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Composites Bioplastic Film for Various Concentration of Zinc Oxide (ZnO) Nanocrystals Towards Physical Properties for High Biodegradability in Soil and Seawater

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Abstract

The effect of various amount of ZnO nanocrystals (NCs) in composite starch/ZnO (PZ) and starch/chitosan/ZnO (PK) to the physical, structural, biodegradation, and antibacterial properties have been synthesized using casting method. The amount of ZnO are 3%, 6%, 10%, 13%, and 16%, and distilled water in acetic acid/glycerin/cassava starch was incorporated into gelatin films. We successfully demonstrated that, the ZnO NCs highly contributing to the crystalline phase and tensile strength of bioplastics. The highest value of tensile strength is for 13% ZnO-bioplastics which could be completely decomposed both in ordinary soil and in seawater for less than 30 days. The packaging tests revealed that an increase in microbial shelf life of sliced bread from 10 to 30 days for PZ and PK samples. All active coatings lessened the number of molds in sliced bread for 30 days, and further improvement in antimicrobial properties obtained for coatings contains 10, 13, and 16% ZnO NCs with no fungal growth for 30 days. In this study shows potential of ZnO NCs to be applied as an environmentally friendly bioplastics starch and starch/chitosan-based to improve shelf life as a food packaging.

Keywords Bioplastics · Zinc oxide · Cassava starch · Chitosan · Seawater

Introduction

Plastics are widely used by people for primary to tertiary needs, such as electronic, food, and drug packaging. In general, raw materials of plastic are polymers [1] which have advantages including strong mechanical properties, cheapness, lightness, and easy in manufacturing and application. However, plastic still has many shortcomings, some of which are not readily biodegradable by the environment, either by rainy or hot weather and microbes that live in the soil [2]. Therefore, a solution is needed to overcome environmental problems by developing biodegradable plastic (bioplastics) materials which can be naturally decomposed by microorganisms in short time of period.

Starch-based bioplastics have several advantages, including renewable, biodegradable, and easy to process from the base materials such as potato starch [3], cassava starch [4,

5], corn starch [6], wheat starch [6], jackfruit seed starch [7], and sago starch [8]. However, bioplastic starch-based has solid hydrophilic properties and lower mechanical properties when compared to synthetic polymers. Starch is also mostly water-soluble and decomposes before undergoing gelatinization [9]. Therefore, it is necessary to have additives such as chitosan that can increase the properties of starch-bioplastic [10]. Chitosan has several valuable inherent traits, such as being non-toxic, biodegradable, renewable, and biocompatible, making it ideal for numerous emerging food applications [11]. Based on research Galaz et al., the bioplastics with the addition of 1% and 2% chitosan shows good mechanical properties, and Yu et al. reported that the bioplastic film with the addition of chitosan degraded for 30 days with a total weight loss of 63% [12]. However, bioplastic films with the addition of chitosan still have poor flexibility [13], so it is necessary to add nanoparticles as reinforcement [14].

In recent years, the incorporation of nanoparticles (NPs) composite materials has attracted much attention for its ability to enhance the thermal, mechanical, and gaseous properties of polymers and act as antibacterial agents [14]. Several metal oxide NPs, such as zinc oxide (ZnO) [14–20], silicon dioxide (SiO₂) [3], titanium dioxide (TiO₂)

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[16], calcium carbonate (CaCO_3) [21] and others. With this addition, composite materials containing nanoparticles can produce high-performance and innovative materials. Inorganic nanoparticles mainly improve interfacial interactions in bioplastics and significantly improve the mechanical properties of the matrix. According to the literature, all mechanical and thermal properties of composites bioplastic will improve by adding inorganic nanoparticles [3].

Zinc oxide (ZnO) nanoparticles have been in great demand as nanofillers because they show good biocompatibility, are harmless and have superior mechanical properties that will result in a better transfer of the mechanical properties of ZnO to the polymer matrix [22]. In addition, ZnO is a multifunctional nanofiller due to its antimicrobial activity [23] and strict UV absorption. ZnO has a more effective antibacterial effect than other metal oxides. The antibacterial activity of ZnO comes from the ability to produce reactive oxygen species, which results in the oxidation of the cytoplasm of bacterial cells, resulting in cell death [19, 20, 23, 24]. Many researchers have investigated the strengthening of polymer composites using ZnO for active food packaging and drug manufacturing [25]. Abdullah et al. reported that, at a concentration of 4%, ZnO showed maximum results for tensile strength, low hydrophobicity, and a strong interaction between ZnO and starch matrix as an increase in the thermal stability of bioplastics [17]. The ZnO also shows barrier activity against enzymatic degradation, which is very suitable for food packaging applications. Anugrahwidya et al. concluded that incorporating the essential ingredients of bioplastics, namely starch, chitosan, and nanoparticles, can improve mechanical properties, good biodegradability, and high tensile strength values [23].

Based on the explanation above, this research focuses on studying the effect of variations of ZnO nanocrystals on bioplastic films in the form of starch/ZnO and starch/chitosan/ZnO. ZnO NCs affects the physical properties, structure, and biodegradation of bioplastics so that they could have high potentials to be applied for mass production as food packaging in the future.

Materials and Methods

Materials

Glycerin ($\text{C}_3\text{H}_8\text{O}_3$) (molecular weight 92.09, boiling point 290°C) (Merck), acetic acid (CH_3COOH) with concentration of 5% (Merck), sulfuric acid (H_2SO_4) with concentration 1 Molar (Merck), chitosan, cassava starch, and aquades (Merck). Material preparation was carried out with 2 sample variations, namely: Starch + ZnO (PZ), and Starch + Chitosan + ZnO (PK).

Bio-composite Sample Preparations

Bioplastic Film Preparation Without ZnO

Based on Mutmainna et al. [26], with some modifications, bioplastic film preparation in this study using casting method. First, mixture 20 ml distilled water in acetic acid/glycerin/starch with the weight ratio of 2/4/6 into a beaker, then homogenized using a magnetic stirrer. The obtained bioplastic gel film was put into a mold and heated for 24 h which was covered with aluminum foil [26] with namely sample PZ.

