

# Synthesis and characterization of chitosan-*Allium sativum* film

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# Synthesis and Characterization of Chitosan-*Allium Sativum* Film

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**Abstract.** Garlic and chitosan are well-known have an antimicrobial activity to terminate or inhibit the growth of bacteria. This study aims to synthesize, characterize chitosan-garlic film using FTIR, SEM, UV-VIS Spectrophotometry, and swelling analysis; and obtain antimicrobial activity during period time. The variation processes were a type of garlic extract, type of bacteria, and the concentration of garlic extract 1%, 2%, 3%, 10%, and 20%. Chitosan-garlic composite film as a product has an elastic and greasy texture. FTIR analysis shows that chitosan-garlic composite film has ionic interaction between chitosan and garlic extract. The water absorption ability of chitosan-garlic composite film cannot be well determined as not using a crosslinking agent that links garlic extract to composite film. SEM analysis shows the heterogeneous morphology surface of the chitosan-garlic film. Chitosan-garlic powder composite film at 20% concentration (b/v) tested against *S. aureus* has the highest antimicrobial activity with OD<sub>600</sub> 0,001342. Chitosan-garlic oil composite film at 20% concentration (v/v) tested against *S. aureus* has the highest antimicrobial activity with OD<sub>600</sub> 0,0013. Spectrophotometry UV-VIS method confirmed that the antimicrobial activity of chitosan-garlic composite film increased compared to the concentration of garlic extract and decreased compared to storage time. The elasticity of chitosan-garlic composite film decreased compared to the concentration of garlic extract.

**Keyword:** film, chitosan-garlic composite, antimicrobial activity

## INTRODUCTION

Indonesia is a tropical country where germs or bacteria are easy to grow and develop. In the case of open wounds, infections often occur due to the entry of germs or bacteria into the wound, so it is necessary to give antiseptic immediately to prevent the wound from getting worse [1].

According to [2], wounds disrupt anatomical structures and the body's physiological functions. The presence of a wound causes a biological response in the form of wound healing which has several phases, such as the hemostasis phase, the inflammatory phase, the proliferative phase, and the remodeling phase. After treatment, there will be vasoconstriction followed by vasodilation, thereby increasing the supply of blood in the wound area. In addition, platelets will de-granulate and secrete Platelet-Derived Growth Factor (PDGF) to attract polymorphonuclear leukocytes (PMN) to the wound area and start the inflammatory phase. PMN leukocytes and macrophages dominate the inflammatory response after four days. After the inflammatory phase, there will be a proliferative phase characterized by the formation of new blood vessels and collagen synthesis. Angiogenesis or the formation of new blood vessels is the key to the wound healing process. This process plays a role in supplying oxygen, nutrients, inflammatory cells, and removing necrotic tissue. Wound healing is a complex process, and incorrect wound treatment can result in more severe wounds, infection, and eventually scars.

Several natural products have been known as drugs in wound healing, such as aloe vera, banana leaf, cocoa, tree bark, turmeric, glucan, honey, and propolis [3]. According to [4], cucumber contains flavonoids, saponins, and polyphenol compounds. The mechanism in the wound healing process in the presence of saponin triggers the

formation of collagen, which is a protein that plays a role in the wound healing process [5]. According to [6], saponin has an antibacterial function. If applied to the injured skin can inhibit bleeding because saponins can form adhesive compounds and cover the injured skin. Flavonoids have anti-inflammatory and anti-allergic properties that can regulate cell function by stimulating the production of Transforming Growth Factor- $\beta$  (TGF- $\beta$ ), which can increase the migration and proliferation of fibroblasts in the scar area and induce Vascular Endothelial Growth Factor (VEGF), which plays a role in the formation of new blood vessels [7]. Polyphenols have functioned as antimicrobials and antivirals [8].

The usage of films from polymers continues to expand in various fields such as biotechnological, pharmaceutical, medical, environmental, and agricultural industries [9]. One of the most critical factors in determining film performance is the selection of film materials. Natural polymers are currently receiving much attention to be used as a film because they are non-toxic, biodegradable, biocompatible, not expensive, and abundant [10]. In particular, alginate and chitosan are natural polymers that have potential as raw materials for composite films.

Chitosan is a polymer that can be used in the pharmaceutical and medical fields. The solubility of chitosan is highly dependent on pH; it often becomes a limitation in the chitosan's utilization. Chitosan is difficult to dissolve in organic solvents and alkaline or neutral solvents. Chitosan can be dissolved well in acidic conditions [11]. Chitosan is also one of the second most significant polysaccharides in nature after cellulose. The cation structure (amine and hydroxyl groups) shows that chitosan has broad antimicrobial ranges against bacteria such as *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Listeria monocytogenes* [12]. In addition, chitosan has film-forming properties, hydrophobic, biodegradable, non-toxic, and transparent [13].

Garlic (*Allium sativum*) has been known as traditional medicine. Garlic has antibacterial properties against both gram-positive and gram-negative bacteria. Garlic (*Allium-Sativum*) has chemical substances such as Allicin, scordinin, allithanin, and selenium. Allicin contained in garlic has functioned as an antibiotic to increase human immunity. Allicin can also inhibit bacteria and yeast growth and has good sensitivity to both gram-positive and gram-negative bacteria. The non-protein amino acid (alliin) and the enzyme alliinase are the most active ingredients contained in garlic. Both compounds will interact and form Allicin which is the main biologically active component in garlic when it is crushed [14].

The addition of garlic extract in chitosan film is supposed to make the chitosan film has a higher antibacterial ability when compared to it the chitosan film.

The purpose of this study was to synthesize and characterize chitosan-allium sativum film, which was produced by combining chitosan with garlic extract (*Allium sativum*) to create a film that has a higher antibacterial ability and could assist in accelerating the wound healing process.

