Barrier and antimicrobial properties of PVA films incorporated with ZnO nanoparticles and stearic acid

Vega Yoesepa Pamela¹, Bayu Meindrawan², Rizal Syarief³, Evi Savitri Iriani⁴, Nugraha Edhi Suyatma³

¹ Department of Food Technology, Faculty of Agriculture, Sultan Ageng Tirtayasa University. Jl Raya Pakupatan Km 4, Serang, 42121. Indonesia
² Food Technology Department, Faculty of Engineering, Bina Nusantara University, Jakarta, Indonesia 11480
³ Department of Food Science and Technology, Faculty of Agricultural Technology, Bogor Agricultural University. Jl. Raya Darmaga, Bogor, 16680. Indonesia
⁴ Research Center for Postharvest Development and Agriculture, Jl. Tentara Pelajar No. 12, Bogor, 16114. Indonesia

Corresponding author: bayu.meindrawan@binus.ac.id

Abstract. The barrier properties of a polymer film is very important for application as packaging material. Moreover, packaging materials with antimicrobial properties give advantages to prolong the shelf life of perishable foodstuff. Polyvinyl alcohol (PVA) is a water-soluble polymer, which has good oxygen barrier properties, but this depends on the humidity, as PVA is sensitive to moisture. The purpose of this study was to assess the characterization of bio-based nanocomposite films of PVA with the addition of ZnO nanoparticles and stearic acid. Bionanocomposite films were prepared by PVA dissolving in distilled and a range of ZnO nanoparticle formulations (and/or stearic acid) was added. Response Surface Methodology data analysis showed all response models qualified as good models with adequate precision (greater than 4). Result revealed that the addition of ZnO nanoparticles and/or stearic acid can reduce the water vapor transmission rate of the resulting film, lower light transmission, as well as results in antimicrobial properties.

Keywords: Polyvinyl alcohol, ZnO nanoparticles, Stearic acid, Barrier properties, Antimicrobial properties

1. Background
Biopolymer-based packaging materials have not been widely used in the packaging industry, mainly due to the inferior mechanical and barrier properties [1]. One way to overcome these deficiencies is to combine it with other materials or a filler. Current research indicates that the homogeneous mixing of polymers with different types of nano-sized filler will lead to improvements in the physical, mechanical and barrier [2]. One of the many biopolymers that are intensively studied is polyvinyl alcohol (PVA), because it can form a good film, which is water-soluble, easy to process, non-toxic, biocompatible and biodegradable [3].
On the contrary, despite these advantages, PVA has the disadvantage that it has poor barrier properties to moisture, so it needs to be combined with fillers to improve the characteristics of the resulting film. One of the nano-sized filler materials that has the potential to improve it is ZnO nanoparticles. ZnO nanoparticles have interesting properties, especially in the food packaging industry because, they have antimicrobial properties [4], a large surface area to volume ratio, chemically alters the physical properties of the film, enhances its surface reactivity, thermal properties, mechanical and is electrical unique, stable to heat and generally recognized as safe (GRASS) by the Food and Drug Administration [5, 6].

Film composites derived from nature or having biodegradable properties have oxygen barrier properties that are relatively good, but which are highly permeable to water, because of their hydrophilic nature. Besides promising nanofillers to improve the gas barrier properties of the nanocomposite films, addition of lipids offers high water barrier properties. Fatty acids, fats, and waxes are used to reduce the water vapor permeability, since the presence of hydrophobic materials can produce a good barrier against moisture migration. The use of lipids as ingredients that can reduce the permeability to water vapor has been reported for several films used on kefir [7], chitosan [8], and Lepidium perfoliatum seed gum [9]. Lipids are most widely used are stearic acid, palmitic acid and some vegetable oils such as soybean oil and sunflower [10].