Bioplastic Film Preparation with ZnO

For preparation bioplastic film using casting method: first, mixture 20 ml distilled water in acetic acid/glycerin/starch with weight ratio of 2/4/6 into a beaker [26] and add 3% of ZnO, then homogenized using a magnetic stirrer [20]. Second, the obtained bioplastic gel put into a mold and heated using a microwave for 24 h covered with aluminum foil. The bioplastic preparation was repeated for various amount of ZnO: 3%, 6%, 10%, 13%, and 16% with namely samples PZ1, PZ2, PZ3, PZ4, and PZ5, respectively.

ZnO Bioplastic Preparation with Chitosan

The preparation bioplastic film by casting method, first, mixture 20 ml distilled water in acetic acid/glycerin/starch with weight ratio of 2/4/6 into a beaker [26] and add 3% of ZnO [20], then homogenized using magnetic stirrer to form a gel. Second, 8% chitosan (of the total weight of starch) was dissolved in sulfuric acid and mixed into the starch-bioplastic gel, then homogenized again [26, 27]. Third, the obtained bioplastic gel was put into a mold and heated using a microwave for 14 h covered with aluminum foil. The bioplastic preparation was repeated for various amount of ZnO: 3%, 6%, 10%, 13%, and 16% are respectively: for namely PK1, PK2, PK3, PK4, and PK5. The ZnO bioplastic film preparation method can be seen in Fig. 1.

Bioplastic Film Sample Testing Preparation

Water Absorption Test

Bioplastics were cut to a size of $2 \times 2 \text{ cm}^2$ and then weighed to get the initial mass. The absorption capacity of bioplastic samples was obtained by immersing the sample in water for 1 h, then weighed and soaked again for 1 h.

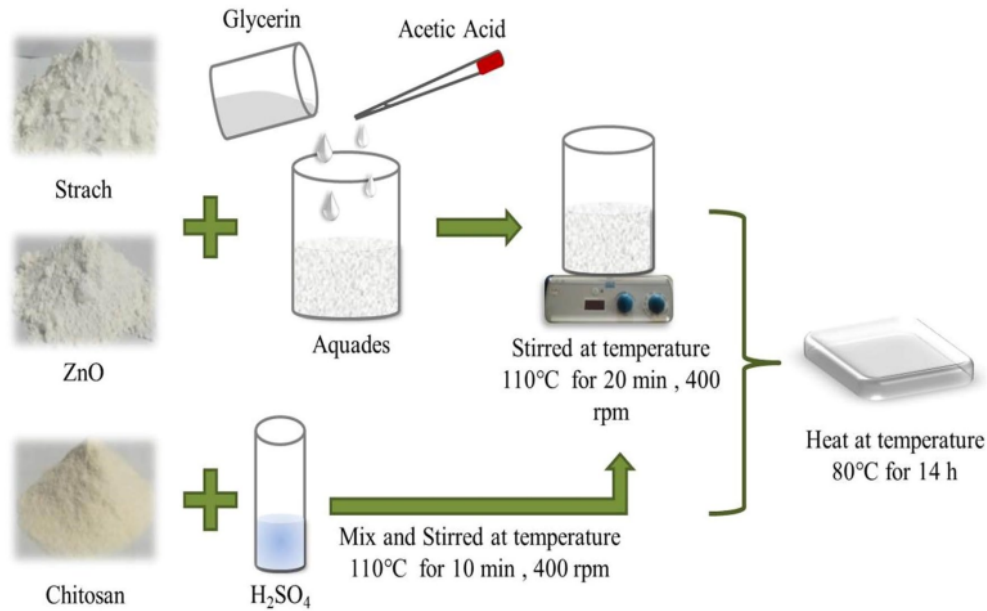


Fig. 1 The preparation scheme of ZnO/starch/chitosan-bioplastics

This cycle lasts until it reaches saturation. The absorption capacity of bioplastics is formulated by Eq. 1 [28]:

$$\text{Water Absorption (\%)} = \frac{m_1 - m_0}{m_0} \times 100\% \quad (1)$$

where m_0 and m_1 are the initial and final masses, respectively.

XRD (X-Ray Diffraction Spectrum) Characterization

The X-Ray diffraction (XRD) was used for analyzing the structural properties, (Shimadzu 7000 X-ray diffraction) with $\text{CuK}\alpha$ radiation ($\lambda = 1.5405 \text{ \AA}$) for 2θ from 5° to 60° , operates at 40 kV and 30 mA. All sample variations were cut in circles with a diameter of 1.5 cm [26, 27].

Packaging Test

The test was carried out to see the feasibility of bioplastic film as food packaging. The bioplastic film is made with a size of $10 \times 10 \text{ cm}^2$, then 6 slices of white bread with a size of $2 \times 2 \text{ cm}^2$ are wrapped using bioplastic films. This test was carried out for 30 days with so modification from reference [14] to see the resistance of the bread.

Tensile Strength Test

The tensile test was carried out to determine the mechanical properties of the bioplastic sample by cut to a size of $5 \times 3 \text{ cm}^2$ [26, 27].

Biodegradation Test

The degradation test serves to determine the level of bioplastic decomposition. The time the sample decomposes can be seen from the mass reduction of each specimen planted in the soil. Samples were cut to a size of $3 \times 3 \text{ cm}^2$, then weigh the sample before planting. The samples were planted in 2 types of soil, namely ordinary soil, and seawater for duration of 7 days, 14 days, 21 days, and 28 days. After the stockpiling period the initial weight and weight after stockpiling were measured and the biodegradation calculated by Eq. 2 [28]:

$$\text{Biodegradation (\%)} = \frac{m_0 - m_1}{m_0} \times 100\% \quad (2)$$

where m_0 and m_1 are the initial and final masses, respectively.

