Antimicrobial Film Food Packaging: Incorporation of Zein Nanoparticles with Clove Essential Oil

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Abstract— **Food packaging is now used for maintaining the quality, healthy and nutritious of the food at the time of consumption and provided safety over long distances of travel or inhibit the contamination of microorganisms. Recently, the food packaging that is developed is not environmental friendly and exposed from external environment that causes the shelf life of the food is reduced. The objectives of this paper are to synthesize and characterize Zein nanoparticles with clove essential oil incorporated in film and to determine the antimicrobial activity in the prepared film food packaging. In this study, antimicrobial agent from the Zein nanoparticles with different concentration of clove essential oil, CEO (2.5,5,7.5,10 and 12.5%) was incorporated during the synthetization of film food packaging. Zein powder are used as nanoparticles and the nanocomposite films was prepared by solvent casting method. There are three analysis that was conducted in this research which are thermal behaviour that was done by using Thermogravimetric Analyzer (TGA), toxicity by using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and antimicrobial activity. As for the thermal behaviour of the film, the incorporation of higher concentration of clove essential oil with Zein nanoparticles prove that it is more thermally stable than other films. As for toxicity, it was surprisingly that the result of Arsenic content exceeded the maximum amount of standard in food packaging but for the Cadmium and Plumbum are in the safety margin. Lastly, the antimicrobial activity of the film showed that all of the composite film inhibits the growth of the tested bacteria, E. coli. The inhibition of bacteria increased remarkably as the concentration of clove essential oil increased. For the control film, Zein nanoparticles consist the antimicrobial agents that can inhibit the growth of the bacteria even in small amount. Therefore, the incorporation of Zein nanoparticles with clove essential oil in the development of antimicrobial food packaging in order to extend the shelf life of food products must be developed.**

Keywords— Nanotechnology, Zein Nanoparticles, Clove Essential Oil, Antimicrobial Food Packaging.

I. INTRODUCTION

In this era of globalization, food technology becomes more advanced as food packaging nowadays not only provides information about the food such as expiration date but saving the environment, enhancing the food quality by promoting the shelf life of the food and able to withstand external forces. The purpose of food packaging has evolved and the innovations in packaging started earlier in the form of traditionally packaging as metallic cans, aseptic packaging, flexible packaging, aluminium foils and flexographic printing had changed towards biodegradable food packaging, antimicrobial food packaging, active packaging and so on [1]. There are a lot of applications of food packaging which involves the participation of packaging technologist, scientist and engineers, packaging material suppliers, packaging converters, food processors, packaging machinery manufacturers, food retailers and regulatory agencies [2]. The packaging helps in food handling convenient from distributor to consumer and other relevant info such as consumers also looking for food that has high quality of protection from worsening in the quality of foods and beverages due to environmental factors.

Packaging materials with antimicrobial function have long been recognized as one of the most promising active packaging system as it helps to improve food safety and shelf life by preventing the contamination from the pathogenic microorganism that causes the food spoilage. Antimicrobial packaging is designed to combine one ingredients into one substances of active agents during package processing for subsequent release during food storage. To design an antimicrobial food package, it is essential to select the right antimicrobial material agent for the right package and food, and to strike a balance between release of the antimicrobial and microbial growth. The incorporation of antimicrobial into a package might alter the mechanical and barrier properties of polymers as certain antimicrobial acts as plasticizers which its improving the tensile properties and flexibility of some polymers [3]. This is due to improve the food quality and safety [4].

Extraction from plant shows better advancement over chemical and physical method as it is less toxic, cost effective and environmental friendly. Clove essential oil is chosen as antimicrobial agent as essential oil is the natural extracts constituted by high amounts of hydrophobic and volatile compounds. The antibacterial effect relies on the hydrophobic character of the oils that might separate the lipids of the bacterial cell membranes and making them more permeable. Essential oils can be incorporated into polymers or into carriers that may be extruded or coated into packaging materials [5]. However, the antimicrobial activity of this essential oil cannot be explained by a single specific mechanism but rather by the combined effect of different substances such as Zein nanoparticles. Zein consist of a group of alcohol-soluble protein that gives low toxic degradable end product. Zein also contribute to water insolubility and improve the water vapor barrier of films. Moreover, clove essential oil constituted by high amount of hydrophobic have the poor water solubility [6]. This can be overcome if the Zein nanoparticles are incorporated with the essential oil to create edible film for food packaging.

