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TiO_2/Chitosan bioplastic as Antibacterial of *Stephylococcus aureus* for Food Preservation

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ABSTRACT

Nowadays, bioplastic development become hot trends to assess environmental issues. Many materials have been purposed to be the best resources for bioplastic manufacturing. Chitosan is one of the most abundant resources in which could derivates from biomaterial waste called chitin. TiO_2 nanoparticles incorporation within biomaterial presumably not only enhance its mechanical properties but also improve biocompatibility of medical characteristic such as bacterial annihilation. From this study, it was shown that small amount of TiO_2 nanoparticles within chitosan bioplastic prove improvement of both characteristic. Nevertheless, it was also slightly increasing material durability to degrade.

Keywords: *TiO2, bioplastic, Antibacterial, Stephylococcos aureus*

1. INTRODUCTION

Recent development of biodegradable plastic manufacturing for food preservation to also solve environmental issues aimed from second derivatives of natural ingredients like chitosan (Diaz, V. et al., 2010; Fathanah, dkk., 2018; Ginting, dkk., 2018; Naito et al., 2016; Nishiyama et al., 1996; Ogawa, et el., 2019; Rohmawati, dkk., 2018, Kustiningsih, I., et. al., 2019). Chitosan is one of the biggest natural biopolymer sources after cellulose (Mallakpour & Madani, 2015) that have excellent biodegradable features, nontoxic, and biocompatible (Haldorai & Shim, 2014; Mazin C., 2015; Nikkhoo et al., 2018). Chitosan also have anti-microbial properties and easy to produce with low price (Kashif & Park, 2019; Logpriya et al., 2018; Panariello, Coltelli, Buchignani, & Lazzeri, 2019). Compare to conventional plastic, chitosan has a back draw predominantly in its mechanical properties such as tensile strength and elongation (Mallakpour & Madani, 2015)..

Manufacturing nanocomposite chitosan with nanofiller such as silica, clay, and TiO_2 particles predominantly tended to improve not only mechanical properties but also enhance biomedical features within nanocomposite material specifically for food preservation (de Azeredo, 2009; Kanmani & Rhim, 2014a; Rhim, 2011; Rhim, Park, & Ha, 2013). Nowadays, nanofiller classified into several specific type based on its shape, including: nanoparticles (Kanmani & Rhim, 2014, Kustiningsih, I., et al., 2019.), nanofibril (Rafieian, Shahedi, Keramat, & Simonsen, 2014) and nanotubes ((Diaz-Visurraga et al., 2010). Nanoparticle TiO2 incorporation within chitosan material proven to alter its antimicrobial apart from other nanofiller by growth-control and invasion of bacteria that leads to elimination of pathogenic microorganism (de Azeredo, 2009).

TiO_2 has been widely developed for its medical features such as virus, fungi, algae and cancer cell elimination (Chawengkijwanich & Hayata, 2008; Tsuang et al., 2008). Furthermore, TiO_2 proven could annihilates several bacteria (*Pseudomonas aeruginosa* and *Enterococcus faecalis* bacteria (Jeffery, Peppler, Lima, & McDonald, 2010); *Lactobacillus helveticus* (Liu & Yang, 2003)). Furthermore, there are few reports on the observation of pure nanoparticles TiO_2 for specified *Staphylococcus aureus* elimination within chitosan bioplastic. The experiment of manufacturing

TiO_2/chitosan bioplastic and its application specifically for food preservation will leads to the opportunity of advancement bio composite material.

2. METHODS

One sample of 1-gram chitosan mixed with 1% (v/v) acetic acid glacial, stirred for 3 hours and homogenized for 30 minutes at room temperature. Followed by TiO_2 incorporation for each variation (TiO_2: 0 g, 0.1 g, 0.2 g, 0.5 g and 1 g), then stirred for 4 hours and homogenized for 1 hour. A solution molded into glass plate and dried at 80°C afterwards.

The characterization of bioplastic was done using Hitachi SU-3500 Scanning Electron Microscope to assess surface morphology, shape smoothness and TiO_2 particle dispersion. Prestige Shimadzu Fourier Transform Infra-Red 8201 was used for chemical functional groups analysis and Universal Testing Machine to analyze mechanical properties such as elongation and tensile strength.