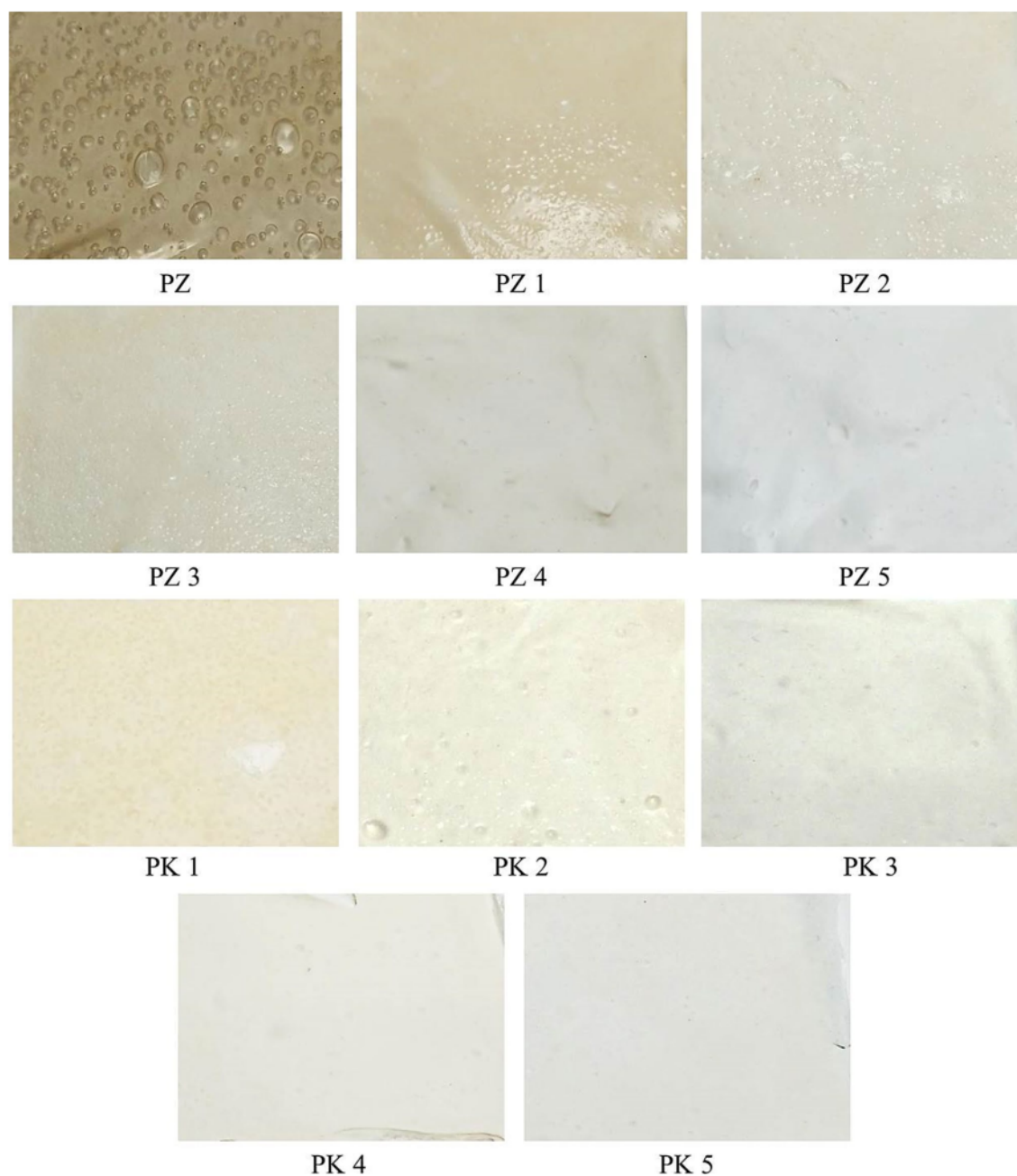


Fig. 2 Optical photograph view of PZ and PK sample variations

Results and Discussion

There are 2 types of bioplastic film samples as shown in Fig. 2 to be discussed, namely PZ samples (Starch + without

and with ZnO), and PK samples (Starch + ZnO + Chitosan) for various ZnO NCs concentration. Bioplastic samples were characterized using XRD, tensile strength test, water absorption test, biodegradation test, and packaging test.

Synthesizing Bioplastic Films

The bioplastic obtained without the addition of ZnO (PZ) produced a darker and transparent color also had many big pores in the bioplastic but had a good level of elasticity compared to other bioplastics (PZ 1–PZ 5).

The whiter the bioplastics produced and became stiffer with the increasing ZnO concentration. ZnO acts as a nano-filler that covers the pores of the starch-bioplastics matrix [29] so that bioplastics containing ZnO concentration tend to be stiffer. Gutierrez et al. reported that the better interaction between ZnO and starch, the more significant transfer of nanoparticles to the starch-based matrix, and the mechanical properties increase [22]. This phenomenon causes the pores in starch-based bioplastics to become less and more negligible.

Starch + ZnO bioplastic obtained by adding chitosan (PK 1) is thin, less transparent, and has many tiny pores than PZ. The resulting bioplastic (PK 2–PK 5) is not transparent, and the pores formed are reduced with the ZnO concentration increases. As the ZnO concentration increases, the resulting bioplastic (PK 2–PK 5) is stiffer, not transparent and

the pores formed are reduced. Bioplastic with chitosan has a solid and sour smell due to the sulfuric acid used as a solvent for chitosan [30]. The addition of chitosan to bioplastics will increase degradation [11, 12] and the mechanical properties of bioplastics [10] so that the chitosan can increase the performance of ZnO as filler in the starch-bioplastic matrix. Figure 2 shows the bioplastic film for the PZ and PK variation.

Water Absorption Test

An absorption test determines the resistance of bioplastic samples to water, measured every hour for 7 h, and Eq. (1) determines the percentage of moisture absorption. A graph of the absorption test results for PZ and PK samples with various ZnO concentrations is in Fig. 3a, b, respectively.

Figure 3a shows, the bioplastic sample is saturated when it enters the 4 h of immersion, and after the 7 h, the bioplastic sample will experience a saturation phase. Thus, for the PZ sample variation, the bioplastic resistance to water absorption lasts for 7 h, whereas the sample lasts only 3 h in the saturation phase before reaching the saturation phase.