With the increase of the world population, consumer and industrial concerns regarding the limited resources and environment protection, the use of materials that drives innovations in environmentally food packaging from renewable resources have gained a special attention. However, the packaging materials that are used in nowadays system is practically undegradable as it representing a major public concern that representing a serious global environmental problem [7]. In order to avoid this problem, antimicrobial food packaging have been studied and researched to develop more environmental friendly packaging system as several biopolymers from natural plant extract contains antimicrobial properties with biodegradable or edible materials.

The most potential or promising developments in the production of functional foods which gave a direct impact on the health of consumer in a beneficial term is by using bioactive packaging that are found in the particular properties exhibit by biopolymer. However, to be concerned in this problem is that the use of biopolymers has been limited because of their usually poor mechanical and barrier properties as this problem could affect the quality and safety of the food. This condition can be improved by adding reinforcing compound (filler), forming composites or the incorporation of antimicrobial into a package that act as carriers [8].

The major concern in food industry that rise in the packaging process is the deterioration by the actions of microorganism, moisture, gases or mechanical forces that contaminating the food. This problem also leads towards affecting the consumer's health related to food-borne disease. Thus, antimicrobial food packaging is recommended as a solution to prevent the microorganism or other contaminants from entering the food by using starch. There are many types of starch that consist of antimicrobial agent which can inhibit the contaminations towards food. Moreover, antimicrobial food packaging is designed to meet the requirement of the foods in term of shelf-life, safety assurance and quality maintenance on food [3]. To emphasize, food quality and safety of food packaging is one of the most important factors during the packaging process.

The objectives of this study are to synthesis and characterize Zein nanoparticles with clove essential oil incorporated in film food packaging and also to determine the antimicrobial activity in the prepared film food packaging by zone inhibition or disc diffusion method. In this research, Zein nanoparticles act as binder and clove essential oil as antimicrobial agent. The amount of binder which is Zein nanoparticles is constant as to determine the best amount of concentration of antimicrobial agent which is clove essential oil. The characterization and antimicrobial activity are the application that studied in this research.

II. METHODOLOGY

A. Materials

Zein (purified in powder form) was purchased from Sigma Aldrich Corp. (Kuala Lumpur, Malaysia). Pure clove essential oil 100% of 15ml bottle was purchased from Young Living Malaysia Sdn Bhd, (Kuala Lumpur, Malaysia). Glycerol (85%), Ethanol (80%) and Nitric Acid (65%) were purchased from Merck Sdn Bhd. As for Hydrochloric Acid (37%) was purchased from Fisher Scientific which is part of Thermo Fisher Scientific. Then, Mueller Hinton Broth for antimicrobial study was purchased from DIFCO® Dehydrated Culture Media.

B. Methods

1) Preparation of nanoparticle films

The preparation of the film-forming solution is prepared by dissolving 16 ± 0.05g of Zein powder in 50 ml of 80% (v/v) of ethanol-water solution and stir at 2rps by using magnetic stirrer for 30 minutes at 80°C and 5 minutes were added as to allow the solution to heat up until 80°C. Next, the solution is cooled down around 10 minutes to reach the temperature of 30°C. Then, Glycerol at 15% (g glycerol/g dry Zein powder) which is 1.9ml is added to the solution and stirred again for 8 minutes at 30°C to form a mixed solution. The mixed solution is then being poured to the rigid polyethylene surface and heated at 80°C for 20 minutes. After complete drying, the films are peeled off. Control films are prepared as prepared above with 0% of clove essential oil. The average thickness of the films was 33 ± 2 mm by using measuring instrument. The preparation of the incorporation film is continued by adding the amount of clove essential oil at 2.5%,5%,7.5%,10% and 12.5% (g essential oil/g dry Zein powder) and stirred for 8 minutes. Table 1 below show the sample preparation of nanoparticles film.

