Many food packages (plastic wrappers) today cannot be broken down by the environment, therefore, it is necessary to add natural substances that can make the food package decompose and be resistant to contamination with bacteria. Development of biodegradable polymers from renewable sources is highly desirable for food preservation and packaging, provided they can be effective as plastics or paper that are currently used in packaging, protecting food against microbial contamination, physical damage and chemical reactions (e.g. oxidation). Poly lactic acid (PLA) is one of the natural polymers produced by several bacteria that grow in crops rich in carbohydrates (such as sugar beets, corn and others). This research aims to insert ZnO nanoparticles and chitosan into a plastic layer of PLA (poly lactic acid) which can improve the antibacterial properties of the resulting packaging. The method used in making PLA-ZnO-chitosan nanocomposite is the precipitation method and the heating method. PLA-ZnO nanocomposites were obtained by varying ZnO nanoparticles 0.5% by weight, 2% by weight, and 3.5% by weight. The results obtained in SEM images show that nanoparticles are homogeneously distributed on the plastic surface. Antimicrobial tests show nanocomposites work effectively in deactivating E. coli and S. aureus, where it was found that E. coli was more susceptible to this type of nanocomposite, where there was a reduction of 3.4 logs to 3.5% ZnO loading in the PLA layer.

Keywords: Nanocomposite, ZnO nanoparticles, PolyLactic Acid, Antimicrobials.

1. Introduction
Materials that produce environmentally friendly materials have developed rapidly. Meanwhile, utilization composites based on vegetable fiber has been mostly introduced. (1, 2, 3). For more, an advantaged approach is being introduced base on a nano-size base material.

The current trend is to direct research into developing innovative solutions for both antimicrobial functional packaging (active packaging and nanocomposite materials) and low environmental impacts (biodegradable materials, packaging can be recycled by reducing size) [4-14]. In this case, developing biodegradable polymers from renewable sources is highly desirable for preservation of food and packaging, provided that they can be effective as plastic or paper currently used in packaging, protecting food against microbial contamination, physical damage and chemical reactions (e.g. oxidation). Poly lactic acid (PLA) is one of the naturally occurring polymers produced by several bacteria grown in carbohydrate-rich agricultural plants (sugar beets, corn). So far, PLA has attracted attention for applications in the plastic and paper industry with the fastest growing market from 10-30% [15-16].

Zinc oxide (ZnO) nanoparticles as antimicrobial agents offer several advantages, such as superior antimicrobial properties, no negative effects on food sensory properties, and compatibility with hard polymer processing conditions [17-20]. Due to the strong antimicrobial activity of ZnO Nanoparticle, it also holds potential applications not only in food decay control [21-23], but also in food safety control by deactivating foodborne pathogens. Among several advantages, it can be increased to use ZnO Nanoparticle for food contact applications, the benefits of which are as follows:

- ZnO non-nano forms have been authorized by EFSA (European Food Safety Authority) as additives for plastic materials and articles, with SML (Specific Migration Limits) of 25 mg / kg of food [24].
- ZnO Nanoparticle shows low toxicity to biological systems (25). In addition, zinc is an important element for human physiological activity; c.a. 10 mg / person / day is needed (CEF EFSA Panel on Food Contact Materials, 2015). Toxicity studies recommend an upper limit of 25 mg / person / day [26].
- In food packaging, transparency remains a determining factor for choosing packaging materials. On the other hand, some foods are susceptible to UV light. To overcome this problem, UV inhibitors are used in polymer processing. Studies show that loading ZnO
Nanoparticle as low as 1% by weight allows good UV blocking performance without interfering with the transparency of the host polymer [27-29].

In this study, we proposed a paper-based packaging material, which was coated with ZnO-PLA nanocomposite. This application can be a paper wrapper for food because there is a risk of relatively high microbiological contamination in cold processed foods. The focus was placed on the in vitro assessment of the antimicrobial activity of ZnO Nanoparticle.

2 Materials and Methods

2.1 Materials

This experiment is divided into three parts. First, zinc oxide nanoparticles (ZnO Nanoparticle) are synthesized. Next, the synthesized ZnO Nanoparticle is used in paper coatings. Finally, both synthesized ZnO Nanoparticle are tested for compatibility and properties. The chemicals and techniques used to synthesize ZnO Nanoparticle and paper coating films will be discussed. All chemicals used in the experiment are grade analytical reagents (AR Grade, 99.9% pure). Dehydrated zinc acetate [Zn(CH₃COO)₂•2H₂O] was obtained from QReC Sdn. Bhd, while sodium hydroxide (NaOH) pellets are obtained from LabChem Sdn. Bhd. Ethanol is obtained from Sigma Aldrich. Poly lactic acid (PLA) (Nature Work TM PLA 3001D) in the form of pellets obtained from Nature Work ® LLC, Minnetonka, MN USA. It has a specific gravity of 1.24 g / cm³ and a long flow index (MFI) of 15 g / 10 minutes (190°C / 2.16 kg). The solvents used in this study were chloroform obtained from R & M Chemistry, de-ionized water is used during the synthesis process.