## EXPERIMENTAL METHODS

### Materials

The medical-grade chitosan purchased from Biotech Surindo (Cirebon, Indonesia) was used directly without any treatment. The degree of deacetylation (DD) and the viscosity-average molecular weight (Mv) of chitosan are 81,81 % and 2,700 kDa, respectively. The acetic acid, Glycerin, NaOH, and nutrient broth were pure analysis grades and purchased from Merck, Germany. Garlic powder was purchased from CV. Internusa, Surabaya. Garlic oil was purchased from Bulk Apothecary, Ohio.

### Preparation of Chitosan-Allium Sativum Film

Chitosan solution 2% (w/w) was prepared by dissolving chitosan powder in 0.75 % (v/v) acetic acid solution. The allium-sativum mixture was made by dispersing garlic powder on aquadest. Chitosan and allium sativum solution were mixed in a magnetic stirrer into a homogenous mixture on a certain ratio. Then, the glycerin was mixed as much as 32% (v/v) while heating at 60°C. The film was produced via the immersion precipitation method. The solution was cast onto the glass surface with a certain thickness, dried on the oven at 65°C for 4 hours, and then soaked in 2% (w/v) NaOH for 24 hours. NaOH solution was used as a non-solvent to precipitate the film.

## Characterization of Chitosan-Allium Sativum Film

The chitosan-allium sativum film was subjected to Fourier Transform Infra-Red spectroscopy to characterize the functional group's chemical changes. Fourier transform infrared spectroscopy (FTIR 8400S Shimadzu, Japan) was done using the KBr disc technique in the range of 4000 – 400 cm<sup>-1</sup>. All of the spectra were recorded at room temperature with a resolution of 4 cm<sup>-1</sup> for 45 scans.

The characterization of Chitosan-Allium sativum film was analyzed by studying morphology, chemical structure, and swelling behavior. Morphological analysis was carried out using Scanning Electron Microscope (SEM). The morphology of the film surfaces was observed using an SEM FEI INSPECT S-50, Netherlands. The film was attached to double-sided carbon tape and subsequently placed in a sample holder.

The swelling analysis was carried out by immersing a 5 x 5 cm film sample in distilled water for a definite time and then weighing it. The procedure was repeated until the film achieved equilibrium. It was assigned by constant weight. Furthermore, the swelling parameters were analyzed by comparing the changes in the film mass at a certain time to the initial dry film mass. The following equation calculates the degree of swelling:

$$\% \text{ swelling} = \frac{\text{wet film mass} - \text{dried film mass}}{\text{dried film mass}} \quad (1)$$

## Antimicrobial Analysis of Chitosan-Allium Sativum Film

Antimicrobial analysis was determined by using UV-VIS spectrophotometry. The analysis was carried out at a wavelength of 600 nm for *S. aureus* and *E. coli* bacteria. Film samples were weighed as MIC value, poured into 20 ml of Nutrient Broth (NB), and then added control bacteria. Then, the samples were incubated for 20 hours in a shaker incubator. The usage of a shaker incubator is to maximize the contact between the film sample and the control bacteria. After 20 h of incubation, the Optical Density (OD) of mixtures were determined at = 600 nm. The OD results indicate the number of bacterial cultures present in the sample mixtures.

## RESULTS AND DISCUSSIONS

The research purpose is to study garlic extract (*Allium sativum*) on the antimicrobial properties of chitosan-*Allium sativum* film. The process variables studied were the mass ratio of the garlic extract to chitosan and the kinds of garlic extract used. The antimicrobial performance was tested on both gram-positive and gram-negative bacteria. It was also tested to know the effective changes of the antimicrobial properties over a lifetime period. The antimicrobial performance of chitosan-allium sativum film was tested by using UV Spectrophotometer. In addition, the physicochemical properties of the films were characterized using FTIR (Fourier Transform Infra-Red Spectroscopy) and SEM (Scanning Electron Microscopy). The water absorption ability of the film was conducted by Swelling analysis.

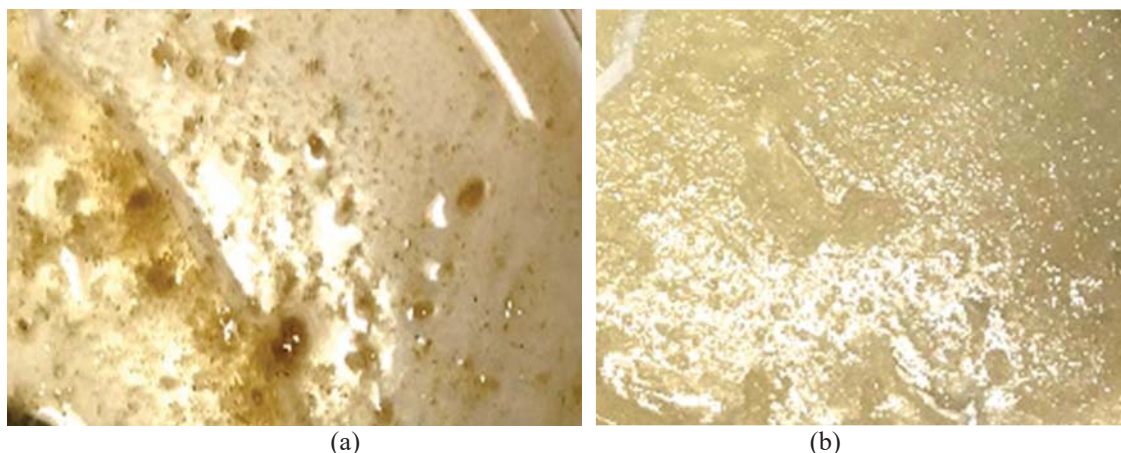
### Chitosan-Allium Sativum Film

In this study, a composite film of chitosan and garlic extract was carried out regarding the research of [15] by modifying the concentration range of chitosan used so that the resulting film structure is not too thick with the presence of chitosan with a semi-powder texture. The solution obtained is clear in color, and bubbles are formed due to stirring. In the process of making chitosan-garlic composite films, chitosan cannot be dissolved entirely, even though chitosan has properties that are soluble in dilute acid solutions.