Sterilized nutrient agar medium has been mixed with sample bioplastic. Followed by blending with rejuvenated Staphyloccus aureus then dissolved with 103 and 104 solution. Thereafter, plating the mixture upon petri dish for 24 hours incubation for different environment: dark and under UV radiation. Bacteria colony counted afterwards by colony forming units (CFU).

3. RESULT and DISCUSSION

3.1. Spectrophotometer FTIR

Fourier Transform Infrared Spectroscopy have observed functional groups analysis involving: O-H bond, N-H bond, C=O bond, C-O bond, and C-N bond for pure chitosan bioplastic sample whereas R-NH-R bond and Cs-TiO_2 were detected for TiO_2/chitosan bioplastic (**Figure 1**).

The graph shows similarity between pure chitosan bioplastic sample and TiO2 incorporated chitosan bioplastic. Characteristics transformation of transmittance peak from FTIR analysis occurred as a result from pure chitosan bioplastic mixed with TiO2 nanoparticles. The mixture has not only physical interaction but also chemical bond (Bourtoom & Chinnan, 2008). Compare to pure chitosan bioplastic, there were shifting of hydroxyl bonds, amino bonds, and amide bonds of TiO2 incorporated chitosan bioplastic. The changes caused by interaction between chitosan molecules and TiO2 nanoparticles (Haldorai & Shim, 2014) which is shown by chitosan – TiO2 bonds on 450 – 950 wavelength range (Amir, Julkapli, & Hamid, 2016).

The spectrum shows that pure chitosan bioplastic has an O-H bonds and N-H bonds at the peak of 3419 cm-1 whereas functional groups of C-H exhibited at 2924 cm-1, C=O at 1616 cm-1, C-O at 1257 cm-1 and C-N at 1039 cm-1 respectively. On the other hand, peak spectrum of TiO2 incorporated chitosan bioplastic is a combination of each pure peak compound's chitosan and TiO2. It could be said with certainty that there was a cross linkage between chitosan and TiO_2 functional groups as for example the overlapped O-H bonds with N-H bonds at 3439 cm-1, R-NH-R at 1600 cm-1, O-H deformation at 1442 cm-1, Chitosan/TiO2 deformation at 947 cm-1.

3.2. Surface layer analysis

The results of Scanning Electron Microscope (SEM) describes the difference upon surface layer of pure chitosan bioplastic and TiO_2 incorporated chitosan bioplastic for each composition (Figure 2). The morphology of pure chitosan bioplastic displays such a homogenous, sleek and clean without any speckles. However, TiO_2 nanoparticles enhancement aimed chitosan bioplastic have a rough structure surface with many grains layered upon.

Tabel 1. FTIR analysys of Cs and TiO_2/Chitosan bioplastic

No	Functional group	Wavelength (cm^{-1}) Reference	Wavelength (cm^{-1}) Experiment
		Cs (Dompeipen, 2017)	
1.	O-H & N-H	3200 - 3500	3419.79
2.	C-H	2850 - 2960	2924.09
3.	C=O	1530 - 1950	1616.35
4.	C-O	1000 - 1300	1257.59
5.	C-N	1030 - 1230	1039.63
		Cs-Ti (Amir et al., 2016)	
1.	-OH *overlapping together with* – NH_2	3300 - 3600	3439.08
2.	R-NH-R	1650 - 1600	1600.92
3.	-OH deformation	1535 - 1400	1442.75
4.	Cs-TiO_2	450 - 950	947.05