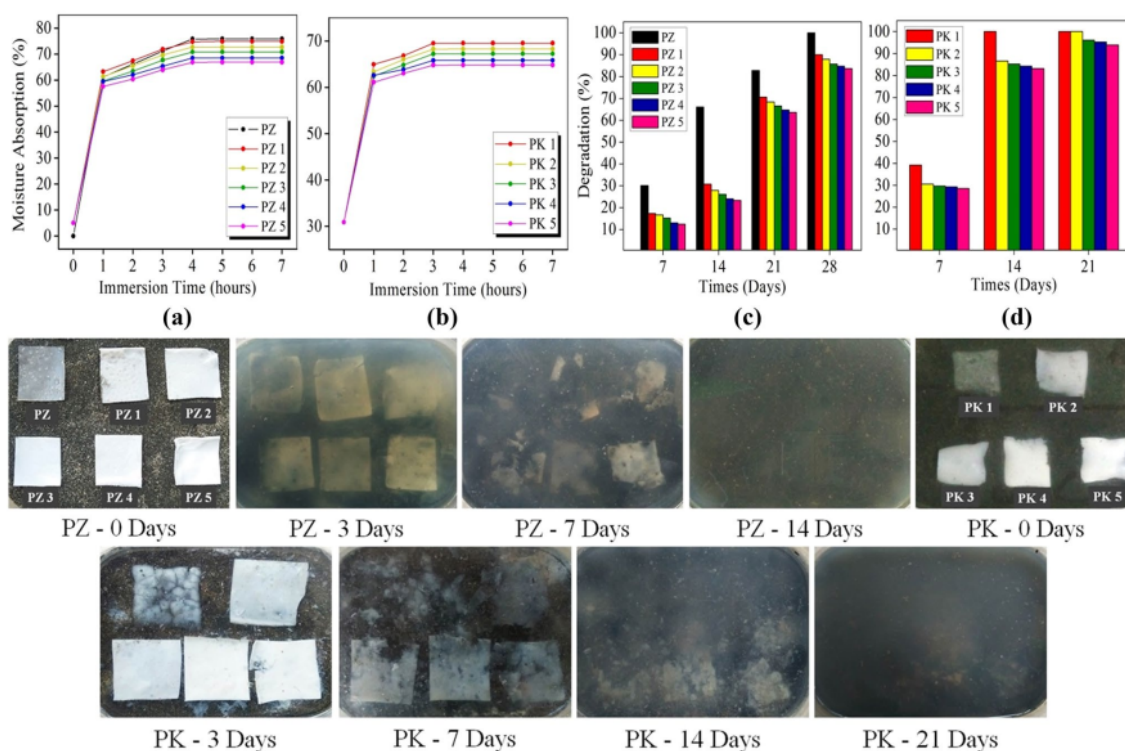


Fig. 3 a The water absorption percentage of PZ sample and b PK sample, c percentage degradation of PZ samples and d PK samples in ordinary soil test, also the photograph visual seawater degrada-

tion test of PZ samples (Starch+ with and without ZnO) for 0 days, 3 days, 7 days, 14 days and PK samples (Starch+Chitosan+ZnO) for 0 days, 3 days, 7 days, 14 days, and 21 days

The maximum value obtained is 75.90% for the PZ composition (0% of ZnO), and the minimum value is 61.88% for the PZ 5 composition (16% ZnO). A significant decrease in absorption value from PZ to PZ 1 is 75.90% to 69.88%, respectively, proving that ZnO has hydrophobic properties.

The increased concentration of ZnO (PK 1–PK 5) contributed to decreasing the percentage of bioplastic to absorb the water [15]. In the variation of PK samples, there was an addition of chitosan composition, which indicated strengthening mechanical properties and reducing the percentage of bioplastic samples' absorption. Based on the results obtained, the absorption capacity of the PK sample variation decreased with the addition of chitosan compared to the PZ samples because the chitosan is hydrophobic [4]. According to Bangyekan et al., hydrophobic acetyl groups of chitosan that is not entirely deacetylated can consequently block water vapor transport, making the material's surface more hydrophobic [31]. Another study also presented results showing a decrease in water absorption with chitosan [32].

Figure 3b shows, the bioplastic sample is saturated when it enters the 3 h of immersion, and after the 7 h, the bioplastic sample will experience a saturation phase. Thus, for the PK sample variation, the bioplastic resistance to water absorption only lasts for 7 h, whereas the sample lasts 4 h in the saturation phase before reaching the saturation phase. The addition of chitosan into bioplastics causes a decrease in the percentage of water absorption, wherein the sample PZ 1, the maximum value obtained is 69.88%. In comparison, PK 1 is 65.73% and continues to experience a decrease in absorption with increasing ZnO concentration. Chitosan has hydrophobic properties and reduces the moisture content of the bioplastic, which affects the intermolecular forces; chitosan will insert between the bioplastic polymers [10, 12, 13]. The lower the percentage of water absorption, the better bioplastic is against water resistance, while the higher the percentage of water absorption, the properties of the bioplastic will be easily damaged. Thus, with the addition of chitosan, it can be indicated that it will help ZnO's performance to produce bioplastics with good mechanical properties.

Soil Degradation Test

Degradation testing on bioplastic samples determines the bioplastic sample's degradation rate or breakdown rate in the environment. The degradation test is used as a parameter to determine the environmentally friendly nature of the bioplastic samples [12] and the percentage of degradation by Eq. (2). The bioplastic degradation data for PZ samples with 7, 14, 21, and 28 days is displayed in Fig. 3c, and the bioplastics for PK samples with 7, 14, and 21 days are shown in Fig. 3d.

Bioplastics without adding ZnO (PZ) lost mass by 30.05% on the 7th day, 66.06% on the 14th day, 82.81% on the 21st day, and 100% after 28 days. However, the PZ 1 sample mass lost 17.35% on the 7th day, 30.74% on the 14th day, 70.53% on the 21st day, and 89.98% after 28 days. The bioplastics without ZnO show a higher level of degradation than those with the addition of ZnO because the starch has an OH hydroxyl group, which ionizes the hydrolysis reaction after absorbing water from the soil. Water absorbed by the sample causes thickening, which accelerates the degradation process [17, 19, 33, 34].

The percentage of degradation of PZ 1–PZ five samples on the seventh day in a row was 17.35%, 16.74%, 15.23%, 13.05%, and 12.36%, after 28 consecutive days 89.98%, 87.97%, 85.65%, 84.70%, and 83.62%, the test results show that the addition of ZnO slows down the degradation process. ZnO can limit the movement of molecules to act as a barrier to the diffusion rate of water into bioplastics and has low hydrophobicity [33]. In addition, the addition of ZnO can increase the interaction between molecules so that the resulting bioplastic has a tight and rigid texture which causes microorganisms not quickly degrade bioplastics [22, 29]. Similar results were also presented by Amni et al. using Jeneng starch with variations of ZnO, revealing that the more ZnO added could affect the biodegradation time of the sample. Bioplastic with a ratio of 1% (by weight of starch) ZnO shows completely degraded on the 30th day, but the bioplastic for 6% ZnO experience more extended degradation until the 36th day may be due to the large amount of ZnO contained in bioplastics, making that more durable [9]. ZnO has metal reinforcing properties that make use of piezoelectric and antimicrobial ceramic. The degradation test using cassava starch with variation of ZnO by Abdullah et al. stated that, after seven days of degradation test, bioplastics with ZnO showed a high growth rate of microorganisms with covered more than 60% microorganisms. Degradation was less noticeable at high ZnO content, indicating that enzymatic degradation inhibited in the samples due to the antimicrobial properties of ZnO [17]. ZnO will produce hydrogen peroxide, which is harmful to microbial cell membranes. Furthermore, the release of Zn²⁺ ions from ZnO nanoparticles can penetrate the microbial cell wall and attack the interior, affecting microbial cell survival [18, 22, 23]. The percentage degradation of PZ samples is in Fig. 3c.