In support of government efforts to run the eco-friendly action as stipulated in Clause 15 of Government Regulation No. 81 of 2012 and Article 15 of Law No. 18 of 2008, presumably our current research may be one alternative to reduce the non-biodegradable packaging. The impact caused by non-biodegradable packaging is not environmentally friendly, because of its widespread use. Based on data from Planning Control Deputy Ministry of Environment, Republic Indonesia, each individual produces an average of 0.8 kilograms of waste a day, of which 15% is plastic [11]. Therefore, we expect the alternatives described in the current research can reduce the amount of non-biodegradable packaging and can be one solution to the problems. However, as eluded to above, biodegradable packaging material needs to be improved.

Therefore, the purpose of this study was to assess the barrier and antimicrobial properties of bionanocomposite film based on polyvinyl alcohol with the addition of ZnO nanoparticles and stearic acid.

2. Materials and Methods

2.1 Materials

The main material used is polyvinyl alcohol commercial type 17K obtained from Surabaya, inorganic matter nanoparticles of zinc oxide (ZnO) obtained from China, and commercially available stearic acid, distilled water, CaCl₂, KCl, NaCl, the growth medium agar (NA), Nutrient Broth (NB), plate count agar (PCA), bacteria Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25 922). The main tool used in this study is a Scanning Electron Microscope (SEM) [Zeiss EVO M10 USA], Differential Scanning Calorimetry (DSC) -60 [Malvern England], X-ray Diffraction (XRD) Bruker D8, [UTMA Comten Industries], Particle Size Analyzer (PSA) [Malvern England], Spectrophotometer Agilent Technologies Cary 60 UV-Vis, and other supporting equipment.

2.2 Preparation of Bionanocomposite Film

The bionanocomposite film was prepared by using solvent casting method developed by Chandrakala et al [12]. An amount of 5 grams of polyvinyl alcohol was dissolved in 95 grams of distilled water using magnetic stirrer at 120°C for 30 minutes. Then the PVA solution was mixed with different concentrations of ZnO nanoparticles that had been sonicated 30 minutes, stearic acid and Tween 80 which has been melted beforehand at 70-80°C. Subsequently, the film was printed and dried in a vacuum oven at 40-45°C for 3 hours. The dried films were stored in aluminum foil and put into the desiccator at relative humidity (RH) (75%) stabilized with silica gel prior to analysis.
2.3 Analysis of Water Vapor Transmission Rate

WVTR value can be determined by calculating the weight difference of cans filled with CaCl\textsubscript{2} at high RH conditions such as in a desiccator which already contains saturated KCl solution. Hygroscopic CaCl\textsubscript{2} will absorb moisture from the outside so that the weight will increase. The more tightly the film, the less weight addition to CaCl\textsubscript{2} will be obtained [13]. The formula to find WVTR is

\[
WVTR = \frac{24}{A} \times \text{slope}
\]

- **WVTR** = water vapor transmission rate (gram/hour/m\textsuperscript{2})
- **Slope** = a linear function of weight and time (gram/hour)
- **A** = film area (m\textsuperscript{2})

2.4 Light Transmission Analysis

Analysis of light transmission was done using an Agilent Technologies Cary 60 UV-Vis spectrophotometer to measure transparency at a wavelength of 420 nm [14].

2.5 Color Analysis

Color analysis was determined by using a Minolta CR 300 Chromameter. The measurement results are converted into CIE LAB system (L*, a* and b*). Total color difference of a sample is known as \[\Delta E = (L^{*2} + a^{*2} + b^{*2})^{1/2}\]

2.6 Analysis of Antimicrobial Properties

The antimicrobial activity of the film was tested using a colony count method [2]. A ml of the two test cultures was inoculated into nutrient broth medium, then incubated at 37 °C for 24 hours. After incubation, dilutions were needed to determine the initial microbial count and adjusting the concentration of microbes. Then, 20 ml diluted solution which had an appropriate amount of microbes (10\textsuperscript{5} FU/ml) was added into a 50 ml erlenmeyer containing 5 gram film sample. Film and media were subsequently incubated at 37°C for 24 hours. Samples were taken at time 0 and after 24 h, serially diluted and plated on NA to determine survival.

