often used in coating food. This technique distributes the coating solution on food surface in the form of droplets with the aid of nozzles [23]. The advantage of this technique is that only a small amount of coating solution is required to coat food due to the high pressure used during the spraying process—up to 60–80 psi [32]. There are still more advantages of coating with spraying techniques such as uniform coating, controllable thickness, possibility of multiple layer coating, minimalized coating solution contamination, controllable temperature of the coating solution, and the possibility to work with a large surface area [23]. However, certain conditions need to be controlled to apply the spraying technique properly. The viscosity of the coating solution should not be high. The characteristics of the spray flow are very dependent on the liquid properties (density, viscosity, and surface tension), operating conditions (flow rate and air pressure), and system conditions (nozzle design and spray angle) [23,33].

The vacuum impregnation technique is usually used when the coating is also used to enrich the food with vitamins and minerals. This technique produces a thicker and more compelling film than the previous coating technique mentioned. The vacuum impregnation is also able to incorporate solutes into foods that have air containing porous matrices such as vegetables and fruits [34,35]. The steps for this technique are similar to the dipping method. However, instead of immersing in the dipping tanks, the food is submerged into a coating solution within two airtight vacuum chambers connected to a vacuum pump. Afterward, the food is conditioned to atmospheric pressure while remained immersed in the coating solution. It is crucial to monitor and control the vacuum period, vacuum pressure, and the atmospheric restoration time for the vacuum impregnation technique [36]. This technique provides enhanced retention, uniformity, and adherence of the coating solution compared to the conventional dipping method [35].

Safe coating can be loaded with additives to modify its mechanical, functional, organoleptic, and nutritional characteristics. Additives can include plasticizer, surfactants, antimicrobials, antioxidants, antibrowning agents, flavor, and or pigments. Among the marvelous additives for food coating is ZnO NPs. It has excellent antibacterial, antifungal, and antibiotic nanoscale agents [8,37]. Therefore, in the following section, the effect of ZnO NPs on properties of some polysaccharides for safe coating are discussed.

3. Effects of Zinc Oxide Nanoparticles on Properties of Polysaccharide-Based Coating

Polysaccharide-based coating selectively allows the transfer of gas, which makes it an excellent barrier to gasses. This advantage property enables the formation of the modified atmospheric condition within the coated food. Even if the gas transfer between the surrounding and the coated food is minimized, it is not completely terminated. The coated fruit is still allowed to respirate aerobically at a lower rate. If the fruit only respires anaerobically, the production of different compounds may occur. These various compounds can affect the coated fruit quality in an unpleasant way. As aerobic respiration occurs at a lower rate, the process of senescence also occurs at a lower rate. Thus, the shelf life of the fruit increases [38].

Even though polysaccharide-based coating provides an excellent barrier against gasses, its hydrophilic nature results in a poor barrier against moisture. Therefore, the polysaccharide-based coating is less suitable for food with high moisture [38]. It is necessary to improve the water barrier capability and other properties of polysaccharide-based coating to increase its functionality as a food coating. Many studies tried to search for a way that increases the functionality of the polysaccharide-based coating. Incorporating ZnO NPs became an alternative to do so. The incorporation of ZnO NPs into polysaccharides provides coating with excellent mechanical, structural, and barrier properties [20,39]. As mentioned in the previous sections, ZnO NPs also provides excellent antimicrobial activities. There are some proposed antimicrobial activity mechanisms for ZnO NPs, such as the induction of oxidative stress through the formation of reactive oxygen species (ROS) and the release of Zn^{2+} ion that could penetrate the cell wall and reacts with the cytoplasmic content [20,39–41] (Figure 2).

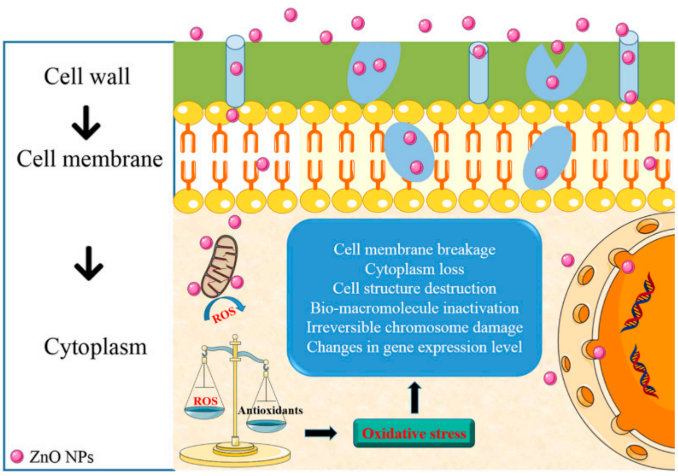

Figure 2. Antimicrobial activities mechanism for ZnO NPs. Reproduced with permission from [20] Copyright 2018, American Chemical Society.

Several studies have investigated the application of polysaccharide nanocomposite safe coating for fruits. The studies are summarized in Table 1 and further discussed in the following subsection.

Table 1. Summary of recent studies in the polysaccharide-ZnO safe coating.