The PK samples degraded faster than PZ samples; PK 1 on the 7th day shows a higher percentage of degradation than the PK 2–PK 5 samples because the ZnO concentration was smaller than chitosan. Thus, PK 1 decomposes faster with a degradation percentage of 39.16%, while for PK 2–PK 5, it is only 30.45%, 29.65%, 29.36%, and 28.59%, respectively. On the 14th days, PK 1 samples were completely degraded compared to other samples with only 83–86%. This phenomenon is due to the presence of ZnO, whose concentration is

greater than that of chitosan, and the antimicrobial properties of ZnO [18] so that microorganisms from the soil are not easy to develop as natural destroyer's bioplastic samples. On the 21th day, the PK 2 samples were 100% degraded, and for PK 3–PK 5, the percentage degradation was 96.15%, 95.29%, and 93.93%, respectively. Compared to the PZ 1–PZ 5 samples, all PK samples degraded faster with the addition of chitosan. Because chitosan is a linear polysaccharide, the deacetylated derivative of this polymer is chitin. Chitin is the second-largest type of polysaccharide in nature after cellulose which is in exoskeleton invertebrates and has several functions on its cell walls. The various absorption test of PK samples in Fig. 3b says that chitosan is hydrophobic [10, 11], so it is difficult to absorb water and can inhibit the degradation process due to the lack of water content in the sample. However, chitosan comes from organic material, easily decomposed by microbes in the soil. Thus, this is causing the PK variation sample to decompose faster than the PZ sample variation, and as the ZnO concentration increases, it will decrease the percentage of degradation of the bioplastic sample [29, 33]. For biological degradation processes of polymers, there are active enzymes extracellular and intracellular depolymerizes [23, 35, 36]. During degradation under aerobic and anaerobic conditions processes, exoenzymes from microorganisms destroy complex polymers by breaking the carbon bond to become smaller oligomers, dimers, or monomers molecules called the depolymerization process [23, 35]. Polymeric materials produced from natural molecule are easier to degrade and mineralize by heterotrophic microorganisms in a wide range of natural environments. The natural environment where anaerobic processes take place where a polymer's complete decomposition will produce CO₂, CH₄, and H₂O. The biodeterioration and degradation of polymer are rarely reaching 100% due to some part of the polymer incorporated into microbial biomass, humus, or other natural product [26].

A similar result is using an agar diffusion test or Kirby-Bauer test on a banana starch/chitosan bioplastic film with various concentrations of ZnO. The results obtained for ZnO 5% (of the total weight of starch) takes 1 h 12 min for degradation, while for a concentration of 3%, it only takes 42 min. Tests were for without ZnO on the banana starch/chitosan bioplastic film, and it only took 34 min to degrade, indicating ZnO affected the degradation process [34]. Mutmainna et al. reported that using corn starch with a variety of chitosan obtained the result that more chitosan concentration than the starch will accelerate the degradation process of the bioplastic sample. From the results obtained, bioplastic samples were degraded about 55% within 14 days without using chitosan. As for the balanced composition between starch/chitosan, which is 50/50, the bioplastic sample decomposes about 70% in 14 days, and the bioplastic sample degrades 80% in just 14 days for the starch/chitosan composition

35/65 [30]. Thus, the higher the chitosan concentration will accelerate the degradation process of bioplastic samples in the soil. The percentage degradation of PK samples is in Fig. 3c.

Seawater Degradation Test

About 80% of marine plastic comes from land, and 20% comes from the sea [37]. The spread of plastic waste into the environment comes from plastic for single-use products, food, and beverage packaging. Plastics in the environment will experience fragmentation due to abiotic processes such as UV radiation from the sun or high temperatures [38]. Single-use plastic waste used several times will continue to spread into the environment, filling the land, rivers, and seas [39]. Therefore, in this study, bioplastic PZ dan PK samples with various ZnO concentrations were tested for degradation using seawater to visually see the sample's level of degradation so that it can be applied as an environmentally friendly plastic and can be degraded both on land and in the ocean. The Photograph Visual Seawater Degradation Test of PZ Samples (Starch + with and without ZnO) for 0 days, 3 days, 7 days, 14 days and PK Samples (Starch + Chitosan + ZnO) for 0 days, 3 days, 7 days, 14 days, and 21 days is in Fig. 3.

PZ Samples (Starch + Without and With ZnO)

Degradation testing is for determining the level of bioplastic degradation in seawater. The degradation test is used as a parameter to determine the environmentally friendly nature of the bioplastic samples [25]. Testing is for 0–14 days, each day with a visual observation of changes in bioplastics.

The observations show that, on the third day, the bioplastic underwent a color change that caused the seawater to become cloudy. The bioplastic reaches saturation when it enters 4 h of immersion and when the immersion is more than 7 h, the bioplastic will reach the saturation phase, which causes a change in the shape of the sample, as shown in Fig. 3a. After being submerged for 24 h, the bioplastic will change its shape and size, which is getting more expansive due to the resistance of the sample in absorbing water that has reached the saturation phase.

The PZ samples decomposed faster than the PK sample because of only consisted of two primary ingredients: ZnO and starch, where starch had a higher concentration than ZnO. Starch has hydrophilic properties [34, 36] and a hydroxyl group of OH, ionizing the hydrolysis reaction after absorbing water. Water absorption by the sample causes thickening, accelerating the degradation process [33, 34]. So, this is what causes the PZ sample variations to decompose more quickly. Based on the results obtained, on the third day, PZ samples degraded faster, which was indicated by the dissolution of half of the samples compared to PZ

1–PZ 5; this was due to the absence of hydrophobic ZnO [17] in PZ samples. On the seventh day, the PZ samples were completely decomposing, and for the PZ 1 bioplastic sample, it had decomposed into small parts compared to the PZ 2 sample, which still had half the bioplastic; the ZnO concentration influenced this in each sample. It appears that the PZ 3–PZ 5 sample is still intact but has been degraded, which is indicated by the depletion of the bioplastic sample layer. Furthermore, on the 14th day, all the bioplastic samples decompose entirely, and there are small white grains, some of them absorbed by the sea sand. The speed of samples for decomposing is due to abiotic processes such as UV radiation from the sun [32] means that the surface of plastics in water will degrade more quickly. The photograph visual seawater degradation test of PZ samples is in Fig. 3.