Table 1: Sample Preparation

Sample	**Component**	
	Zein (g)	**Clove Essential Oil (%)**
A	16	0
B	16	2.5
C	16	5
D	16	7.5
E	16	10
F	16	12.5

2) Zein film properties

Characterization and antimicrobial activity have been conducted in this research which the film is tested with TGA to monitor the thermal behavior, ICP-OES to check the toxicity material in the food film packaging and also antimicrobial activity that are using the E. coli as sample to observe the growth of bacteria on the film.

3) Thermal behavior

Thermal analysis is important as to comprehend the quality of film under cooling or heating rate in inert, reduction, oxidation atmosphere or under gas pressure [9]. The thermal behavior of the film was determined by using Thermogravimetric analysis spectra that were obtained by Metler Toledo TGA SDTA851. The samples were weighed and was heated from starting temperature of 30°C to end temperature of 750°C with a heating rate of 10°C/min in an ambient air environment. For the method of thermal behavior of the film, the mass of a sample is measured over time as the temperature changes. This is to record for the decomposition and the stability of the film of Zein nanoparticles with incorporation of clove essential oil.

4) Toxicity

a) Digestion Method

For the analysis of toxic metals by ICP-OES instrument, acid digestion procedure is done. This procedure is recommended by Environmental Protection Agency (EPA, Method 3050B was used as the conventional acid extraction method. The acid digestion procedure is used to prepare the film of film which is in solid sample for analysis of toxic metals by ICP-OES. 1g of film is weighed and placed in 250ml flask which 10ml of Nitric Acid, HNO_3 and 10ml of Hydrochloric Acid, HCl is added to the sample. Then, the sample was heated to 95°C for 15 minutes without boiling [10]. Next, the sample is filtered by using membrane paper. After cooling, the sample is diluted up to 100ml with deionized water. When the sample is labelled accurately, the analysis of toxicity through tracing the toxic metal is ready to analyze.

b) Inductive Coupled Plasma Optical Emission Spectroscopy

To determine and to trace the toxic element which are Arsenic, As, Cadmium, Cd and Plumbum, Pb in the film of the food packaging, the instrument of ICP-OES is used. The ICP-OES model iCAP6000 (Thermo Fisher Scientific, Waltham, MA, USA) was operated in axial and radial viewing modes and was equipped with simultaneous charge injection device (CID) detector and Echelle grating, concentric nebulizer, cyclonic spray chamber, peristaltic pump and a quartz demountable plasma torch with a central tube injector of 2.0mm internal diameter for quantify analytes and dissolved organic carbon (DOC). This DOC refers to remained carbon compounds after the digestion. The operational parameters of ICP-OES are presented in Table 2.

Table 2: Operating parameters of ICP-OES

Instrument parameter	Operating Condition
RF applied power (kW)	1.15
Plasma gas flowrate ($Lmin^{-1}$)	12
Auxiliary gas flowrate ($Lmin^{-1}$)	0.5
Nebulizer gas flowrate ($Lmin^{-1}$)	0.7
Number of replicates	3
Emission lines monitored (nm) Pump rate (rpm)	As I 189.0; C I 193.7 As I 189.0; C I 193.7; Cd II 228.8; Pb II 220.3 75

5) Antimicrobial Analysis

a) Preparation of Mueller Hinton agar

For the preparation of agar for antimicrobial activity analysis, 20.0g of Mueller Hinton agar was dissolved in 1 liter of purified water. Then, the mixture was heated and mixed with magnetic stirrer frequently until completely dissolved. Then, the mixture undergoes autoclave at 121°C for 15 minutes. After the mixture is cool down, the mixture is pour to plates and agar is formed after 20 to 30 minutes.

b) Antimicrobial Activity

The antimicrobial activity of produced food packaging materials was determined by disc-diffusion method or inhibition zone method. 0.1ml of the inoculation of Escherichia coli (E. coli) bacteria was spread to the surface of Mueller-Hinton (MH) agar petri dishes by spread plate technique. Then, 6mm of food packaging films that was prepared were placed onto petri dishes. The disc film containing petri dishes and control samples were incubated at 37°C for 24 hours by using incubator shaker. After the incubation, petri dishes were checked for bacterial growth which the inhibition zones around the disc films were evaluated quantitatively and qualitatively.