Polysaccharide	Additives	Coating Method	Fruit	Storage Condition	Effect of Coating	References
Chitosan 5% *w*/*v*	ZnO 1% *v*/*v* gel	Dipping	Guava	20 days at 21 ± 1 °C and 80% RH	Reduced weight loss, color, and firmness are better maintained; no external injuries until end of storage; and reduced ripening index ratio (SS/TA)	[10]
Chitosan (3 g in 0.4 L coating solution)	ZnO 0.005%, 0.006%, 0.010%, 0.020%, and 0.027% *w*/*w* coating solution (611.30 nm)	Dipping	Fresh-cut papaya	12 days, 10 °C	Reduced microbial growth	[42]
Alginate 1.5% *w*/*v*	ZnO 0.25, 0.75, and 1.25 g/L (30–50 nm)	Dipping	Strawberry	20 days, 1 °C, RH 95%	Reduced microbial growth, reduced weight loss, better maintained firmness, lower increases in soluble solid, lower decreases in acidity, lower decreases in anthocyanin, phenolic, and antioxidant activities, lower increases in peroxidase activity, and lower decreases in superoxide dismutase activity	[43]
Alginate 5% *w*/*v*	ZnO 1% *w*/*v* gel	Dipping	Guava	20 days at 21 ± 1 °C and RH 80	None	[10]
Alginate–chitosan (90%–10%) 5% *w*/*v*	ZnO 1% *w*/*v* gel	Dipping	Guava	20 days at 21 ± 1 °C and RH 80	Firmness are better maintained and prevent external injuries	[10]
Carrageenan 0.8 g in 0.1 L solution	ZnO 0.5% and 1% *w*/*w* of carrageenan	Dipping	Mango	20 °C and RH 61%	Reduced weight loss, reduced CO_2 production, better maintained total acidity, better maintained color, and better maintained textural appearance	[15]
CMC 0.5% *w*/*v*	ZnO 0.1% and 0.2% *w*/*v* (30–100 nm)	Dipping	Pomegranate arils	12 days, 4 °C and RH 90%	Reduced weight loss, reduced vitamin C loss, reduced anthocyanin and phenolic content loss, and higher antioxidant activities	[44]
Pectin 10 g in 1 L solution	ZnO 0.1 g inside 1 L solution	Dipping	Star fruit	8 days at 25 °C	Reduced weight loss, reduced browning index and redness value, and reduced physical damage	[45]

3.1. Chitosan

Chitosan is a copolymer of N-acetyl-D-glucosamine and D-glucosamine with linear and semicrystal polymer structures [46,47]. The presence of amine groups in chitosan contribute to its excellent solubility in dilute acid solution (pH < 6) [48,49]. Chitosan has a lot of interesting properties such as biodegradability, natural origin, abundance, and antimicrobial and antifungal activities, which led to its utilization in many fields, such as in the food industry [50,51]. Chitosan film can be utilized as food packaging with excellent protection for diverse kinds of food, especially when it is combined with other film-forming material [52–54].

The research conducted by Arroyo et al. investigated chitosan-ZnO NPs coating toward the shelf life of guava (*Psidium guajava* L.) [10]. Safe coating was prepared with a composition of chitosan 5% *w/v* dissolved in acetic acid 0.5% *v/v*, glycerol 2% *v/v*, and ZnO NPs 1% *v/v* gel. The coating formulations were applied to guavas by dipping inside the coating solution for 3 min and dried at 25 °C. Guavas were then stored in a controlled environment for 20 days, with a temperature of 21 ± 1 °C and 80% relative humidity (RH). The result of the experiment showed that the chitosan coating with ZnO NPs could reduce weight loss, indicating reduced water loss and ripening process. The firmness and green colour of the coated fruit were better maintained, also indicating a reduced ripening process. The ripening index ratio (soluble solid/titratable acid) was reduced compared to the uncoated fruit. External injuries were not detected on the coated guavas until the end of storage time, while the uncoated guavas already showed lesions from day 12. The authors concluded that coating with chitosan enhanced by ZnO NPs could delay the fruit ripening process and protect the fruit from weight loss and damaged appearance, resulting in the guavas still being edible until 20 days of storage.

The study by Lavinia et al. also explored the application of chitosan-ZnO NPs as fruit coating, but mainly focused on microbiological activities toward fresh-cut papaya [42]. The coating formulations were prepared by mixing 0.1 L ZnO NPs solution (0.005%, 0.006%, 0.010%, 0.020%, and 0.027% *w/w* of total solution weight inside 1% acetic acid; size 611.30 nm) with 0.3 L chitosan solution (3 g chitosan dissolved in acetic acid 1%). The coating was applied to fresh-cut papaya (size 3–4 cm^3) through dipping for 10–20 s and drained afterward. The coated papaya was then stored in a refrigerator for 12 days at 10 °C. The result showed that chitosan-ZnO NPs coating could retard microorganism growth. Based on the maximum limit of microbial count (5.00 log CFU/g) set by the Indonesia National Agency of Drug and Food Control (BPOM), the coated fresh-cut papaya was still safe to consume up until four days after storage. At the same time, the uncoated one was no longer safe to consume. The author mentioned that the antimicrobial activities of the coating were the result of synergistic interaction between chitosan and ZnO NPs, and concluded that the nanocomposite coating can maintain the quality of the fresh-cut papaya through the reduction in microbial growth.

3.2. Alginate

Alginate is one of the most abundant polysaccharides that can be found on brown algae and seaweed. Alginate is a polymer composed of (1→4)-linked α-L-guluronic acid (G) and β-D-mannuronic acid (M) monomeric unit. Alginate has the potential to be utilized as active packaging due to its biodegradability, biocompatibility, and null toxicity [55–57].

Emamifar and Bavaisi observed the effect of alginate-ZnO NPs coating, together with cold storage temperature, toward the shelf life of fresh strawberries (*Fragaria × ananassa Duch.*) [43]. Coating solutions were composed of sodium alginate 1.5% *w/v*, glycerol 1% *v/v*, and ZnO NPs (size 30–50 nm) with different concentrations (0.25, 0.75, and 1.25 g/L). Coatings were applied to fresh strawberries by dipping inside the coating solution at 25 °C for 5 min. The coated strawberries were then dried for an hour under fans. The coated strawberries were then stored at 1 °C and 95% RH for 20 days. The result showed that coated strawberries had reduced microbiological growth, including yeast, mold, and aerobic bacteria. It was mentioned by the authors that reduced microbiological growth may be attributed to the antibacterial properties of ZnO NPs or the improved barrier properties to oxygen and moisture by ZnO NPs. The coated strawberries also reduced weight loss, indicating reduced fruit surface evaporation and respiration rate. The coated strawberries also exhibited better-maintained firmness, resulted from CO_2 buildup inside the fruit that caused inhibition of enzymes responsible for fruit softening. The strawberries with coating had lower increases in total soluble solid and lower decreases in acidity, showing that the coating can control the fruit maturity. The coated strawberry exhibited lower decreases in ascorbic acid, anthocyanin and phenolic content, and antioxidant activity, showing that the coating has antioxidant and antimicrobial properties. The coated strawberries showed lower increases in peroxidase activity and lower decreases in superoxide dismutase activity, which indicated the strawberries were better maintained from various damages or stresses. The sensory evaluation also showed that the coated strawberries had better acceptance compared to the uncoated. The authors concluded that alginate coating could improve the physicochemical, sensory, and microbial qualities of fresh strawberries within cold storage conditions. The addition of ZnO NPs into alginate coating showed a synergistic effect toward the shelf life of the strawberries. Increasing concentration of ZnO NPs, up to 1.25 mg/L, showed improvement in the reduction in microbial numbers, physicochemical properties, and overall sensory acceptance. The authors also highlighted that cold storage temperature was also an important factor for strawberry preservation.