PK Samples (Starch + Chitosan + ZnO)

The degradation test is used as a parameter to determine the environmentally friendly nature of the bioplastic samples [25]. The test is for 0–21 days with visual observations on changes in bioplastics every day. The observations show that the bioplastic changes in shape are getting more expansive on the third day. Due to the resistance of the sample to absorb water, this phenomenon has reached the saturation phase. The bioplastic reaches saturation when it enters a 3-h immersion and when the immersion is more than 7 h, the bioplastic will reach the saturation phase, which causes a change in the shape of the sample, as shown in Fig. 3b.

On the third day, PK 1 samples degraded faster, indicated by splitting all parts of the sample compared to PK 2–PK 5, due to the small concentration of hydrophobic ZnO [17]. On the seventh day, the PK 1 sample almost completely decomposed, and the PK 2 bioplastic sample had decomposed in half and looked thinner than the PK 3–PK 5 sample, which only lost a quarter of the bioplastic but had been degraded, which was characterized by increasing the thinning of the layer of bioplastic samples, the ZnO concentration also influences this in each sample. Then, on the 14th day, the PK 1 and PK 2 samples were decomposed entirely, and there were tiny white granules that were partially absorbed or absorbed by the sea sand. Meanwhile, the PK 3–PK 5 sample has degraded but has broken down into small pieces and is getting thinner, but on the 21st day, all samples were 100% degraded. For the seawater environment, O_2 is available, and aerobic microorganisms are primarily responsible for breaking down polymer to become small pieces with the final products, microbial biomass, CO_2 , and H_2O [26]. The photograph visual seawater degradation test of PK samples is in Fig. 3.

The explanation above shows that chitosan and ZnO can inhibit the degradation process of bioplastic samples. The PK sample took longer to decompose than the PZ sample

because of chitosan, which is also hydrophobic. Compared to soil degradation, PK samples degraded faster because chitosan is an organic material, although it is difficult to absorb water, which is one of the factors that cause bioplastics to degrade quickly.

XRD (X-Ray Diffraction Spectrum) Characterization

The X-Ray Diffraction (XRD) characterization is for analyzing the structural properties of bioplastics samples (Shimadzu 7000 X-ray diffraction) with $CuK\alpha$ radiation ($\lambda = 1.5405 \text{ \AA}$) for 2θ from 5° to 60° , operates at 40 kV and 30 mA. The results are presented in graphical form to see the pattern of each bioplastic sample. The diffractogram pattern of each sample has different intensity at each angle and has peaks marked with sharp curves. These peaks are known as crystal regions [40–42]. The PZ and PK sample's diffraction pattern can be seen in Fig. 4a, b, respectively.

Starch is semi-crystalline, consisting of crystalline and amorphous units [40–46]. The PZ sample is a bioplastic sample without using amorphous ZnO, which were starch consists two components; the first is amylose with a linear chain structure, and the second is amylopectin with a branched-chain structure [43]. The branch structure is the main contribution to forming amorphous structures [44, 45]. There are three prominent peaks in the 2θ range: 18.08° , 19.54° , and 20.98° which indicated as diffraction peaks of cassava starch. Similar results were obtained in the range 2θ : 15° – 23° [5, 47, 48].

In sample PZ 1, showing an amorphous structure in the range of 2θ : 15° – 25° with values of 19.64° , 20.50° , and 18.90° , and in the range of 2θ : 30° – 40° , three new peaks in the diffraction peak of ZnO with values of 2θ : 36.11° , 31.62° , and 34.27° , this is evidenced by some research, that the three prominent peaks of ZnO are in the range of 2θ : 30° – 40° [23, 49, 50]. For sample PZ 2, the diffraction peaks in the range 2θ : 15° – 25° , decreased compared to samples PZ and PZ 1, namely 18.15° , 20.90° and 19.46° , and the intensity of the diffraction peaks in the range of 2θ : 30° – 40° increased by 36.46° , 31.97° and 34.63° . The ZnO concentration influences this diffraction pattern in each bioplastic sample; the more ZnO concentration given, the higher the intensity formed. Based on the results obtained on the PZ 3 sample, it shows that the intensity of the starch structure decreases in the range of 2θ : 15° – 25° , and the intensity of ZnO increases. Along with the addition of ZnO, the bioplastic structure from the semi-crystalline phase became crystalline, as can be seen in samples PZ 4–PZ 5. There were also diffraction peaks 2θ : 46° and 56° in samples PZ 1–PZ 5 which were diffraction peaks of ZnO [23] with less intensity than at 2θ : 30° – 40° . The XRD characterization results for PZ samples are in Fig. 4a.