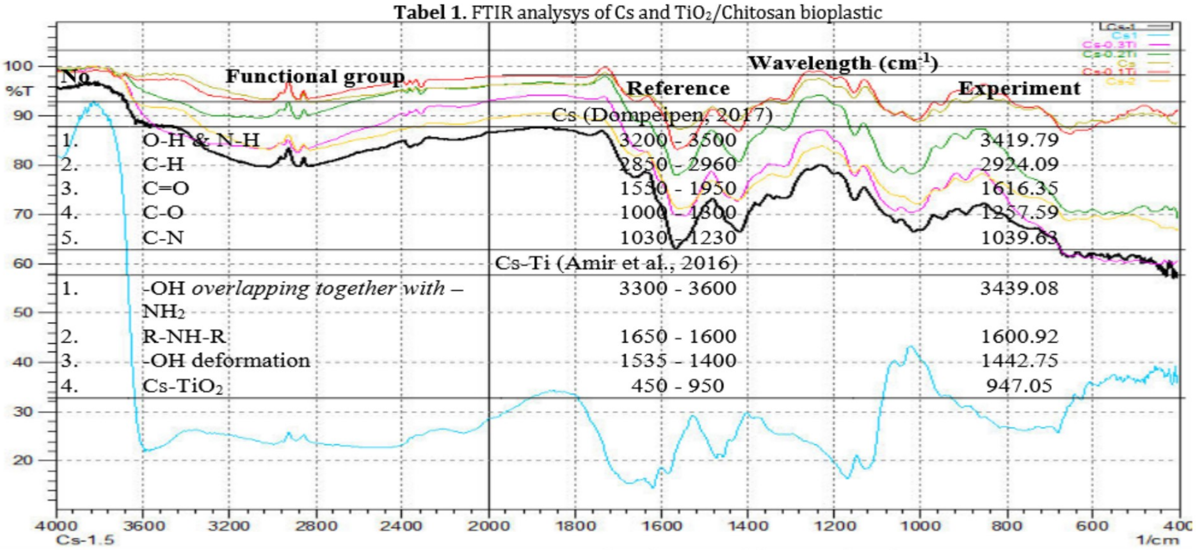

Figure 1. FTIR spectrum of Cs dan TiO_2/chitosan bioplastic

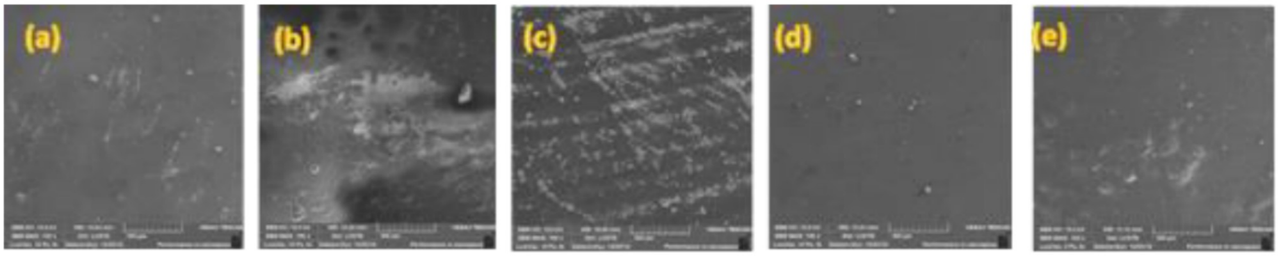

Fig. 2. . Scanning Electron Microscope (SEM) image of chitosan bioplastic and TiO2 incorporate chitosan bioplastic (a) Cs (b) Cs-OH (c) Cs-0.1 Ti (d) Cs-0.2 Ti (e) Cs-0.3 Ti.

These speckles reveal $\mathrm{TiO_2}$ nanoparticles dispersion within chitosan bioplastic matrix (Figure 2b to Figure 2e). $\mathrm{TiO_2}$ nanoparticles started to exhibit regular pattern when the composition of $\mathrm{TiO_2}$ at 1 : 0.1 within chitosan bioplastic (Figure 2c). At ratio of 1 : 0.2 and 1 : 0.3 (Cs : $\mathrm{TiO_2}$), the distribution of $\mathrm{TiO_2}$ nanoparticles within chitosan bioplastic manifests such a fine cluster compare to the chitosan bioplastic surface (Figure 2d and Figure 2e). A proper distribution of $\mathrm{TiO_2}$ nanoparticles within matrix polymer is one of key indicator of a good TiO2 incorporated chitosan bioplastic (Diaz-Visurraga et al., 2010).