In the research conducted by Arroyo et al. (2020), the coating solution used consists of sodium alginate 5% *w*/*v*, glycerol 2% *v*/*v*, and ZnO NPs 1% v/v_{gel} to coat guavas by dipping for 3 min, and later dried at 25 °C [10]. The coated and uncoated guavas were then stored for 20 days with a temperature of 21 ± 1 °C and 80% RH. The experiment demonstrated that weight loss of coated and uncoated guavas was not significantly different after 20 days of storage. Hence, the author highlighted that the incorporation of ZnO NPs in the alginate coating system did not provide a water vapor barrier to prevent water and weight loss of guavas. The poor barrier of alginate coating was due to the little adherence of the coating solution toward the guavas epicarp, which might be caused by the low viscosity of the coating solution or the concentration of alginate used in the coating solution.

Aside from pure alginate coating with ZnO NPs, Arroyo et al. also fabricated a coating solution that consists of mixed alginate and chitosan (90–10%) in the concentration of 5% *w/v* to coat guavas [10]. The coating system showed improvement in reducing weight loss. Additional parameters observed including firmness, color, and ripening index ratio were also tested. The coated guavas had better maintained firmness, but the color was not significantly different to control guavas. The ripening index ratio was not significantly different to controlled guavas. The author concluded that the coating could delay the ripening process, reduce weight loss, and maintain the appearance of the guavas more effectively. However, it was not the best formulation pointed out by the authors.

3.3. Carrageenan

Carrageenan is a polysaccharide with gel-forming and thickening abilities originated from certain species of red seaweed. Carrageenan structure consists of an alternating unit of D-galactose and 3,6-anhydrogalactose (3,6-AG), which is connected through α-1,3 and β-1,4 glycosidic links. Based on its solubility on potassium chloride solution, carrageenan is classified into various types such as kappa (κ), iota (ι), and lambda (λ) carrageenan. Carrageenan can be utilized as an safe coating due to its potential as a film-forming material [58].

Meindrawan et al. investigated the application of carrageenan incorporated with ZnO NPs toward mangoes' (*Mangifera indica* L.) shelf-life [15]. Coating solutions were made with composition of the ZnO NPs (0%, 0.5%, and 1% *w/w* of carrageenan), 0.8 g carrageenan, and 0.5 mL of glycerol inside a 100 mL solution. The coatings were then applied to the selected mangoes by dipping inside the coating solution for 30 s and drained afterwards. Mangoes were then subjected to a controlled environment with a temperature of 20 °C and 61% RH. The incorporation of ZnO NPs into the carrageenan coating could reduce the weight loss of the coated mangoes, resulted from decreased water loss due to improved moisture barrier. However, carrageenan nanocomposite could not maintain the firmness of the coated mangoes compared to the uncoated mangoes. The authors mentioned that this may happen because the formation of ZnO NPs aggregation during production of the coating may influence the barrier ability of the coating, and there are many factors that cause mango softening that is not inhibited by the coating. Carrageenan coating with ZnO NPs reduced CO_2 production and maintained total acidity. These results showed the decreased in respiration rate. The appearance of coated mangoes was also better maintained in terms of color and textural appearance. The coated mangoes maintained their color better and showed less emergence of black color due to microbiological damages. Textural appearance of the coated mangoes tended to be more rigid, compared to the wrinkled texture of the uncoated. Overall, the author declared that carrageenan coating, fortified with ZnO NPs, could maintain the shelf life of mangoes. The author highlighted that the best formulation of the coating was 1% *w/v* ZnO NPs.

3.4. Carboxymethyl Cellulose

Cellulose is one of the most abundant organic compounds mainly found on biomass. However, utilization of cellulose itself is quite limited due to its low reactivity and solubility [59]. Hence, chemically modified cellulose is invented to improve its utilization and application. One of the most common industrially produced chemically modified cellulose is carboxymethyl cellulose (CMC). CMC is made through the carboxymethylation process of cellulose. CMC has good solubility and reactivity that are accredited to the reactive carboxyl and hydroxyl groups that cellulose do not have [59]. CMC application mainly includes thickener, emulsifier, and suspension agent [59]. It can also be utilized as a gelling agent in some circumstances. The advantages of CMC includes its non-toxicity, biodegradability, and biocompatibility [59]. Hence, CMC has potential to be utilized as safe coating.

Corporations of ZnO NPs in carboxymethyl cellulose (CMC) coatings for pomegranate (*Punica granatume* L.) arils were proposed by Saba and Amini [44]. The arils were dipped in ZnO NPs solution (0.1% and 0.2% *w/v*; size 30–100 nm) at 20 °C for 4 min, and excess water was removed afterwards. ZnO NPs treated arils were then dipped in CMC 0.5% *w/v*. As a control experiment, the arils were only dipped 4 min in distilled water. Arils were then air-dried at room temperature and stored for 12 days at 4 °C and 90% RH. The results showed that yeast, mold, and aerobic mesophilic bacteria growth were detected lower on coated arils until the end of storage time. The authors mentioned that the antimicrobial activities of the coating were resulted from ZnO NPs antimicrobial properties. Soluble solid concentration (SSC) of the arils decreased regardless of the treatments and the TA value increased with no significant difference between coated or uncoated arils at the end of storage time. The coating managed to reduce weight loss of the arils, indicating less water loss. However, amount of water loss did not cause a difference in juice content between coated and uncoated arils. Vitamin C loss across storage time was lower in coated arils compared to uncoated ones (Figure 3A). This was due to reduce endogenous oxygen by the coating, resulting in less ascorbic acid oxidation. Anthocyanin and phenolic content reduced along the storage time. However, the coated arils exhibited lower reduction than uncoated arils (Figure 3B,C). Antioxidant activities of arils increased along storage time, but coated arils showed higher antioxidant activities than the uncoated one (Figure 3D).