The addition of glycerin and garlic extract with the ratio of glycerin to acetic acid-chitosan solution refers to the research of [16]. The amount and type of garlic extract added were variables used in this study, with the types of extracts being garlic oil and garlic powder. The amount of garlic extract added will correlate with variations in the concentration of garlic extract in the chitosan-garlic composite film, namely concentrations of 1%, 2%, 3%, 10%, and 20% (v/v) for garlic oil and 1 %, 2%, 3%, 10%, and 20% (w/v) for garlic powder. Garlic extract will dissolve in acetic acid (CH<sub>3</sub>COOH) because garlic extract is polar and because the structure of Allicin will be stable in compounds containing hydroxyl groups [17].

The film solution was heated for 4 hours at 70°C to remove the solvent, referring to the research of [15]. After drying, the appearance of the film will change the color of the film to be browner when compared to before drying.

Then the film was immersed in a 2% (w/v) NaOH solution to release the film. The use of 2% (w/v) NaOH solution serves as a non-solvent solution, where NaOH has OH<sup>-</sup> ions that can bind to H<sup>+</sup> ions from the acetic acid solvent that is remaining on the chitosan-garlic composite film. OH<sup>-</sup> ions will release Na<sup>+</sup> and bind to H<sup>+</sup> ions due to hydrogen bonds, which are the strongest ionic bonds, so that H<sup>+</sup> ions will be released from the chitosan-garlic composite film, leaving marks on the film will become film pores. The chitosan-garlic composite film that has been formed and released from the mold has a thick, chewy, and slippery texture.



**FIGURE 1.** Chitosan-*Allium Sativum* Films (a). Garlic Powder; (b) Garlic Oil

Figure 1 shows that the chitosan-garlic powder film has a heterogeneous surface compared to the chitosan – garlic oil film. Garlic powder has the characteristics of being easy to clot and cannot dissolve completely, so the film formed has a more brittle structure.

It shows that the higher concentration of garlic oil added to the chitosan-*allium sativum* film, the film was white-yellowish and reduced the film elasticity. Otherwise, the higher the concentration of garlic powder added to the chitosan-*allium sativum* film, the film elasticity decreased. The optimum film elasticity is a film which a garlic concentration of 3% (w/v).

### **FTIR Characterization of Chitosan-*Allium Sativum* Film**

The characterization of the physical properties of the chitosan-*allium sativum* film was carried out using the FTIR Spectrophotometer analysis to indicate the functional group of the compound. The active substance in garlic extract that is responsible as an antibacterial agent is Allicin. It has the primary functional group S = O (sulfoxide). The S=O (sulfoxide) functional group has a wavenumber between 1030 cm<sup>-1</sup> – 1070 cm<sup>-1</sup>. The presence of sulfoxide on the film indicates that the film has the ability of an antimicrobial.

Figure 1 and Figure 2 show the presence of the S=O functional group in the garlic and composite film, it was a shift from 1074.02 cm<sup>-1</sup> to 1043.61 cm<sup>-1</sup> in the 10% garlic oil-chitosan film (v /v), and there was a shift from 1026.13 cm<sup>-1</sup> to 1035.13 cm<sup>-1</sup> on the 2% (w/v) chitosan-garlic powder film. The shift in wavenumber on the 2% (w/v) chitosan-garlic powder composite film indicates an ionic interaction between the S=O functional group in chitosan and garlic powder indicated by the widening of the functional group peak. The S=O functional group in garlic oil/powder has a relatively small peak compared to several other peaks.



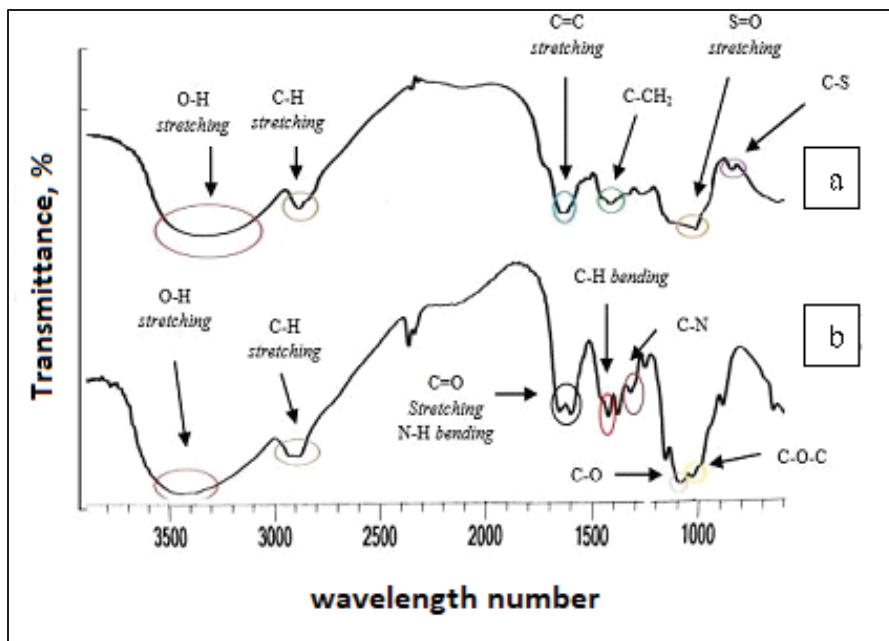


FIGURE 2. IR-spectrum of (a) Allium Sativum; (b) Chitosan

Garlic has properties that are not heat-resistant so that during the extraction and drying process of garlic at high temperatures ( $\geq 100^{\circ}\text{C}$ ), the active substance (Allicin) is partially converted into allyl sulfide where this compound does not contain the S=O functional group. This compound also has antimicrobial properties but is not as effective as Allicin. In Fig. 3 (c), there is no absorption of the O-H stretching functional group. Since making garlic oil with a temperature of 100 C, the sulfur compounds contained in garlic, namely allyl cysteine, evaporate. This allyl cysteine compound contains the O-H functional group.