III. RESULTS AND DISCUSSION

A. Thermal behavior

Based on the Figure 1, it shows that the plotting data of thermogravimetric analysis by TGA instrument. According to the Figure 1, it can be seen that, the effect of higher concentration of clove essential oil with Zein nanoparticles, Sample 12.5% CEO resulted in most stable thermal behavior as the amount of sample left as residue, 30% is higher compared to another samples. It is known that the incorporation of CEO has form a good film as the thermal stability of compounding is increased. Moreover, sample 10% CEO is slightly differed with sample 12.5% CEO as the sample left as residue is 28%. In the other hand, it was much different for the control sample and sample 2.5% CEO which there is no sample left as residue at temperature of 400°C and 500°C respectively. The thermal decomposition curves for control sample and sample 2.5% CEO shows slightly the same.

For the sample of Sample 5.0%, 7.5% and 10.0% of CEO, this three samples slowly degraded and almost similar to the temperature at which Sample F start to degrade. This proves that above 5% concentration of clove essential oil incorporated with Zein nanoparticles, the film is thermally stable and can withstand until 180°C for the film start degrading. The temperature range at which these samples was degraded completely is not present in the spectra in Figure 2. The increasing concentration that incorporated with starch film increasing the thermal resistant of the film.

From Figure 2, the degradation of film of all samples shows slightly the same but 2.5% CEO sample is the fastest rate of mass change. It indicates the weak thermal degradation has taken place [11]. However, sample with incorporation of CEO exhibited different thermal degradation profiles when compared to the control sample, indicating that the presence of CEO has thermally stabilized the nanocomposites even sample of 2.5% of CEO is the fastest rate of mass change. In Figure 2, the peak start to decreased at 30-750°C as all of the samples had high amount of moisture content. For this analysis, the incorporation of clove essential oil with Zein nanoparticles are proved that it is more thermally stable than control film. The concentration of clove essential oil also plays a major role in improving the film of food packaging. So, the divergence in the temperature of films decomposition could be the result of volume added nanoparticles [12].

B. Toxicity

From Table 3, it shows that 0.02 ppm of Arsenic is found in the control film. Based on equation (1), the real value of the toxic elements content is found. The purpose of calculating this is because of the solid sample is going through the digestion method before the analysis in ICP-OES is run.

$$Content = \frac{Result\ x\ Volume}{Mass\ of\ Sample\ in\ Digestion\ Method} \tag{1}$$

From ICP-OES analysis, the Arsenic content is 0.02ppm while for the real content by using equation (1) is 2ppm. The calculation is shown in equation (2):

$$Content = \frac{\left(0.02\ ppm \left| \frac{\frac{1mg}{L}}{1\,ppm} x\ 0.1\ L\right.\right)}{0.001\,kg} = 2\ mg/kg \tag{2}$$

This calculation is repeated for Cadmium, Cd and also Plumbum, Pb.

Table 3: Content of Toxic Elements in the film of food packaging

Element(s)	Content, mg/kg	Limit, mg/kg
Arsenic, As	2	<0.01
Cadmium, Cd	0.03	<2.00
Plumbum, Pb	1	<2.00

According to the Codex Standard 193-1995, the limit and maximum amount of toxic metals for Arsenic, As, Cadmium, Cd and Plumbum, Pb is less than 0.05mg/kg and less than 2 mg/kg respectively [13]. From the content of As in the food film food packaging, it exceed the maximum amount as this toxin metals could danger from lung or urinary bladder cancer or damages in the skin. Meanwhile for the Cadmium and Plumbum, the amount of these contents in the film is in the safety margin.

From the previous research, Zein is component that gives low toxic degradable end product and clove essential oil is natural plant extract that majorly contain phenol and does not contain Arsenic. Moreover, there is a study conducted on reevaluation of glycerol as food additives as this researcher recommended that the maximum limits for impurities of toxic element in glycerol should be revised to ensure that glycerol is safe to be as food additives. The most probability of the existence of Arsenic metal with supported from previous research is come from glycerol at the period of sample preparation [14].