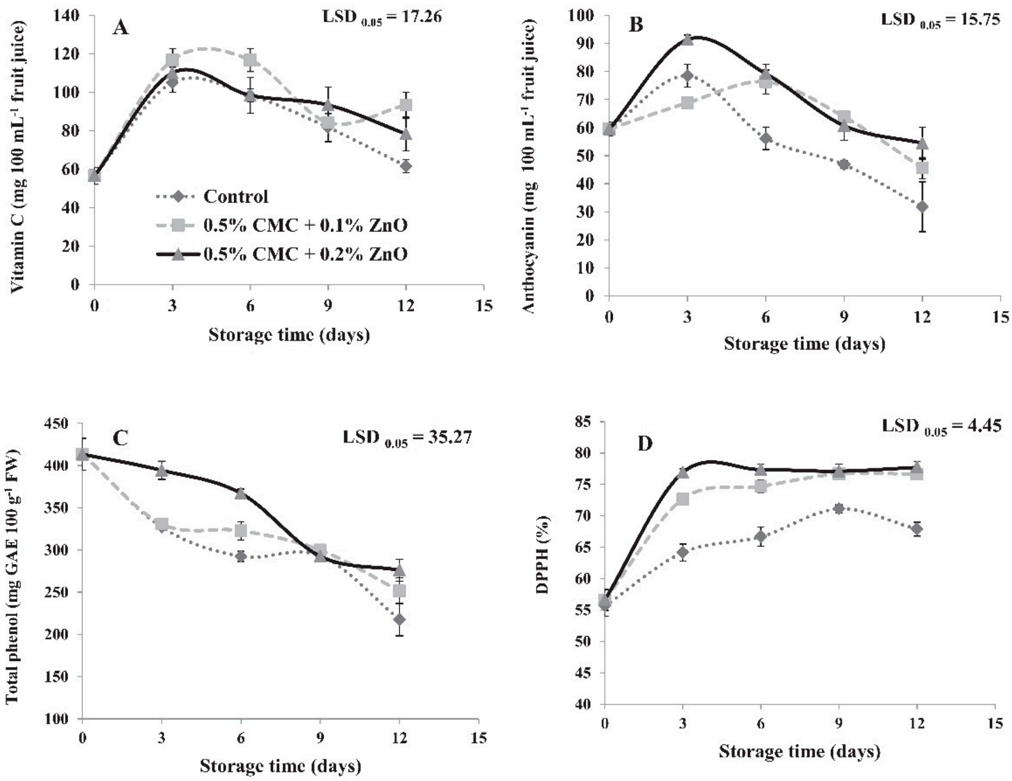

Figure 3. Storage time vs. total value of Vitamin C (**A**), anthocyanin (**B**), total phenol (**C**), and antioxidant capacity (**D**) in pomegranate arils either uncoated or coated with CMC-ZnO NPs. Reproduced with permission from [44]. Copyright 2017, Elsevier.

3.5. Pectin

Pectin is a complex polysaccharide found in plant cell walls that acts as a hydrating agent and cement for the cellulosic network. It is located on the primary cell wall and the main middle lamella, contributing to the firmness of plant tissue. Pectin is a block copolymer, which consists of block containing heteropolymer of rhamnogalacturonan ([1à2]α-L-rhamnosyl-[l-L]α-D-galactosyluronic acid) with side chains such as galactan and arabinan on rhamnose unit, and block of homopolymer galacturonic acid with varying degrees of methyl esterified carboxyl group. Pectin became among the growing interests in the food packaging industry due to its non-toxic, biocompatible, and biodegradable properties [60].

The application of pectin-ZnO nanocomposite safe coating for star fruit (*Averrhoa carambola*) has been investigated by Romadhan and Pujilestari [45]. The safe coating was composed of 0.1 g ZnO NPs, 10 g pectin, and 1 mL of glycerol inside 1 L aquadest. The coating was applied to star fruits by dipping for 20 s, followed draining the excess solution from the star fruit. The star fruits were then subjected to storage at room temperature (25 °C). The result of the experiment showed that the coated star fruits did not exhibit significant difference in weight loss compared to uncoated star fruits. The authors mentioned that single polysaccharide could give significant differences in maintaining weight loss because it contains many hydrophilic groups. The addition of ZnO NPs in small amounts only gave small reduction in weight loss (~1% compared to uncoated star fruits). The star fruits exhibited reduced browning index and redness value increases, showing decreases in ripening process. Physical damage on coated star fruit are also reduced. Until the end of storage time, the coated star fruits were still edible because physical damage was minimal. On the other hand, the uncoated star fruits were not edible at the end of storage time. The authors mentioned that damages on the star fruit may be caused by brown rot diseases that are caused by mold. The authors concluded that safe coating could increase the star fruit shelf life up to 2–3 days longer than the uncoated one.

4. Safety Issue of Zinc Oxide Nanoparticles as a Safe Coating Material

Food packaging is an essential part of food manufacturing. Due to the expanding mindfulness of consumers in terms of health issues, the migration level of food packaging materials is a crucial point in commercial food packaging. Consumers may hesitate and worry about consuming foods that are treated with ZnO NPs. However, little study about the migration of ZnO NPs from polysaccharide is available.

ZnO NPs migration from low-density polyethylene (LDPE)-ZnO nanocomposite film reported by Bumbudsanpharoke et al. in varying conditions [61]. Conditions used were distilled water to simulate aqueous food, acetic acid 4% *w/v* to simulate acidic food, ethanol 50% *v/v* to simulate alcoholic food, and n-heptane to simulate fatty food. The result showed that ZnO NPs performed the highest migration on acetic acid 4% *w*/*v*. The high migration rate of ZnO NPs might be accredited to its solubility on acetic acid, which was the highest compared to other simulating solutions used. On the other hand, there was no zinc detected on n-heptane simulating solution.