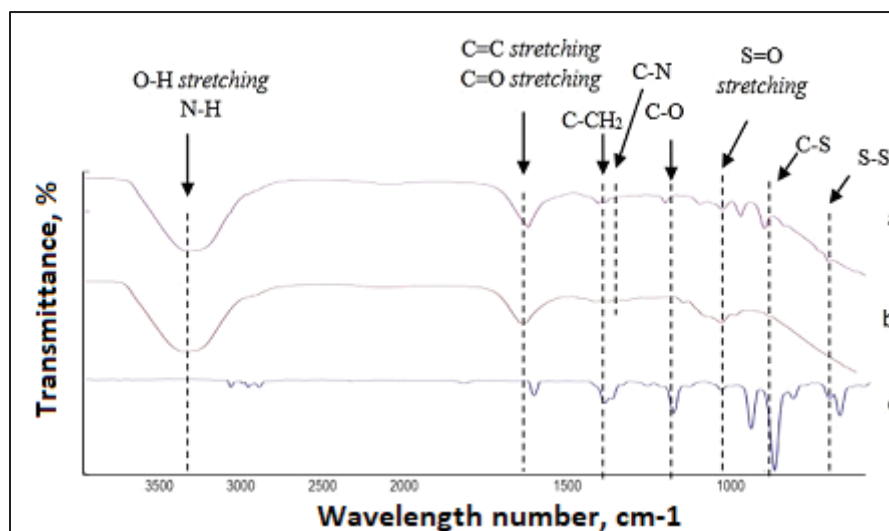
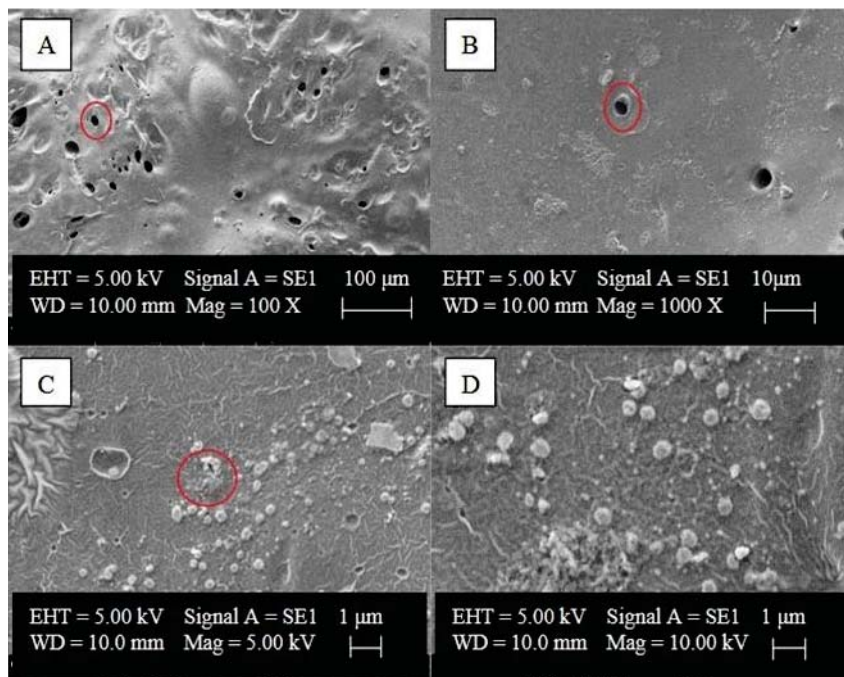


FIGURE 3. IR-spectrum of (a) Chitosan-Allium Sativum from oil garlic 10 % (v/v); (b) Chitosan-Allium Sativum from garlic powder 2%; (c) garlic oil

During the extraction and drying process of garlic into garlic powder, most of the Allicin is converted to other sulfur compounds such as diallyl sulfide and diallyl disulfide. This sulfur compound does not contain the S=O functional group. This shows that Allicin contained in garlic powder is less than Allicin contained in garlic oil.

## Scanning Electron Microscopy (SEM) Characterization of Chitosan-Allium Sativum Film

Morphological analysis was carried out on the surface of the chitosan-garlic composite film using Scanning Electron Microscopy (SEM). Based on Figure 4, the 10% (v/v) chitosan-garlic oil composite film has an uneven, random, and hollow structure. In Fig. 4 (A), (C), and (D), the surface of the film is irregular due to the presence of bubbles. The bubbles on the film's surface are garlic oil that does not bind to chitosan, so it does not mix homogeneously. In this study, we did not use a crosslinker that functions as a binder between garlic oil and chitosan.



**FIGURE 4.** SEM Analysis of Chitosan-Garlic Oil Film 10% (v/v):  
(A) 100x magnification; (B) 1000x magnification; (C) Magnification 5,000x; (D) Magnification 10,000x

There is a smooth surface on the film, a chitosan matrix, while the heterogeneous and irregular surface is caused by the nature of the chitosan-garlic composite film, which is not resistant to heating film undergoes a drying process, there are parts that shrink.