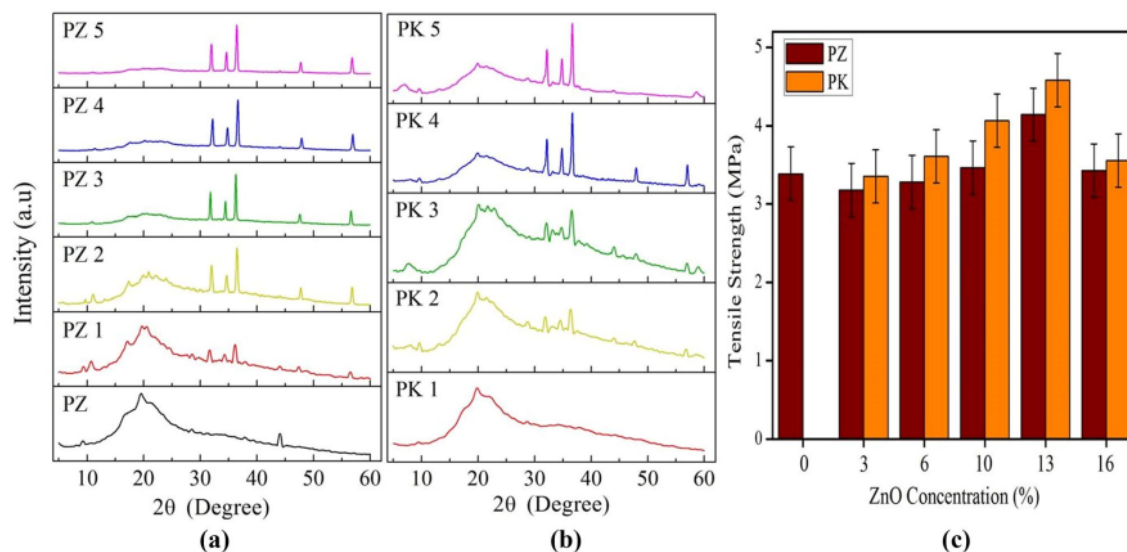


Fig. 4 a XRD spectra PZ for 0% ZnO and PZ 1–PZ 5 for various amount of ZnO and b PK samples, PK 1–PK 5 for various amount of ZnO also c The average values of tensile strength of PZ and PK

samples for various concentration of ZnO (PZ for 0% ZnO, PZ 1–PZ 5 and PK 1–PK 5) with the error bar was included

The diffraction peak of cassava starch is in the range of $2\theta = 18^\circ$ – 20° , which is an amorphous structure. The PK 1 sample in the range of $2\theta = 30^\circ$ – 40° , which is the crystal structure of ZnO, shows a minimal intensity and the diffraction peak of chitosan. Based on the result [21] research by Mutmainna et al., chitosan showed broad peaks in the range $2\theta = 25^\circ$ – 35° and small sharp peaks in the range $2\theta = 48^\circ$ – 55° [30], while research by Jagong et al., chitosan showed broad peaks in the range $2\theta = 30^\circ$ – 40° , 40 as well as research by Amalia et al., the diffraction spectrum of the chitosan film showed the presence of two diffraction peaks at $2\theta = 15.35^\circ$ and 20.80° [32]. The diffraction peak near the angle of $2\theta = 20^\circ$ indicates that chitosan has a semi-amorphous structure.

The results obtained in the sample PK 1 there are three diffraction peaks with the highest intensity, namely at $2\theta = 19.78^\circ$, 20.72° and 21.68° , where these peaks are a combination of starch and chitosan. There is also a tiny diffraction peak in the range of $2\theta = 28^\circ$ – 30° for samples PK 1–PK 5, which is the diffraction peak of chitosan. In sample PK 2, there are three diffraction peaks of starch/chitosan with the highest intensity, namely at $2\theta = 19.90^\circ$, 21.32° and 22.44° and in the range of $2\theta = 30^\circ$ – 40° a sharp crystal structure is from ZnO at $2\theta = 31.85^\circ$, 34.52° and 36.34° . The ZnO concentration increases will be increasing the peak intensity at $2\theta = 31^\circ$ – 36° , which can be seen in the sample PK 3–PK 5 in Fig. 4b.

Sample PK 3, the diffraction peak intensity in the 2θ range for starch/chitosan decreases at 18.22° , 20.04° , and

22.80° while the intensity of the 2θ diffraction peaks for ZnO increased at 32.06° , 34.74° and 36.55° . Similar results for the PK 4 sample, the intensity of the two diffraction peaks for starch/chitosan decreased at 19.75° , 20.16° , and 21.18° . In contrast, the diffraction peak intensity for ZnO increased at 32.82° , 35.56° , and 36.02° and for PK 5, the diffraction peak intensity for starch/chitosan decreased at 19.14° , 19.90° , and 21.32° . In contrast, the intensity of the 2θ diffraction peak for ZnO increased at 32.14° , 34.80° , and 36.63° . These results indicated that the crystallinity of the starch/chitosan mixture decreased with the addition of ZnO concentration in the bioplastic samples.

Based on the exposure of the two variations of bioplastic samples, PZ and PK samples, it can conclude that the greater the concentration of ZnO given, the lower the intensity of the diffraction peaks of starch and chitosan as well as the structure of bioplastics formed into semi-amorphous crystals. The 2θ range for cassava starch is at 18° – 20° , the 2θ range for chitosan is 21° – 22° and 28° – 30° , and the 2θ range for ZnO is 33° – 37° .

Tensile Strength Test

The tensile strength test shows the mechanical properties of the bioplastic samples. The tensile strength results for PZ samples for 0% ZnO and PZ 1–PZ 5 for various amounts of ZnO and PK Samples PK 1–PK 5 for various ZnO is in Fig. 4c.

Based on the results obtained for the PZ and PK samples, the addition of 13% ZnO showed the highest tensile strength of 4.142 MPa and 4.583 MPa, respectively. While bioplastic without ZnO showed the lower tensile strength of 3.388 MPa. According to Wahyuningtias et al., bioplastics made from cassava starch have low tensile strength [33], which is similar to several reported studies with the average tensile strength of cassava starch: 1.14–5.65 MPa [4, 5, 17, 47, 48, 51, 52] [19].

Bioplastics with the addition of 3% ZnO were lower than without ZnO, which was 3.180 MPa, but as the ZnO concentration increased, the tensile strength of bioplastics increased. ZnO tensile strength values from 3–13% are 3.180, 3.283, 3.465 and 4.142 MPa. The increase in tensile strength indicates that a good interfacial bond between ZnO and the starch matrix occurs. This interfacial bond is an important aspect that affects mechanical properties [53]. The strong interaction between the starch matrix and ZnO nanofillers was due to the homogeneous dispersion of ZnO in the starch matrix affected to the mechanical properties increased. However, when the ZnO concentration is above 13%, the tensile strength value decreases to 3.427 MPa. This phenomenon could be due to the agglomeration of ZnO; inhomogeneous ZnO dispersion causes a weakening of the starch-ZnO matrix interaction [4, 28]. Thus, it concluded that the variation of PZ samples with the addition of ZnO could improve the mechanical properties of bioplastic samples. The variation of the PK sample bioplastic has a higher

tensile strength value than the PZ sample variation, as can be seen in Fig. 4c.

The tensile strength values of PK 1–PK 4 are 3.354, 3.613, 4.068, and 4.583, respectively, proves that chitosan also plays a role in improving the mechanical properties of bioplastic samples [10, 12, 13, 30]. Bergel et al. explained that the application of chitosan to the starch-bioplastic matrix would form a three-dimensional covalent bond network [4], consequently the material stiffer and stronger [54]. The addition of chitosan also increases its mechanical strength. However, for 16% ZnO, the tensile strength value decreases, almost the same for the PZ 5 sample; due to ZnO experiencing agglomeration, the tensile strength tends to decrease.