Another study also focused on ZnO NPs migration from commercially available polypropylene (PP)-ZnO nanocomposite food containers (Nano center, Ltd., Shanghai, China). Simulating solution placed on the container for migration test was distilled water, acetic acid 4% *v*/*v*, and n-heptane. The test was conducted with varying temperatures (30, 45, and 60 °C). The result showed that as storage time increased, the amount of migrated ZnO NPs also increased [62]. The rate of migration was higher under high-temperature conditions [62]. The migration of ZnO NPs was found to be higher on acetic acid and n-heptane [62]. The authors mentioned that organic food simulating solution had a swelling effect on polypropylene which caused a high migration concentration of ZnO NPs.

Even though ZnO is GRAS substance which is approved by the Food and Drug Administration (FDA) [63], the nano size of ZnO may generate toxic actions. Therefore, an assessment of ZnO NPs toxicity is needed. Research by Barkhordari et al. specifically tested the cytotoxicity of ZnO NPs (size 30–70 nm) toward the human spermatozoa cells [64]. The authors mentioned that ZnO NPs caused sperm cell death in a dose- and time-dependent manner. The result showed that ZnO NPs concentration up to 100 µg/mL, incubated up to 90 min with the spermatozoa, resulted in cell death less than 10%. Elsewhere, Wahab's research group introduced ZnO NPs to malignant cells (T98G Gliomas, and KB) and non-malignant cell (HEK) [65]. The result showed that ZnO NPs presented a depressing effect of the T98G cell growth, moderately effective on KB cells and least toxic on normal HEK cell. Namvar et al. also showed the similar effect of ZnO NPs on mice cells [66]. Namvar et al. tested ZnO against normal fibroblast cell of mice. For comparison, ZnO NPs were also tested against various cancer cells (4T1, CRL-1453, CT-26, and WEHI-3B), and cancer drugs (paclitaxel/PTX) were tested against cancer cell WEHI-3B. The results showed that the presence of ZnO NPs inhibited the proliferation of the various cancer cells (Figure 4a). Meanwhile, the presence of PTX also showed the inhibitory effect on WEHI-3B cancer cells (Figure 4b). On the other hand, ZnO NPs (100 µg/mL) did not show any toxicity toward normal fibroblast cell lines of mice using the MTT assay (Figure 4c).

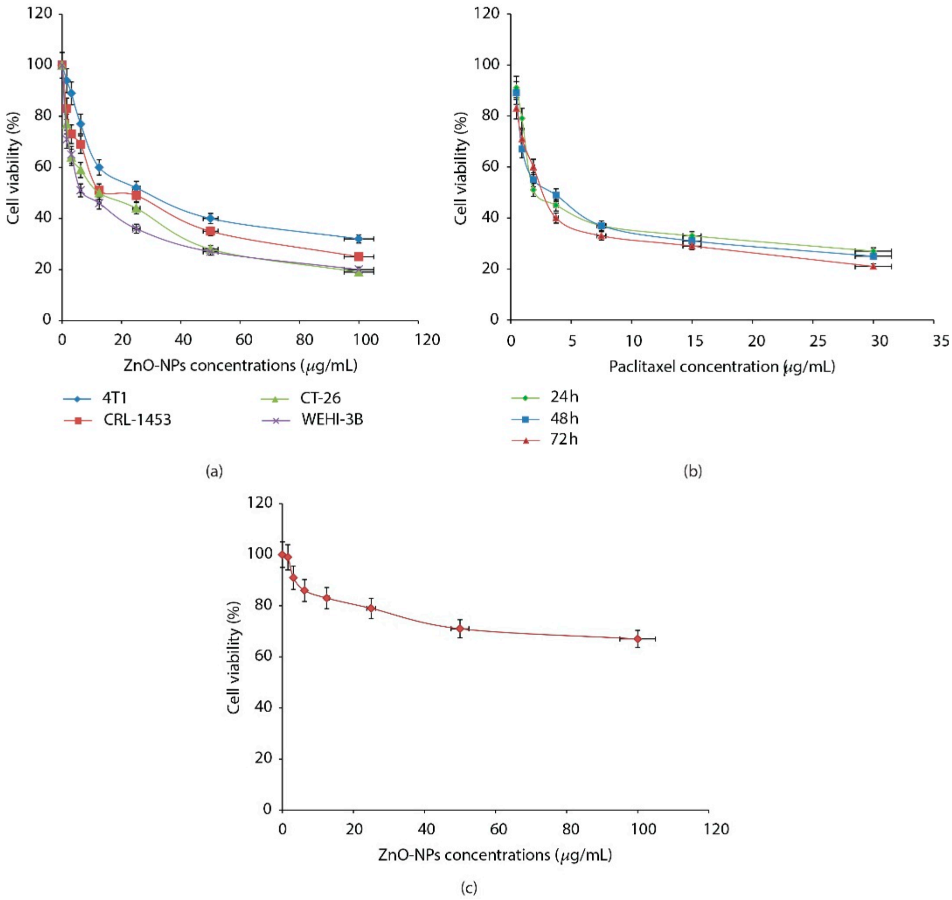

Figure 4. Cell viability evaluation of various cancer cells against ZnO NPs (**a**), cell viability evaluation of WEHI-3B cells against PTX (**b**), cell viability evaluation of normal mouse fibroblast cell against ZnO NPs (**c**). Reproduced with permission from [66] (copyright 2015, Hindawi).

5. Conclusions

There is a lot of added value in safe coating incorporated with ZnO NPs. Polysaccharide-ZnO nanocomposite can inhibit the ripening process. Varying polysaccharides have been used, including chitosan, alginate, carrageenan, cellulose. The deterioration process due to water loss and oxidation can also be prevented due to the improved gas and water barrier properties of nanocomposite material. The antimicrobial activity of ZnO NPs from the nanocomposite has also proven to be effective on coated fruits. The migration of ZnO NPs into the food does not pose a dangerous risk, as various cytotoxicity tests showed low toxicity of ZnO NPs. Hence, there is great potential for the utilization of ZnO NPs as a component for food packaging, as it has proven its ability to extend the shelf life of the food products, and safety issues are minimal. As for the future development of polysaccharide nanocomposite safe coating, it is necessary to investigate the migration of ZnO NPs from biopolymer instead of synthetic petroleum-based polymer to address the safety issue concerning even more accurately ZnO NPs migration.