The FTIR results validated the SEM results, which show that the addition of garlic oil to chitosan gives a smaller wavenumber of the hydroxyl group so that it can be proven that garlic oil and chitosan do not mix homogeneously because of the small interaction between the -OH group in garlic and the chitosan group -NH<sub>2</sub> in chitosan.

An inhomogeneous mixture causes many areas that are uneven, hollow, and irregular. Therefore, the garlic attached to the film may come off.

### The Swelling Analysis of Chitosan-Allium Sativum Film

Based on Table 1 and Table 2, 3 % Swelling for chitosan-garlic oil and garlic powder composite films showed negative results. It is because, in this study, there was no crosslinker / chemical binding agent between chitosan and garlic extract oil or powder, so that when immersed in distilled water, there were some garlic extracts that were released from the chitosan-garlic composite film, which affected the weighing results of the film in wet conditions.

In Table 2, the Swelling test can only be carried out on chitosan-garlic powder films with 1%, 2%, and 3% (w/v). It is because, at concentrations of 10% and 20% (w/v), the film formed has no elastic properties at all, and when immersed in distilled water, it will crumble.

**TABLE 1.** The Degree of Swelling of Chitosan-Allium Sativum (oil) Film

| <b>The Concentration of Garlic on Chitosan-Allium Sativum Film</b> |        |        |        |        |        |
|--|--------|--------|--------|--------|--------|
|  | 1 %    | 2 %    | 3 %    | 10 %   | 20 %   |
| <b>% Swelling</b>  | -0.178 | -0.188 | -0.228 | -0.199 | -0.276 |

**TABLE 2.** The Degree of Swelling of Chitosan-Allium Sativum (powder) Film

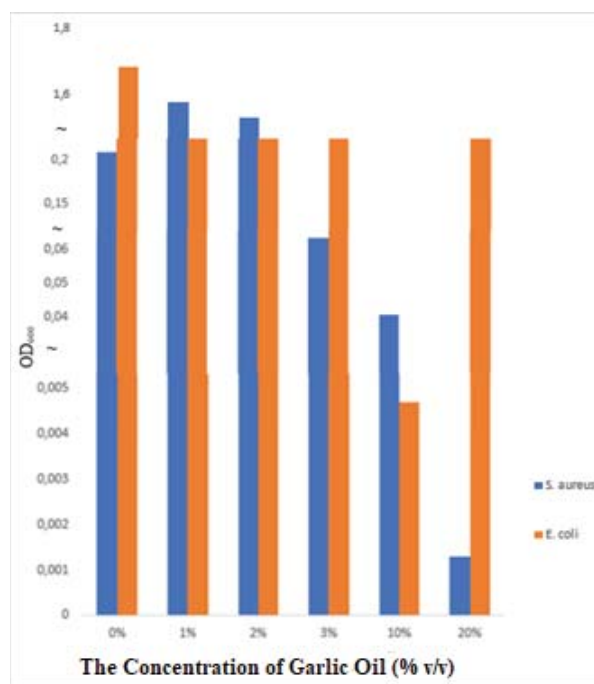
| <b>The Concentration of Garlic Powder on Chitosan-Allium Sativum Film</b> |        |        |        |      |      |
|---|--------|--------|--------|------|------|
|   | 1 %    | 2 %    | 3 %    | 10 % | 20 % |
| <b>% Swelling</b>   | -0.102 | -0.104 | -0.089 | -    | -    |

In Table 2, the Swelling test can be carried out on chitosan-garlic powder films with concentrations of 1%, 2%, and 3% (w/v) only. It is because, at concentrations of 10% and 20% (w/v), the film formed has no elastic properties at all, and when immersed in distilled water, it will crumble.

### Antibacterial Analysis of Chitosan-Allium Sativum Film

The results of the UV-VIS spectrophotometer analysis at = 600 nm or OD<sub>600</sub> indicate the number of bacterial colonies contained in the sample.

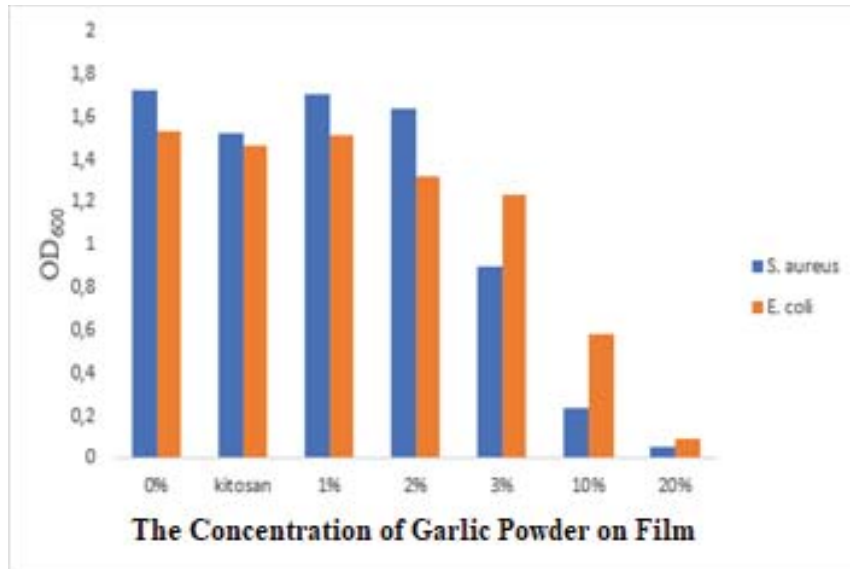
Based on Fig. 5 and Fig. 6, it can be seen that in the composite film-garlic powder and composite film-garlic oil, the higher the concentration of garlic extract, the more the antimicrobial properties of the composite film increased, which was indicated by a decrease in the value of OD<sub>600</sub>.