C. Antimicrobial activity

Based on the Table 4, it shows that all of the composite film inhibits the growth of the tested pathogenic bacteria, E. coli. The photographs of the inhibitory effect of films incorporated with Zein nanoparticles with different concentration of essential oil against the microorganisms of E. coli is observed. The previous study stated that a larger zone of inhibition shows that the antimicrobial is more effective [15].

The results show that control film which is pure Zein inhibit the bacterial growth. The diameter of inhibition zone in incubation for 24 hours at 37°C is 0.3cm. This prove that Zein contain antimicrobial agent that can inhibit the growth of bacteria. The zone inhibition of bacteria can be affected by the release rate of Zein nanoparticles that were embedded in the control film, surface to volume ratio that had a contact with bacteria, characteristic of organic substances and also the mobility of antimicrobial agent in the film. Zein which is mainly constituted from protein can causes the significant interference towards the inhibition of pathogenic bacteria.

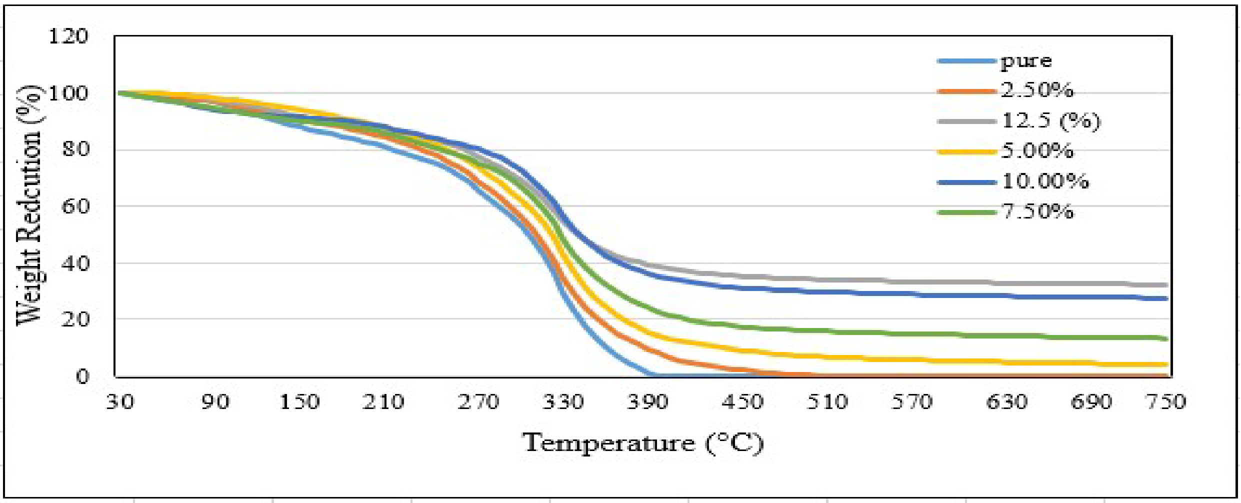

Figure 1: Decomposition curves of Food Film Samples

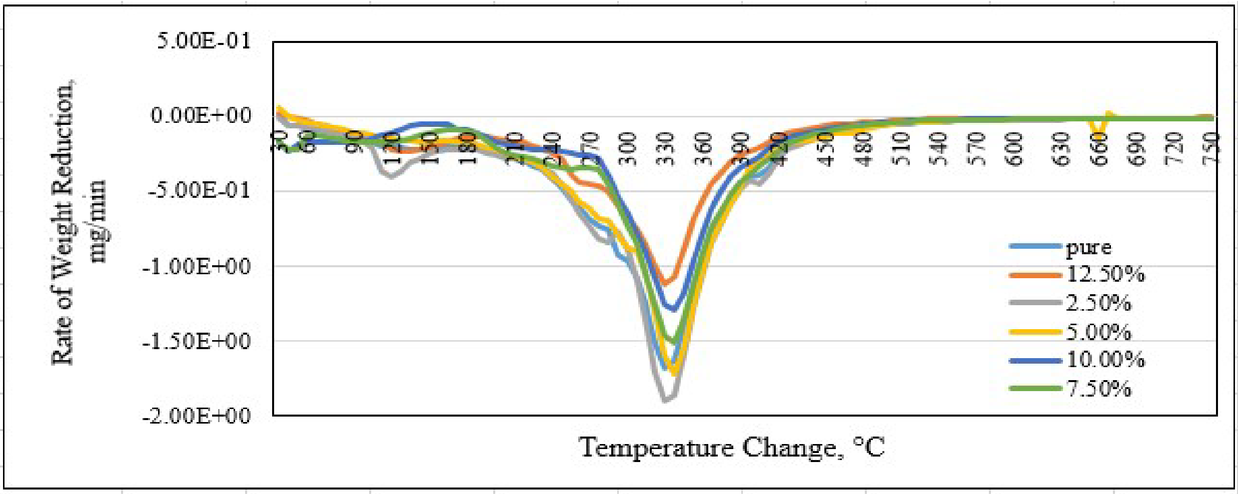

Figure 2: Rate of Weight Reduction of Food Film Samples.

Based on Table 4, the highest concentration (12.5%) of clove essential oil show the highest diameter of zone of inhibition compared to other film. The diameter of inhibition zone for the concentration of clove essential oil at 12.5% after incubation is 1.0cm. As for the zein film with 2.5% and 5.0% clove essential oil is similar with control film, 0.3cm. The incorporation of Zein nanoparticles with clove essential at concentration of 2.5% and 5.0% does not change the result of diameter of inhibition zone of bacteria as compared with control film after 24hours incubation. High concentration of clove essential oil allowing the antimicrobial agent to dissolve more easily towards the target bacteria's lipid cell membrane [16].

This result may be due to the presence of an additional external membrane that surrounding the cell wall which restrict diffusion of hydrophobic compounds through the lipopolysaccharide layer.