3.3. Mechanical Properties analysis (Tensile Strength and Elongation)

Tensile strength and elongation tests are involved on mechanical property analysis of chitosan bioplastic and $\mathrm{TiO_2}$ incorporated chitosan bioplastic (Table 2, Figure 3). $\mathrm{TiO_2}$ enhancement on chitosan bioplastic reduce the both of mechanical features. The amount of $\mathrm{TiO_2}$ nanoparticles composition within chitosan bioplastic change the matrix from homogenous into heterogeneous as appears upon surface layer. The phenomena occurred caused by the irregularity of $\mathrm{TiO_2}$ nanoparticles distribution within chitosan bioplastic matrix as shown in Figure 2b to Figure 2e. These finding also align from others that discovered the effect of $\mathrm{TiO_2}$ nanoparticles on mechanical properties of chitosan (Mazin C., 2015).

Enhancement of $\mathrm{TiO_2}$ nanoparticles within chitosan bioplastic gave a positive results of mechanical properties improvement. For a sample with 0.1 g $\mathrm{TiO_2}$ composition, tensile strength exhibited is 16.93 MPa (decreasing 10.55 MPa) with observed elongation is 8.67 % (increasing 6.02%) from control sample which made from pure chitosan bioplastic. Furthermore, for 0.2 g $\mathrm{TiO_2}$ composition, observed tensile strength is 29.46 (increasing 1.98 MPa) with elongation exhibited was 8.67 % (same with 0.1 g TiO2).

Afterwards, increasing $\mathrm{TiO_2}$ nanoparticles into chitosan bioplastic mixture seems to be declining its mechanical properties. It was shown that for TiO2 composition at 0.3 g, 1 g, 2 g and 3 g, all of them have smaller mechanical properties rather than control sample of pure chitosan. These phenomena happened due to influence of $\mathrm{TiO_2}$ nanoparticles that made a change in matrix structure of chitosan. Pure chitosan bioplastic has a homogeneous matrix structure compare to TiO2 incorporated chitosan bioplastic (Figure 2a to Figure 2e).

Table 2. Influence of $\mathrm{TiO_2}$ nanoparticles to mechanical properties chitosan bioplastic

No	Mixture	Tensile Strength (MPa)	Elongasi (%)
1	Cs	27.48	2.65
2	Cs-1	2.07	3.33
3	Cs-1.5	4.79	2.33
4	Cs-2	11.93	5.33
5	Cs-0.1 Ti	16.93	8.67
6	Cs-0.2 Ti	29.46	8.67
7	Cs-0.3 Ti	15.39	2.33

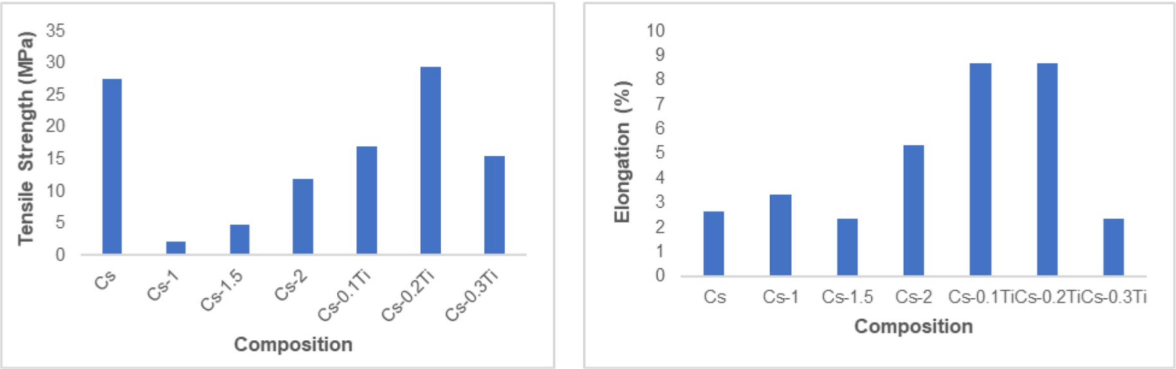

Fig. 3 Mechanical properties analysis (a) Tensile strength; (b) Elongation

3.4. Biodegradation and Antibacterial activity analysis

Biodegradation analysis results after 2 months burial is shown in Table 3. TiO_2 enhancement did not inhibit degradation process of chitosan bioplastic. Mass reducing of bioplastic sample still exhibited due to natural ingredient of bioplastic material (Figure 4). Mass reducing showed up because natural ingredient prone to easier to be digested by several microorganism. Thus, the more TiO_2 nanoparticles enhanced within chitosan bioplastic apt to prevent the process that leads to the longer time required for degradation. This antimicrobial activity from TiO_2 nanoparticles is become one of the features that could effectively prolong chitosan bioplastic endurance from aging.