**FIGURE 5.** The Effect of garlic oil concentration on antibacterial ability for S. aureus and E.Coli

Based on Fig. 5 and Fig. 6, it can also be seen that both types of composite films have antimicrobial properties that are more effective against S. aureus bacteria. It is because gram-negative bacteria such as E. coli have a more complex wall structure consisting of 20% lipid and an outer membrane layer so that Allicin tends to be more difficult to diffuse into E. coli, while gram-positive bacteria such as S. aureus only have a wall structure consisting of 2% lipids, thereby increasing the permeability of Allicin and accelerating the process of cell destruction (Abiy & Berhe, 2016). Therefore, it can be concluded that the antibacterial properties of the chitosan-garlic powder composite film were more effective against gram-positive bacteria.

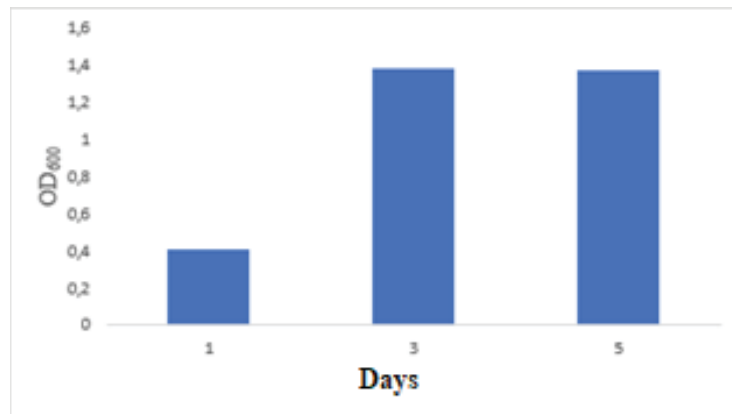




**FIGURE 6.** The Effect of garlic powder concentration on antibacterial ability for S. aureus and E.Coli

This is following the research of Salima, Jeanna (2015), which states that the antimicrobial ability of garlic extract is more effective against gram-positive bacteria than gram-negative bacteria.

The characterization of the antimicrobial properties of the chitosan-garlic oil composite film was carried out by analyzing the effectiveness of the antimicrobial properties of the composite film concerning time, with a selection of 7 days. The composite film used was 10% (v/v) chitosan-garlic oil composite film using S. aureus bacteria.



**FIGURE 7.** Effect of storage time on the effectiveness of antimicrobial properties of chitosan-garlic oil 10% (v/v) Film Using S. aureus Bacteria

Based on Fig. 7, it can be seen that the longer the storage time of the 10% (v/v) chitosan-garlic oil composite film, the lower the antimicrobial properties of the film, which is indicated by the decrease in the OD<sub>600</sub> value of the composite film. It is due to the nature of Allicin which is unstable and easy to re-metabolize into other sulfur compounds such as vinylidithiines and diallyl disulfide (ajoene), which also have antimicrobial power with less activity (Londhe, 2011). Therefore, the antimicrobial activity will decrease with the longer storage time of the 10% (v/v) chitosan-garlic oil composite film. It is following the research of Shokrzadeh et al. (2006), which states that the antimicrobial properties of garlic extract will decrease with the longer storage time.

## CONCLUSION

1. The chemical characteristics of the chitosan-*Allium sativum* (garlic) film based on the FTIR results showed a chemical interaction between several functional groups found in chitosan and garlic. The widening of the peak on specific functional groups in the film have validated the analysis. However, the interaction between the two components did not produce new peaks. Meanwhile, based on the swelling test results on the chitosan-*Allium sativum* composite film, the results showed a negative result because no crosslinker functions as a binder between chitosan and garlic. The results had a decreasing trend.
2. The antimicrobial properties of the chitosan-*Allium sativum* (garlic) composite film increased effectiveness when the amount of garlic extract was more significant than the amount of chitosan in the composite film.
3. The antimicrobial properties of the chitosan-*Allium sativum* (garlic) composite film has effectiveness, whose value decreases with time.

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# Preface

WELCOME FROM InCITE 2021 STEERING COMMITTEE

It is a great pleasure to welcome all of you to the 3rd Bi-Annual International Conference on Informatics, Technology, and Engineering 2021 (InCITE 2021) held by the Faculty of Engineering, University of Surabaya (UBAYA). The first and second InCITE have been successfully held in Bali, Indonesia in 2017 and 2019. Hence, now we are delighted to host the third InCITE through online media due to the Covid-19 pandemic situation.

There are 37 papers have been selected to be presented in InCITE 2021. The papers were written by experts not only from Indonesia, but also from different parts of the world. The main theme of this conference is Leveraging Smart Engineering in response to the current and future Industrial Revolution 4.0 that should be handled by every country in the world. We hope through this conference, all participants will be able to know each other and interact to develop future collaboration.

We would like to express our sincere gratitude to the Keynote speakers, International Scientific Committee, Steering Committee, and Organizing Committee for their huge efforts to make this conference successful.

Thank you all for your support and attendance at InCITE 2021. Please enjoy the conference!

Asst. Putu Doddy Sutrisna, Ph.D.  
Chair, InCITE 2021 Steering Committee



# Preface

WELCOME FROM InCITE 2021 ORGANIZING COMMITTEE

Welcome to InCITE 2021! The third bi-annual international conference on engineering domain conducted by the Faculty of Engineering, The University of Surabaya (UBAYA). Due to the COVID-19 pandemic, InCITE 2021 is held as an online conference. Online conference opens the opportunity for many researchers around the globe to share their findings and learn from other global researchers with less restrictions.

InCITE 2021 invites three keynote speakers, well reputable global researchers in their research domain from Australia and Taiwan. Following each keynote session are two presentation sessions run in parallel.