2.7 Statistical analysis

The Response Surface Methodology (RSM) is used to analysis the data obtained. This method supported by Design Expert 7 © with mixture method D-optimal design of experiments.

| Table 1 The combination of ZnO nanoparticles formulations and stearic acid in the design of RSM |
|---------------------------------|-----------------|-----------------|
| **Standard** | **ZnO Nanoparticle %** | **Stearic Acid %** |
| 1           | 4.0              | 6.0             |
| 2           | 0.0              | 10.0            |
| 3           | 2.0              | 8.0             |
| 4           | 3.0              | 7.0             |
| 5           | 1.0              | 9.0             |
| 6           | 0.5              | 9.5             |
| 7           | 3.5              | 6.5             |
| 8           | 1.5              | 8.5             |
| 9           | 0.0              | 10.0            |
| 10          | 4.0              | 6.0             |
3. Results and Discussions

3.1 Water Vapor Transmission Rate (WVTR)
A quadratic polynomial model was obtained as the result of analysis response of the water vapor transmission rate. The resulting model is significant with insignificant mismatches. The predicted and adjusted $R^2$ indicate that the model can represent the actual and prediction data up to 78.9% and 72.2%, respectively. The adequate precision for water vapor transmission rate response is 9.692, which is greater than 4. So overall, the response model for water vapor transmission rate qualifies as a good model. The actual equation shows that the water vapor transmission rate value will increase as ZnO nanoparticles increase, stearic acid and the interactions between stearic acid ZnO nanoparticles.

Figure 1 shows that the WVTR leads to a decrease with the presence of ZnO nanoparticles and stearic acid. The area which leads to a low WVTR is an area that had high nanoparticles and stearic acid value. This is presumably because high amounts of the two components will generate a strong interaction through hydrogen bonding. While the graph which leads to a great WVTR value is an area that is not optimum in moisture blocking. It means that these two components are not in the same high value. This is presumably due to the weak interaction between the nanoparticles ZnO and stearic acid, resulted in less powerful blocking moisture. The weakness of the bond between the two components is because one of them has lowest value.

Water vapor barrier properties will increase with the physical barrier of a hydrophobic fatty acid. This behavior can be explained because of high hydrophobicity and low mobility of fatty acids [15]. Stearic acid, when well distributed and homogeneous within the matrix of the film, condensed on the temperature control, and contributed to a decrease in the rate of water vapor [16]. Similarly, for ZnO nanoparticles, a decrease in water vapor transmission rate is observed due to ZnO nanoparticles which are distributed homogeneously and will form a tortuous path to block water molecules into the polymer matrix [2].

Water vapor permeability difference may be related to differences in molecular diffusion of water vapor and hydrophilic-hydrophobic interactions [16]. Water vapor permeability also depends on other factors such as the ratio between crystalline and amorphous, polymer chain mobility and specific interactions between the functional groups of the polymer [17].

|   |   |   |
|---|---|---|
| 11 | 2.0 | 8.0 |
| 12 | 0.0 | 10.0 |
| 13 | 4.0 | 6.0 |
Figure 1 Graphs of two component mix bionanocomposite films on the response of the water vapor transmission rate (WVTR).

### 3.2 Light transmission

A cubic polynomial model was resulted as outcome of the analysis of light transmission. The resulting model is significant with unsignificant mismatches. Both prediction and adjusted $R^2$ show that the model can represent the actual data and prediction data up to 97.6% and 96.3%, respectively. The adequate precision for light transmission response is 30,400, greater than 4. Thus, overall response model for the transmission of light qualifies as a good model. Based on Figure 2, both fatty acid stearic and ZnO nanoparticles gave a negative effect on the transmission of light films. The decreasing of light transmission value indicate that film is less transparent (or more opaque). ZnO nanoparticles influenced the light transmission of nanocomposite films as reported by Augustine et al. [18] and Gupta et al. [19] who reported that there was a substantial yield in absorption intensity of PVA-ZnO nanocomposite films compared with pure PVA film. The percent of transmittance is lower compared to control. Similar to the above, Chandrakala et al. [12] also stated that the optical transmittance decreases with the increasing of nanoparticles concentration. The reduction of transmittance intensity with increasing nanoparticles filler might be due to the formation of charge transfer complexes between ZnO-Ce$_2$O$_3$ and hydroxyl groups of PVA [20]. Similarly, stearic acid also affects the turbidity of PVA film produced in this study. Previous studies indicated that the addition of a hydrophobic substance produces films with high opacity due to a strong dispersion of the solid phase [21, 22]. Fatty acid particle formation during drying of the film is thought to give a polymer matrix interference, thereby increasing internal heterogeneity and reducing the transparency of the film.
3.3 Color