Table 3. Influence of TiO_2 nanoparticles to mass losses of chitosan bioplastic and TiO_2/chitosan

Sample	Initial weight (g)	Final weight (g)	Mass losses (%)
Cs	0.05	0.02	60.00
Cs-0,1 Ti	0.10	0.05	50.00
Cs-0,2 Ti	0.10	0.05	50.00
Cs-0,3 Ti	0.14	0.09	35071
Cs-0,4 Ti	0.15	0.10	33.33
Cs-0,5 Ti	0.17	0.12	29.41

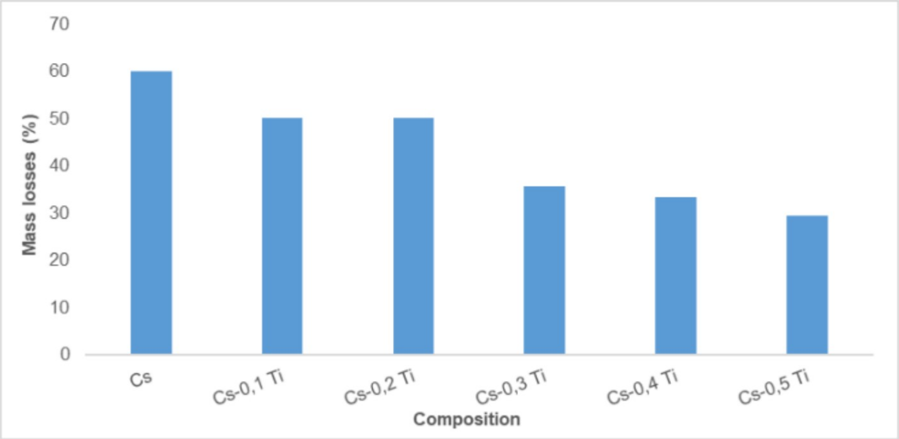

Figure 4 Biodegradation analysis of chitosan bioplastic and TiO_2/chitosan bioplastic

Photocatalytic TiO_2 by UV irradiation has been done to verify antibacterial bioplastic characteristic in order to annihilate gram-negative bacteria and gram-positive bacteria including: endospore, fungi, algae, protozoa and also virus (Paspaltsis et al., 2006). Staphylococcus aureus has been used as a representation of gram-positive bacteria. The results of antibacterial activity presented in Table 4.

As by nature, chitosan bioplastic has an antibacterial activity features but not to eliminate the numbers of Stephylococcus aureus. The antibacterial properties come from amino functional groups that has positive charge which could interact with membrane cell of microorganism which has negative charge. This interaction would cause a destruction of protein and other intracellular constituent from microbes. Chitosan has been tested effectively eradicate gram-negative bacteria rather than gram-positive bacteria (Diaz-Visurraga et al., 2010; Zheng et al., 2000)

It has been proven by these experiment and the results that shown in Table 4, TiO2 nanoparticles enhancement could significantly reduce the amount of s.auerus bacteria that has been incubated for 24 hours. Interestingly, elimination of bacteria s.aureus could be done either by irradiation or without irradiation by UV lights. These kinds of characteristics also observed in other experiments (Diaz-Visurraga et al., 2010; Tsuang et al., 2008). Photocatalytic activity of TiO2 nanoparticles can be initiated by UV irradiation that will release positive ion charge within chitosan bioplastic. These positive ion charge then will interact with lipid membrane of s.aureus bacteria in which have negative ion.