This year, we received 66 papers submitted by researchers from four distinct countries (i.e., first author's country of origin): Indonesia, Australia, Taiwan, and Kazakhstan.

We employed a double-blind review to ensure a high standard and a minimum level of bias in the reviewing processes. This resulted in 56% of the submissions were accepted and will be published to the AIP Conference Proceedings.

Authors of all accepted papers are to disseminate their findings during InCITE 2021 conference between 25 to 26 of August 2021. This presents a great opportunity for everyone, including the researchers, to discuss and further improve current achievements.

We thank all keynote speakers, presenters, and reviewers/scientific committees for the generous supports. We thank the University of Surabaya, the Faculty of Engineering UBAYA, and all InCITE 2021 committees that enable InCITE 2021.

We wish you a very pleasant and rich conference experience in InCITE 2021 and looking forward to seeing you again on InCITE 2023! Thank you.

Yours sincerely,  
Asst. Prof. Dr. Jimmy  
Chair, InCITE 2021 Organizing Committee

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The InCITE 2021 Organizing Committee would like to thank the following names who have served as the:

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# TABLE OF CONTENTS

|                            |     |
|----------------------------|-----|
| Preface .....              | i   |
| Conference Organizer ..... | iii |
| Table of Contents .....    | vii |

## SMART DESIGN AND INNOVATION

|   |      |
|---|------|
| How energy efficiency, smart factory, and mass personalization affect companies in Industry 4.0<br><i>Yu-Chung Tsao, Felix Arril Simbara Barus, Chien-Wei Ho</i> .....  | A-1  |
| Kansei Engineering and Product-Service Systems (KEPSS) for Customer-centered Experience<br><i>Markus Hartono</i> .....  | A-10 |
| The Role of Supply Chain Performance to Determine the Firm Performance<br><i>Yoni Kristiawan, Edi Purwanto, and Rustono Farady Marta</i> .....  | A-15 |
| Real-time Monitoring Design for Make-To-Order Industry<br><i>Senapati C. Adisasmito, Pradipta Deffinika Pamungkas, and Anas Ma'ruf</i> .....  | A-25 |
| Reducing Production Process Lead Time Using Value Stream Mapping and Kaizen Approaches:<br>A Case Study in the Musical Instrument Industry<br><i>Indra Setiawan and Hernadewita</i> .....                       | A-35 |
| Simple Heuristics for Scheduling Apache Airflow: A Case Study at PT. X<br><i>Hans Tanto, Indriati N. Bisono, and Hanijanto Soewandi</i> .....   | A-44 |
| Quality Improvement with PDCA Approach and Design of Experiment Method in Single Socks<br>Industry in Indonesia<br><i>Hibarkah Kurnia, Choesnul Jaqin, and Humiras Hardi Purba</i> .....                        | A-54 |
| Integration of Corporate Social Responsibility for Improved Business Performance: Evidence<br>from the Indonesian Manufacturing Industry<br><i>Esti Dwi Rinawiyanti, Xueli Huang, and Sharif As-Saber</i> ..... | A-65 |
| Community Mobility during Covid-19 Pandemic and Tourism Performance: Data Mining Approach<br><i>Gunawan</i> .....   | A-76 |
| Inventory System Improvement for Short-lived Item<br><i>Noverta Brilly Leksana Putra, Indri Hapsari, Dina Natalia Prayogo</i> .....   | A-85 |
| Users' Perception of Digital Prototypes in Indonesian Fashion Industry: A Qualitative Study<br><i>Christabel Parung and Prayogo Waluyo</i> .....  | A-92 |

## SMART MANUFACTURING AND PROCESSES

- Dynamic Study of Batch Milk Cooling Process at KUD SAE Pujon  
*Rudy Agustriyanto, Puguh Setyoprato, and Endang Srihari*..... B-1
- Recent advances and application of Selective Laser Melting (SLM) in the aerospace industry  
*Anel Yerubayeva, Essam Shehab, and Md. Hazrat Ali* ..... B-8

## POWER SYSTEM AND SMART ENERGY MANAGEMENT

- Immobilization of Xylanase on Acid Pretreatment Bentonite as Green Biocatalyst  
*Lieke Riadi, Ruth Chrisnasari, Joshua Kristanto, Cahaya Caesar Bigravidia, Meyta Sanoe* ..... C-1
- Rheological Behavior and Antioxidant Activity of Carrageenan Extracted from Green Seaweed (*Eucheuma cottonii*) Using Alkaline Solution at Low Temperature  
*Puguh Setyoprato and Lanny Sapei* ..... C-9
- Simulation of Impact Azimuth Angle on Specific Energy Output of a Fixed Mounting Rooftop PV System in Jakarta, Indonesia  
*Elieser Tanigan*..... C-18
- Rice Husk Ash for the Stabilization of the Outer Interfacial Layer of W/O/W Double Emulsion  
*Lanny Sapei, Rudy Agustriyanto, Endang Wahyu Fitriani, Zerravym Levy, and Cindy Sumampouw*..... C-25
- The Effects of Material to Solvent Ratio on The Performances of Natural Dyes Extraction  
*Putu Doddy Sutrisna, Meyta Sanoe, Rifando Gogo Adiyaksa, Kristina Wahyu Agustine, Hadiyatni Rita Priyantini, Prayogo Widyastoto Waluyo, and I Made Ronyastra* ..... C-33
- Carbon Based Sulfonated Catalyst as an Environment Friendly Material: A Review  
*Putu Padmareka Deandra, Herry Santoso, and Judy Retti B. Witono*..... C-43
- A Performance Study of Magnetite-Lignin Composites as Photothermal Materials in Solar Steam Generation System  
*Marta Devega Yuharma, W. Widiyastuti, Ni Made Intan Putri Suari, and Heru Setyawan*..... C-54
- Organosolv Lignin from Coir Fibers as Potential Biomaterials for Sunscreen  
*Diana Novita Sari, Mahardika F. Rois, W. Widiyastuti, and Heru Setyawan*..... C-61
- The Effect of Sodium Lauryl Sulfate on Silica Nanofluid Stabilization Using Microbubble Method  
*Ratri Sekaringsalih, I Made Joni, W. Widiyastuti, and Heru Setyawan* ..... C-69
- One-Step Electrochemical Synthesis of Silica-coated Magnetite Nanofluids  
*Delyana Ratnasari, W. Widiyastuti, and Heru Setyawan* ..... C-77
- Textile Waste Purification Using Corona Discharge Method  
*Rezha Carina Rachmady, Ari Rahman, and Wahyu Kunto Wibowo* ..... C-85
- Synthesis and Characterization of Chitosan-Allium Sativum Film  
*Emma Savitri, Maria Irine Tjahayani, and Anastasia Apriyani Ngene Say* ..... C-92