Recently, the previous study reported that the increasing concentration of *Zataria* Essential oil to whey-protein film generally produced larger growth inhibtion zoned for pathogenic bacteria [4]. Thus, high concentration of antimicrobial agent which is clove essential oil incorporated with Zein nanoparticles increase the antimicrobial activity of the film food packaging.

Table 4: Antimicrobial Activity of Food Film with E. Coli

Sample	Diameter of Inhibition Zone, cm	Inhibition Behavior
Zein (Pure Film)	0.3	
Zein + 2.5% CEO	0.2	
Zein + 5.0% CEO	0.3	
Zein + 7.5% CEO	0.4	
Zein + 10.0% CEO	0.5	
Zein + 12.5% CEO	1.0	

IV. Conclusion

In conclusion, this study synthetized and characterized Zein nanoparticles incorporated with clove essential oil in producing film food packaging. In addition, this study also determined the antimicrobial activity of the film by using disc diffusion method. The results concluded that incorporation of higher concentration of clove essential oil in Zein nanoparticles will improve the antimicrobial food packaging. This is proven from the results of antimicrobial activity that the diameter of inhibition zoned of 12.5% concentration of clove essential oil is 1.0cm which is the highest. In thermal behavior analysis, the highest concentration of sample film had a strongest thermal behavior that also prove the improvisation of antimicrobial food packaging. The thermal decomposition of the film at 12.5% concentration had 30% sample residue left while other concentrations degraded lower than this sample. As for the toxicity analysis, the metals of Cadmium and Plumbum contents in the film is in the safety margin. As for Arsenic component, this toxin metals are surprisingly high in the film that could endanger the human health. The Arsenic content might be due to the involvement of Glyercol in preparation of sample. For recommendation, further study should be done to characterize and analyze the incorporation of Zein nanoparticles with clove essential oil in Scanning Electron Microscopy, water and oxygen permeability and so on. The plasticizer used which is Glycerol also need to change to natural plasticizer such as alkyl citrates. The scope of antimicrobial activity analysis should also be explored as the film food packaging should be tested against the S. aureus, P. aeruginosa and B. subtilis to check the inhibition of those bacteria by the food film.

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