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References

1. Marsh, K.; Bugusu, B. Food packaging—Roles, materials, and environmental issues: Scientific status summary. *J. Food Sci.* **2007**, *72*, R39–R55. [CrossRef] [PubMed]
2. Arfat, Y.A.; Ejaz, M.; Jacob, H.; Ahmed, J. Deciphering the potential of guar gum/Ag-Cu nanocomposite films as an active food packaging material. *Carbohydr. Polym.* **2017**, *157*, 65–71. [CrossRef] [PubMed]
3. De Silva, R.T.; Mantilaka, M.M.M.G.P.G.; Ratnayake, S.P.; Amaratunga, G.A.J.; Nalin de Silva, K.M. Nano-MgO reinforced chitosan nanocomposites for high performance packaging applications with improved mechanical, thermal and barrier properties. *Carbohydr. Polym.* **2017**, *157*, 739–747. [CrossRef] [PubMed]
4. Niu, X.; Liu, Y.; Song, Y.; Han, J.; Pan, H. Rosin modified cellulose nanofiber as a reinforcing and co-antimicrobial agents in polylactic acid /chitosan composite film for food packaging. *Carbohydr. Polym.* **2018**, *183*, 102–109. [CrossRef] [PubMed]
5. Guilbert, S.; Gontard, N.; Cuq, B. Technology and applications of edible protective films. *Packag. Technol. Sci.* **1995**, *8*, 339–346. [CrossRef]
6. Ncama, K.; Magwaza, L.S.; Mditshwa, A.; Tesfay, S.Z. Plant-based edible coatings for managing postharvest quality of fresh horticultural produce: A review. *Food Packag. Shelf Life* **2018**, *16*, 157–167. [CrossRef]
7. Murmu, S.B.; Mishra, H.N. The effect of edible coating based on arabic gum, sodium caseinate and essential oil of cinnamon and lemon grass on guava. *Food Chem.* **2018**, *245*, 820–828. [CrossRef]
8. Joshy, K.S.; Jose, J.; Li, T.; Thomas, M.; Shankregowda, A.M.; Sreekumaran, S.; Kalarikkal, N.; Thomas, S. Application of novel zinc oxide reinforced xanthan gum hybrid system for edible coatings. *Int. J. Biol. Macromol.* **2020**, *151*, 806–813. [CrossRef]
9. Gamage, T.V.; Rehman, M.S. Post harvest handling of foods of plant origin. In *Handbook of Food Preservation*; Rehman, M.S., Ed.; Marcel Dekker: New York, NY, USA, 1999; pp. 11–46.
10. Arroyo, B.J.; Bezerra, A.C.; Oliveira, L.L.; Arroyo, S.J.; de Melo, E.A.; Santos, A.M.P. Antimicrobial active edible coating of alginate and chitosan add ZnO nanoparticles applied in guavas (*Psidium guajava* L.). *Food Chem.* **2020**, *309*, 125566. [CrossRef]
11. Maftoonazad, N.; Ramaswamy, H.S.; Marcotte, M. Evaluation of factors affecting barrier, mechanical and optical properties of pectin-based films using response surface methodology. *J. Food Process Eng.* **2007**, *30*, 539–563. [CrossRef]
12. Moalemiyan, M.; Ramaswamy, H.S.; Maftoonazad, N. Pectin-based edible coating for shelf-life extension of Ataulfo mango. *J. Food Process Eng.* **2012**, *35*, 572–600. [CrossRef]
13. Hassan, B.; Chatha, S.A.S.; Hussain, A.I.; Zia, K.M.; Akhtar, N. Recent advances on polysaccharides, lipids and protein based edible films and coatings: A review. *Int. J. Biol. Macromol.* **2018**, *109*, 1095–1107. [CrossRef]
14. Sahraee, S.; Milani, J.M.; Regenstein, J.M.; Kafil, H.S. Protection of foods against oxidative deterioration using edible films and coatings: A review. *Food Biosci.* **2019**, *32*, 100451. [CrossRef]
15. Meindrawan, B.; Suyatma, N.E.; Wardana, A.A.; Pamela, V.Y. Nanocomposite coating based on carrageenan and ZnO nanoparticles to maintain the storage quality of mango. *Food Packag. Shelf Life* **2018**, *18*, 140–146. [CrossRef]
16. Abdul Khalil, H.P.S.; Tye, Y.Y.; Saurabh, C.K.; Leh, C.P.; Lai, T.K.; Chong, E.W.N.; Nurul Fazita, M.R.; Hafiidz, J.M.; Banerjee, A.; Syakir, M.I. Biodegradable polymer films from seaweed polysaccharides: A review on cellulose as a reinforcement material. *Express Polym. Lett.* **2017**, *11*, 244–265. [CrossRef]
17. Romanazzi, G.; Feliziani, E.; Baños, S.B.; Sivakumar, D. Shelf life extension of fresh fruit and vegetables by chitosan treatment. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 579–601. [CrossRef]
18. Cakmak, I.; Kutman, U.B. Agronomic biofortification of cereals with zinc: A review Roles of Zn in human physiology and health. *Eur. J. Soil Sci.* **2018**, *69*, 172–180. [CrossRef]
19. Mwangi, M.N.; Oonincx, D.G.A.B.; Stouten, T.; Veenenbos, M.; Melse-Boonstra, A.; Dicke, M.; van Loon, J.J.A. Insects as sources of iron and zinc in human nutrition. *Nutr. Res. Rev.* **2018**, *31*, 248–255. [CrossRef]
20. Sun, Q.; Li, J.; Le, T. Zinc Oxide Nanoparticle as a Novel Class of Antifungal Agents: Current Advances and Future Perspectives. *J. Agric. Food Chem.* **2018**, *66*, 11209–11220. [CrossRef]
21. Pavlath, A.E.; Orts, W. Edible films and coatings: Why, what, and how? In *Edible Films and Coatings for Food Applications*; Huber, K.C., Embuscado, M.E., Eds.; Springer: New York, NY, USA, 2009; pp. 1–23.
22. Nussinovitch, A. Hydrocolloids for coatings and adhesives. In *Handbook of Hydrocolloids*; Phillips, G.O., Williams, P.A., Eds.; Woodhead Publishing Series: Shaston, UK, 2009; pp. 760–806.