Thus, based on the previous explanation, bioplastics based on cassava starch have a lower tensile strength value than starch/chitosan bioplastics. The addition of ZnO also significantly affects the improvement of the mechanical properties of bioplastics. The two samples found that the PK sample had the highest tensile strength values.

Packaging Test

The growth of bacteria or fungi and staling (stale/hardening of the bread) are the two main problems in bakery products that can cause economic losses. As a solution, ZnO-based bio-composites or bioplastics increase the microbial shelf life of sliced white bread. Visual photograph results of the

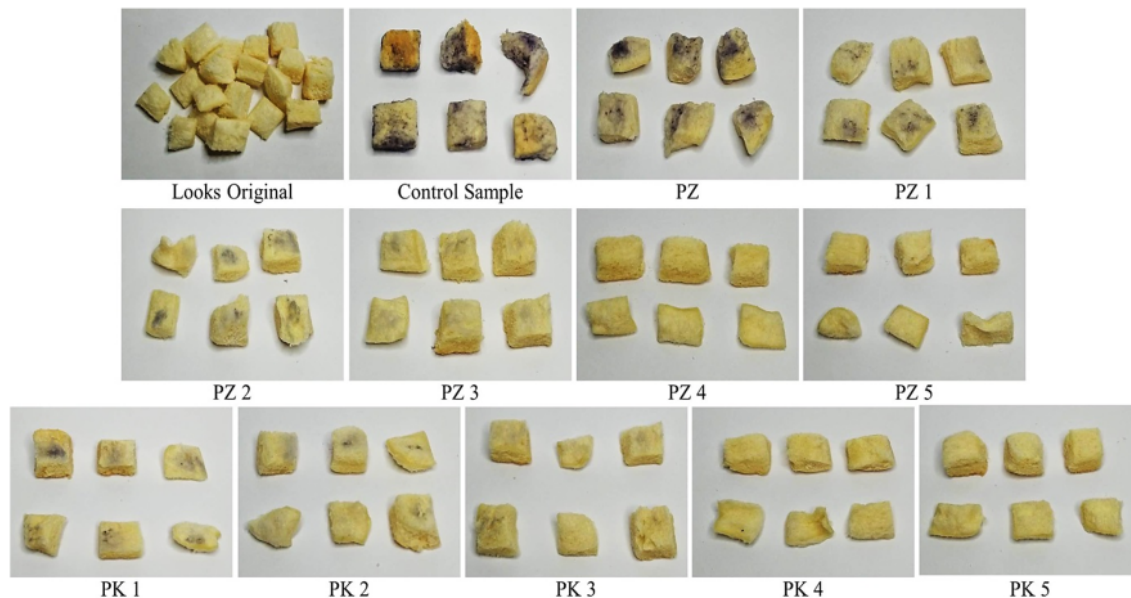


Fig. 5 Visual photograph results of bioplastics packaging test on bread. First rows from the left are original bread, control sample without packaging for 10 days, PZ for 20 days, PZ 1–PZ 5 continue to the second rows for 30 days, and third rows for PK1–PK5 for 30 days

microbial shelf life of white bread wrapped in bioplastic are in Fig. 5.

For bread as a control sample (without being wrapped in bioplastic) after ten days have been overgrown by microbes identified by blackening of the bread, and the texture of the bread is rigid (staling) than usual or shows high amylopectin retrogradation. Compared to the PZ sample without ZnO, the sample was overgrown by microbes after 20 days; this was because the type of starch affected the rate of retrogradation [14]. As ZnO concentration increases, microbes are difficult to grow, indicated by fewer fungi that grow in the PZ and PK sample.

The visual results of the variations sample PZ 1–PZ 5 show that, as the concentration of ZnO increases, microbes are difficult to grow. For PZ 1–PZ 3 samples, microbes began to grow on the 30th day. Compared to PZ 1–PZ 3, microbial growth was faster in PZ 1 samples and decreased in PZ 2 and PZ 3 samples. As for PZ 4–5 has not been overgrown with microbes, but the texture of the whole bread tends to dry out, indicating that the antimicrobial activity increases rapidly with the presence of ZnO in the polymer matrix. Electrostatic binding of Zn²⁺ ions to the cell surface of microorganisms causes changes in cell membrane permeability, interactions with respiratory enzymes and their inactivation, reactions with cell components, and depletion of cytoplasmic contents. Thus, microbes will be challenging to live [23, 55].

Figure 5 also shows the visual results of the variation of PK 1–PK 5 samples. As ZnO concentration increases, microbes are difficult to grow, indicated by fewer fungi that grow in the PK sample than the PZ sample. For samples PK 1–PK 3, microbes began to grow on day 30; compared to PK 1–PK 3, microbial growth was faster in samples PK 1 and continued to decrease in samples PK 2 and PK 3. As for PK 4–5 not overgrown with microbes. However, as the concentration of ZnO increases, the shelf life of bread is more extended than without ZnO coating. Thus, the starch/chitosan bioplastic packaging test variations in ZnO concentration showed an increase in the shelf life of bread. The best results for PZ-PK 4 and 5 samples. In general, the results of this study indicate that the application of ZnO-based bioplastic films is a valuable method for increasing the microbial shelf life of bread and slowing the rate at which bread reaches staling.

Conclusion

The bioplastic films based on cassava starch/chitosan for the various ZnO nanocrystals were synthesized with the casting method. The bioplastics ZnO-based was successfully degraded easily in soil and seawater. Bioplastics containing chitosan need 21 days in seawater and the ground, but for

bioplastics without chitosan is needed 28 days in the ground and 14 days in the seawater. The crystalline phase of ZnO increased the tensile strength and extended food life due to the antimicrobial of ZnO. ZnO can be potentially applied as an environmentally friendly bioplastics starch and starch/chitosan-based to improve shelf life as a food packaging.

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Author Contributions RA: Investigation, Methodology, Data curation, Writing—original draft & editing. BA: Investigation, Methodology, Resources. DT: Conceptualization, Methodology, Writing—original draft, Writing—review & editing, Resources, Supervision, Project administration.

Declarations

Conflict of interest The author declare that they have no known competing financial or personal relationship that could have appeared to influence the work reported in this paper.

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