Under UV irradiation, TiO_2 nanoparticles will generate electron (e-) on conduction band and exhibit the hole (h+) on valence band. The hole will interact with water molecule to produce reactive radical hydroxyl (·OH) and ·O2 which lead to degradation of any organic substance like membrane barrier of cell bacteria (Gumiero et al., 2013). Without UV irradiation, the mechanism of s.aureus bacteria elimination within TiO_2 nanoparticles incorporated mixture remains unknown. It was presumably done by similar process in which Ag elements become an antibacterial agent. Both of Ag and TiO_2 have a positive charge that can interact with negative charge which comes from membrane barrier of cell bacteria (Amrulia, 2012). After 24 hours incubation, the number of control variable bacteria tended to be decreased (Figure 6) for both methods (photocatalytic and non-photocatalytic). On pure chitosan bioplastic, bacteria only occurred in several colony. In addition, for every sample tested using TiO_2/chitosan bioplastic there is none of Staphylococcus aureus bacteria prone to be lived.

Table 4 Antibacterial activity analysis

Sample	UV irradiation		Without radiation	
	CFU (mL^{-1})	Survival Ratio (%)	CFU (mL^{-1})	Survival Ratio (%)
Control	2.9×10^5	100.0	2.9×10^5	100.0
Cs	2.0×10^4	6.9	2.0×10^4	3.5
TiO_2	0.0	0.0	0.0	0.0
Cs-0,1 Ti	0.0	0.0	0.0	0.0
Cs-0,2 Ti	0.0	0.0	0.0	0.0
Cs-0,3 Ti	0.0	0.0	0.0	0.0
Cs-0,4 Ti	0.0	0.0	0.0	0.0
Cs-0,5 Ti	0.0	0.0	0.0	0.0

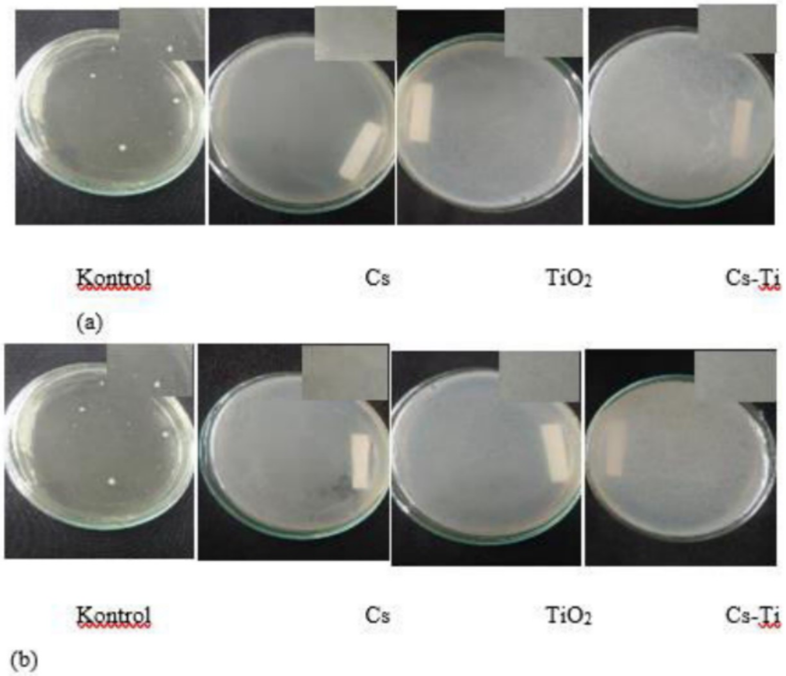

Figure 5. Antibacteria analysis (a)non photocataltic (b) photocatalytic under UV

4. CONCLUSIONS

TiO_2 incorporation within chitosan bioplastic is viable to be manufactured. For specified application of food preservation, TiO_2 nanoparticles enhancement exhibits improvement of not only mechanical properties features such as tensile strength and elongation but also able to annihilates the number of Staphylococcus aureus bacteria to zero either by using photocatalytic of non-photocatalytic method. Nano filling TiO_2 within chitosan bioplastic also made material slightly more durable to degradation compare to conventional plastic.

5. ACKNOWLEDGMENTS

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