23. Andrade, R.D.; Skurtys, O.; Osorio, F.A. Atomizing spray systems for application of edible coatings. *Compr. Rev. Food Sci. Food Saf.* **2012**, *11*, 323–337. [CrossRef]
24. Sipahi, R.E.; Castell-Perez, M.E.; Moreira, R.G.; Gomes, C.; Castillo, A. Improved multilayered antimicrobial alginate-based edible coating extends the shelf life of fresh-cut watermelon (*Citrullus lanatus*). *LWT Food Sci. Technol.* **2013**, *51*, 9–15. [CrossRef]
25. Weiss, J.; Takhistov, P.; McClements, D.J. Functional materials in food nanotechnology. *J. Food Sci.* **2006**, *71*, R107–R116. [CrossRef]
26. Poverenov, E.; Danino, S.; Horev, B.; Granit, R.; Vinokur, Y.; Rodov, V. Layer-by-layer electrostatic deposition of edible coating on fresh cut melon model: Anticipated and unexpected effects of alginate–chitosan combination. *Food Bioprocess Technol.* **2014**, *7*, 1424–1432. [CrossRef]
27. Skurtys, O.; Acevedo, C.A.; Pedreschi, F.; Enrione, J.; Osorio, F.; Aguilera, J.M.C. *Food Hydrocolloid Edible Films and Coatings*; Hollingworth, C.S., Ed.; Nova Science Publishers, Inc.: New York, NY, USA, 2010.
28. Duan, J.; Wu, R.; Strik, B.C.; Zhao, Y. Effect of edible coatings on the quality of fresh blueberries (Duke and Elliott) under commercial storage conditions. *Postharvest Biol. Technol.* **2011**, *59*, 71–79. [CrossRef]
29. Youssef, A.M.; Assem, F.M.; El-Sayed, S.M.; Salama, H.; Abd El-Salam, M.H. Utilization of Edible Films and Coatings as Packaging Materials for Preservation of Cheeses. *J. Packag. Technol. Res.* **2017**, *1*, 87–99. [CrossRef]
30. Ahmadian-Yazdi, M.R.; Zabihi, F.; Habibi, M.; Eslamian, M. Effects of Process Parameters on the Characteristics of Mixed-Halide Perovskite Solar Cells Fabricated by One-Step and Two-Step Sequential Coating. *Nanoscale Res. Lett.* **2016**, *11*, 408. [CrossRef]
31. Yang, J.; Blawert, C.; Lamaka, S.V.; Snihirova, D.; Lu, X.; Di, S.; Zheludkevich, M.L. Corrosion protection properties of inhibitor containing hybrid PEO-epoxy coating on magnesium. *Corros. Sci.* **2018**, *140*, 99–110. [CrossRef]
32. Tharanathan, R.N. Biodegradable films and composite coatings: Past, present and future. *Trends Food Sci. Biotechnol.* **2003**, *14*, 71–78. [CrossRef]
33. Norton, T.; Sun, D.W. Computational fluid dynamics (cfd)—An effective and efficient design and analysis tool for the food industry: A review. *Trends Food Sci. Biotechnol.* **2006**, *17*, 600–620. [CrossRef]
34. Guillemin, A.; Degraeve, P.; Noel, C.; Saurel, R. Influence of impregnation solution viscosity and osmolarity on solute uptake during vacuum impregnation of apple cubes (var. Granny smith). *J. Food Eng.* **2008**, *86*, 475–483. [CrossRef]
35. Vargas, M.; Chiralt, A.; Albors, A.; Gonzalez-Martinez, C. Effect of chitosan-based edible coatings applied by vacuum impregnation on quality preservation of fresh-cut carrot. *Postharvest Biol. Technol.* **2009**, *51*, 263–271. [CrossRef]
36. Parreidt, T.S.; Schmid, M.; Muller, K. Effect of dipping and vacuum impregnation coating techniques with alginate based coating on physical quality parameters of cantaloupe melon. *J. Food Sci.* **2018**, *83*, 929–936. [CrossRef]
37. Trandafilović, L.V.; Božanić, D.K.; Dimitrijević-Branković, S.; Luyt, A.S.; Djoković, V. Fabrication and antibacterial properties of ZnO-alginate nanocomposites. *Carbohydr. Polym.* **2012**, *88*, 263–269. [CrossRef]
38. Yousuf, B.; Qadri, O.S.; Srivastava, A.K. Recent developments in shelf-life extension of fresh-cut fruits and vegetables by application of different edible coatings: A review. *LWT Food Sci. Technol.* **2018**, *89*, 198–209. [CrossRef]
39. Kanmani, P.; Rhim, J.W. Properties and characterization of bionanocomposite films prepared with various biopolymers and ZnO nanoparticles. *Carbohydr. Polym.* **2014**, *106*, 190–199. [CrossRef]
40. Mohammadi, H.; Kamkar, A.; Misaghi, A. Nanocomposite films based on CMC, okra mucilage and ZnO nanoparticles: Physico mechanical and antibacterial properties. *Carbohydr. Polym.* **2018**, *181*, 351–357. [CrossRef] [PubMed]
41. Oun, A.A.; Rhim, J.-W. Preparation of multifunctional chitin nanowhiskers/ZnO-Ag NPs and their effect on the properties of carboxymethyl cellulose-based nanocomposite film. *Carbohydr. Polym.* **2017**, *169*, 467–479. [CrossRef]
42. Lavinia, M.; Hibarturrahman, S.N.; Harinata, H.; Wardana, A.A. Antimicrobial activity and application of nanocomposite coating from chitosan and ZnO nanoparticle to inhibit microbial growth on fresh-cut papaya. *Food Res.* **2019**, *4*, 307–311. [CrossRef]