A cubic polynomial models were chosen as a result of the color response analysis. The resulting model is significant with insignificant mismatches. The predicted and adjusted $R^2$ indicate that the model can represent the actual data and prediction data 61.9% and 43.9%, respectively. The adequate precision for light transmission response is 6.893 is, greater than 4. So overall response model for the transmission of light qualifies as a good model.

As can be seen on Figure 3, the effect given by ZnO nanoparticles and stearic acid is not very significant because the model depicted in the graph shown leads to both decreases and increases in the total color difference value. It means that both ZnO nanoparticles and stearic acid gave positive effect on the response of the total color difference. The smaller the value of total color difference, the more transparent film is. Compare with control ($ZnO$ % and Stearic acid 0%), the addition of fatty acids increased the total color difference. These findings are consistent with those reported by Wang et al. [17] and Seyedi et al. [9] who stated that the opacity increased with the addition of a fatty acid. This is because of the physical properties of the fatty acids, which solidifies at room temperature so that the fat or oil in the film matrix will affect its transparency. Coalescence and creaming of fat during drying can also trigger the roughness of the film surface [9]. Increased opacity of the film is also suspected due to dispersion of stearic acid into the emulsion and their continuous distribution throughout the polymer network [17].

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**Figure 2** Graphs of two component mix nanocomposite films on the response of light transmission

*Design-Expert® Software*

| Light Transmission (nm) |
|-------------------------|
| Actual ZnO Nanoparticle |
| Actual Stearic Acid     |

- **DesignPoints**
  - X1 = A: ZnO Nanoparticle
  - X2 = B: Stearic Acid
3.4 Antimicrobial Properties
The polynomial models were chosen as a result of analyzing the response of the antimicrobial properties is quadratic, as suggested. The predicted and adjusted coefficient determination show that the model can represent the actual data and prediction data up to 95.6% and 94.0% for *E.coli*, 86.0% and 81.2% for *S.aureus*. The adequate precision for the response antimicrobial properties for *E. coli* and *S.aureus* are 23.278 and 12.511, respectively. Thus, overall model for the response antimicrobial properties qualifies as a good model. Similarly with result of Kanmani et al. [2] which worked on agar, carragenan and CMC films, the actual equation for the response antimicrobial properties showed a decreasing number of microbes as ZnO nanoparticles increase (Figure 4). According to Mayachiew et al. [23], the mechanism of antimicrobial activity of ZnO nanoparticles is bactericidal. The antimicrobial effects on ZnO nanoparticles can be caused by three main mechanisms, namely: 1) ZnO will release the ions to be antimicrobial, 2) the interaction of nanoparticles with a microorganism that can damage the integrity of the bacterial cell, 3) the ability to form reactive oxygen species (ROS) with the effect of light radiation. The decline in the number of viable counts is more drastic for *S. Aureus*, probably due to the structure of their cell walls (Gram-positive) that are not as complex as for Gram-negatives, so it can be easily damaged by ZnO nanoparticles.

Figure 3 Graphs of two component mix nanocomposite films on the response of total color difference
Figure 4 Graphs of two component mix to the response of antimicrobial nanocomposite films for *E. coli* (above); *S. aureus* (below)
4. Conclusion
The addition of ZnO nanoparticle and stearic acid is able to improve the functional properties of the film PVA, including a decrease water vapor transmission rate, a lower light transmissions, as well as having good antimicrobial properties against Gram-negative and Gram-positive bacteria. With the increase of functional properties it is expected that this could be developed into active packaging that is sustainable, environmentally friendly and has a capacity as an antimicrobial.

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