2.2 Preparation of zno nanoparticle

Preparation of ZnO Nanoparticle is carried out using the precipitation method mentioned in Figure. 1. First, 0.2 M of zinc acetate dihydrate is made by dissolving zinc acetate dihydrate with the molecular formula Zn(CH₃COO)₂•2H₂O in de-ionized water. Then, 0.6 M of sodium hydroxide, NaOH is prepared and added drop by drop in zinc solution dihydrate acetate with constant stirring at room temperature for 2 hours. White deposits are formed from the reaction between zinc acetate dihydrate and NaOH solution. The white sediment is filtered and rinsed with ethanol followed by de-ionized water. This is to ensure that the remaining NaOH in the white deposits is lost. Finally, the white precipitate is dried overnight in an oven at 60 °C. The white sediments obtained are sent to XRD and FTIR analysis to ensure that pure ZnO Nanoparticle is obtained. ZnO Nanoparticle is then stored to be used to prepare the composite.

2.3 Nanokomposit pla / zno preparation

15 g PLA pellets (Polyactic Acid, 4060D, Natureworks) were dissolved using chloroform. Then the dissolved PLA is added with ZnO Nanoparticles at a concentration of 0.5% by weight, 2% by weight and 3.5% by weight (% by weight of PLA) and stirred with strong stirring at room temperature until it is fully dissolved. Furthermore, white kraft paper is bleached (base weight 106 g / m, 7.7% ash content, upper side size) is used as a substrate for coating. Coating is done on the size side. Coating is carried out at the film lab applicator (Elcometer 4340) using a fine rod to store wet film 50 microns to the substrate. After coating, the sample is left to dry overnight at room temperature. The structure of the final packaging material is illustrated in Figure.2.
bacteria. The sample was incubated for 8 hours at 37ºC.

3 Result and Discussion
3.1 Characteristics of ZnO nanoparticles

Figure 3. Spectrum of FTIR ZnO Nanoparticle

Figure 3.1 shows the FTIR spectrum of the synthesized ZnO nanoparticles obtained in the range of 4000-500 cm⁻¹. The band at 3378.49 cm⁻¹ is caused by stretching the vibration of OH groups in water, alcohol and phenol, possibly due to atmospheric humidity. The peak at 1695.5 cm⁻¹ is related to C=O group of carboxylic derivatives, which may be due to residues of zinc acetate used in the reaction. The peak at 2328.6 cm⁻¹ is related to the symmetrical stretching vibrations of the carboxylate group (C=O) [30]. Absorption at 832.20 cm⁻¹ is due to the formation of Zn tetrahedral coordination [31]. At a wavelength of 1500.71 cm⁻¹, it shows the hexagonal group of ZnO [32].

3.2 Characteristics of PLA-zno paper coating nanocomposite

SEM (scanning Electron Microscope)

Figure 3.2 shows the morphology of the layer surface using representative SEM taken from each sample. Note that images are presented in pairs when captured at the same point of view but are produced with different detectors: secondary electrons provide more information about the morphology of the sample, while the fibrous electrons contrast with the composition of the material. In general, the surface of the paper is well covered by the coating, showing good fineness and the absence of surface pores. This is important for the antimicrobial test following the JIS Z 2801. In addition, ZnO is distributed homogeneously, which is clearer in backscatter images (small white dots); on the other hand, some ZnO aggregates persist in the layer (larger white dots). Obviously, with increasing Nanoparticle loading, more Nanoparticle appears on the surface, and thus stronger antimicrobial activity than material can be expected.

3.3 Characteristics of antibacterial tests

The S. aureus (Gram-positive) susceptibility test with respect to the active ingredient is shown in Figure 5. For convenience of comparison, the control bacterial concentration at 0 hours was used as the baseline. As expected, the susceptibility of S. aureus to follow the concentration of ZnO - higher concentrations provide a stronger antimicrobial effect. If in log reduction, this corresponds to 1.16, 1.66, and 5.18. Reduction of log greater than 2 indicates success of killing greater than 99%, and hence the material can be described as "antimicrobial" [33-39]. So, in this sense, only a sample of 3.5% -NOO Nanoparticle can be said to be antimicrobial against S. aureus. These results are comparable to those reported in previous publications; for example, in the study [40], starch-based coatings containing 1.37% by weight ZnO Nanoparticles induced 1.6 log reductions to S. aureus; in a similar study [41], 3% of Nanoparticle weight achieved a 4.3 log reduction against the same bacterial strain; while in work [28], it takes longer (7 days, not 24 hours) to achieve a satisfactory log reduction. The difference between results from different sources can be related to microbe activity. ZnO Nanoparticle in certain morphologies has stronger activity than others [42]. In addition, the smaller the particle size the stronger the antimicrobial efficacy [43].

For the E. coli susceptibility test, as shown in Figure 6, a stronger inhibitory effect was observed with all samples containing ZnO Nanoparticle, which each induced 3.15, 3.58 and 4.30 log reduction. Obviously, this test provides evidence that E. coli (Gram-negative) is more susceptible to ZnO Nanoparticles than S. aureus (Gram-positive). Note that the detection limit of the coating method used is 3.4 log (CFU / mL).
4 Conclusion

The results obtained are in SEM images showing that the nanoparticles are distributed homogeneously on the surface. Antimicrobial tests showed that nanocomposite worked effectively in deactivating E. coli and S. aureus. Furthermore, E. coli was found to be more susceptible to this type of nanocomposite, showing a reduction of 3.4 log to 3.5% ZnO loading in the PLA layer. Based on the results, it can be explained that the ZnO nanoparticles have several active mechanisms, and different antimicrobial testing methods can only activate some of the mechanisms.

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