43. Emamifar, A.; Bavaisi, S. Nanocomposite coating based on sodium alginate and nano-ZnO for extending the storage life of fresh strawberries (Fragaria × ananassa Duch.). *J. Food Meas. Charact.* **2020**, *14*, 1012–1024. [CrossRef]
44. Koushesh Saba, M.; Amini, R. Nano-ZnO/carboxymethyl cellulose-based active coating impact on ready-to-use pomegranate during cold storage. *Food Chem.* **2017**, *232*, 721–726. [CrossRef]
45. Romadhan, M.F.; Pujilestari, S. Sintesis nanopartikel ZnO dan aplikasinya sebagai edible coating berbasis pektin untuk memperpanjang umur simpan buah belimbing. *Jurnal Agroindustri Halal* **2019**, *5*, 030–038. [CrossRef]
46. Jayakumar, R.; Menon, D.; Manzoor, K.; Nair, S.V.; Tamura, H. Biomedical applications of chitin and chitosan based nanomaterials—A short review. *Carbohydr. Polym.* **2010**, *82*, 227–232. [CrossRef]
47. Venkatesan, J.; Kim, S.-K. Chitosan composites for bone tissue engineering—An overview. *Mar. Drugs* **2010**, *8*, 2252–2266. [CrossRef]
48. Croisier, F.; Jerome, C. Chitosan-based biomaterials for tissue engineering. *Eur. Polym. J.* **2013**, *49*, 780–792. [CrossRef]
49. Rinaudo, M. Chitin and chitosan: Properties and applications. *Prog. Polym. Sci.* **2006**, *31*, 603–632. [CrossRef]
50. Campos, C.A.; Gerschenson, L.N.; Flores, S.K. Development of edible films and coatings with antimicrobial activity. *Food Bioprocess Technol.* **2010**, *4*, 849–875. [CrossRef]
51. Ferreira, C.O.; Nunes, C.A.; Delgadillo, I.; Lopes-da-Silva, J.A. Characterization of chitosan-whey protein films at acid pH. *Food Res. Int.* **2009**, *42*, 807–813. [CrossRef]
52. Khor, E.; Lim, L.Y. Implantable applications of chitin and chitosan. *Biomaterials* **2003**, *24*, 2339–2349. [CrossRef]
53. Li, Q.; Dunn, E.T.; Grandmaison, E.W.; Goosen, M.F.A. Applications and properties of chitosan. *J. Bioact. Compat. Polym.* **1992**, *7*, 370–397. [CrossRef]
54. Romanazzi, G.; Feliziani, E.; Santini, M.; Landi, L. Effectiveness of postharvest treatment with chitosan and other resistance inducers in the control of storage decay of strawberry. *Postharvest Biol. Technol.* **2013**, *75*, 24–27. [CrossRef]
55. Anugrah, D.S.B.; Ramesh, K.; Kim, M.; Hyun, K.; Lim, K.T. Near-infrared light-responsive alginate hydrogels based on diselenide-containing cross-linkage for on demand degradation and drug release. *Carbohydr. Polym.* **2019**, *223*, 115070. [CrossRef]
56. Lee, K.Y.; Mooney, D.J. Alginate: Properties and biomedical applications. *Prog. Polym. Sci.* **2012**, *37*, 106–126. [CrossRef] [PubMed]
57. Parreidt, T.S.; Müller, K.; Schmid, M. Alginate-based edible films and coatings for food packaging applications. *Foods* **2018**, *7*, 170. [CrossRef] [PubMed]
58. Shojaee-Aliabadi, S.; Hosseini, H.; Mohammadifar, M.A.; Mohammadi, A.; Ghasemlou, M.; Hosseini, S.M.; Khaksar, R. Characterization of κ-carrageenan films incorporated plant essential oils with improved antimicrobial activity. *Carbohydr. Polym.* **2014**, *101*, 582–591. [CrossRef]
59. Pettignano, A.; Charlot, A.; Fleury, E. Carboxyl-functionalized derivatives of carboxymethyl cellulose: Towards advanced biomedical applications. *Polym. Rev.* **2019**, *59*, 510–560. [CrossRef]
60. Nešić, A.; Gordić, M.; Davidović, S.; Radovanović, Ž.; Nedeljković, J.; Smirnova, I.; Gurikov, P. Pectin-based nanocomposite aerogels for potential insulated food packaging application. *Carbohydr. Polym.* **2018**, *195*, 128–135. [CrossRef] [PubMed]
61. Bumbudsanpharoke, N.; Choi, J.; Park, H.J.; Ko, S. Zinc migration and its effect on the functionality of a low density polyethylene-ZnO nanocomposite film. *Food Packag. Shelf Life* **2019**, *20*. [CrossRef]
62. Liu, J.; Hu, J.; Liu, M.; Cao, G.; Gao, J.; Luo, Y. Migration and characterization of nano-zinc oxide from polypropylene food containers. *Am. J. Food Technol.* **2016**, *11*, 159–164. [CrossRef]
63. Jin, T.; Sun, D.; Su, J.Y.; Zhang, H.; Sue, H.-J. Antimicrobial efficacy of zinc oxide quantum dots against Listeria monocytogenes, Salmonella enteritidis, and Escherichia coli O157:H7. *J. Food Sci.* **2009**, *74*, 46–52. [CrossRef]
64. Barkhordari, A.; Hekmatimoghaddam, S.; Jebali, A.; Khalili, M.A.; Talebi, A.; Noorani, M. Effect of zinc oxide nanoparticles on viability of human spermatozoa. *Int. J. Reprod. Biomed.* **2013**, *11*, 767–771.

65. Wahab, R.; Kaushik, N.K.; Kaushik, N.; Choi, E.H.; Umar, A.; Dwivedi, S.; Musarrat, J.; Al-Khedhairy, A.A. ZnO Nanoparticles Induces Cell Death in Malignant Human T98G Gliomas, KB and Non-Malignant HEK Cells. *J. Biomed. Nanotechnol.* **2013**, *9*, 1181–1189. [CrossRef] [PubMed]
66. Namvar, F.; Rahman, H.S.; Mohamad, R.; Azizi, S.; Tahir, P.M.; Chartrand, M.S.; Yeap, S.K. Cytotoxic effects of biosynthesized zinc oxide nanoparticles on murine cell lines. *Evid. Based Complement. Altern. Med.* **2015**, *2015*. [CrossRef] [PubMed]