## THE ROLE OF IT IN INNOVATION ENHANCEMENT

|   |       |
|---|-------|
| Hate Speech Content Detection System on Twitter using K-Nearest Neighbor Method<br><i>Vincentius Riandaru Prasetyo and Anton Hendrik Samudra</i> .....                                    | D-1   |
| School Finder, Intelligent Recommendation System for Elementary School Selection<br><i>Susana Limanto, Endah Asmawati, and Yusuf Wira Kencana Putra</i> .....                             | D-11  |
| Designing a Recommender System based on the Application of Decision Tree Algorithm in Data Mining with KNIME<br><i>Yenny Sari, Vincentius Riandaru Prasetyo, and Kevin Liyansah</i> ..... | D-20  |
| Weather Image Classification using Convolutional Neural Network with Transfer Learning<br><i>Mohammad Farid Naufal and Selvia Ferdiana Kusuma</i> .....                                   | D-28  |
| Student Performance Prediction in Higher Education: A Comprehensive Review<br><i>Ellysa Tjandra, Sri Suning Kusumawardani, and Ridi Ferdiana</i> .....                                    | D-38  |
| Sentiment Analysis on Feedback of Higher Education Teaching Conduct: An Empirical Evaluation of Methods<br><i>Jimmy and Vincentius Riandaru Prasetyo</i> .....                            | D-48  |
| Implementing Directed Pairwise Judgment Approach in Web-Based AHP Survey Application to Reduce Inconsistency Ratio<br><i>Daniel Hary Prasetyo</i> .....                                   | D-57  |
| Automatic Leaf Geometric Properties Measurement Based on Camera Parameters<br><i>Joko Siswantoro, Ida Bagus Made Artadana, and Muhammad Z. F. N. Siswantoro</i> .....                     | D-64  |
| A Design of Secure Supply Chain Management System with Blockchain Technology<br><i>Alexander Yohan, Nai-Wei Lo, and Kevin Valentino</i> .....   | D-72  |
| Implementation of Behavior Tree for Creating an In-Game Cut-Scene<br><i>Delta Ardy Prima</i> .....  | D-82  |
| The Online Attendance System Models for Educational Institutions<br><i>Andre and Marcellinus Ferdinand Suciadi</i> .....  | D-92  |
| Genetic Algorithm with Adaptive Diversification and Intensification for the Vehicle Routing Problem<br><i>Eric Wibisono, Iris Martin, and Dina Natalia Prayogo</i> .....                  | D-102 |

# Smart Design and Innovation

# Smart Design and Innovation

## TABLE OF CONTENTS

|   |      |
|---|------|
| How energy efficiency, smart factory, and mass personalization affect companies in Industry 4.0<br><i>Yu-Chung Tsao, Felix Arril Simbara Barus, Chien-Wei Ho</i> .....  | A-1  |
| Kansei Engineering and Product-Service Systems (KEPSS) for Customer-centered Experience<br><i>Markus Hartono</i> .....  | A-10 |
| The Role of Supply Chain Performance to Determine the Firm Performance<br><i>Yoni Kristiawan, Edi Purwanto, and Rustono Farady Marta</i> .....  | A-15 |
| Real-time Monitoring Design for Make-To-Order Industry<br><i>Senapati C. Adisasmito, Pradipta Deffinika Pamungkas, and Anas Ma'ruf</i> .....  | A-25 |
| Reducing Production Process Lead Time Using Value Stream Mapping and Kaizen Approaches:<br>A Case Study in the Musical Instrument Industry<br><i>Indra Setiawan and Hernadewita</i> .....                       | A-35 |
| Simple Heuristics for Scheduling Apache Airflow: A Case Study at PT. X<br><i>Hans Tanto, Indriati N. Bisono, and Hanijanto Soewandi</i> .....   | A-44 |
| Quality Improvement with PDCA Approach and Design of Experiment Method in Single Socks<br>Industry in Indonesia<br><i>Hibarkah Kurnia, Choesnul Jaqin, and Humiras Hardi Purba</i> .....                        | A-54 |
| Integration of Corporate Social Responsibility for Improved Business Performance: Evidence<br>from the Indonesian Manufacturing Industry<br><i>Esti Dwi Rinawiyanti, Xueli Huang, and Sharif As-Saber</i> ..... | A-65 |
| Community Mobility during Covid-19 Pandemic and Tourism Performance: Data Mining Approach<br><i>Gunawan</i> .....   | A-76 |
| Inventory System Improvement for Short-lived Item<br><i>Noverta Brilly Leksana Putra, Indri Hapsari, Dina Natalia Prayogo</i> .....   | A-85 |
| Users' Perception of Digital Prototypes in Indonesian Fashion Industry: A Qualitative Study<br><i>Christabel Parung and Prayogo Waluyo</i> .....  | A-92 |