




OPTIMIZATION OF ANTIBACTERIAL EDIBLE FILM FORMULATION BASED ON CHITOSAN, VELVET BEAN ETHANOL EXTRACT, AND CINNAMON ESSENTIAL OIL

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ARTICLE INFO	ABSTRACT
<p>Keywords: Antibacterial; Cinnamon Essential Oil; Edible Film; Velvet Bean; Ethanol Extract</p> <p>Article History: Received: 2024-07-13 Accepted: 2024-12-25 Published: 2024-12-25 doi:10.20961/jkpk.v9i3.90152</p>  <p>© 2024 The Authors. This open-access article is distributed under a (CC-BY-SA License)</p>	<p>The existing research on edible films as packaging materials has maintained that they are better in constituent materials, composition, and functionality. Due to their good bioactivity, edible films are utilized for packaging, which is considered bioactive. The recent study focused on using chitosan-velvet bean (<i>Mucuna pruriens</i> (L.) DC.) based edible bioactive packaging formulation for antibacterial activity. Velvet bean ethanol extract (V) accompanied by cinnamon (<i>Cinnamomum burmannii</i> (Ness) BL) essential oil (C) which is effective as an antibacterial against <i>Escherichia coli</i> ATCC 25922 and <i>Staphylococcus aureus</i> ATCC 25923. Based on this study, the ascertained concentrations of V and C can generate the most optimum edible film associated with antibacterial activity and characterize the physical properties and morphology of the most optimum formulation of antibacterial edible film. It was proved that by addition of 30% V and 3.0% C, the most optimum edible film can be produced, which has maximum antibacterial activity against <i>E. coli</i> ATCC 25922 (19.36 mm strong) and <i>S. aureus</i> ATCC 25923 (18.94 mm strong). Moreover, this formulation boosts the thickness, tensile strength, and solubility of the film and simultaneously reduces the film's elongation, WVTR, and WVP. The edible bioactive packaging formulation produced thickness, tensile strength, solubility, percentage elongation, WVTR, and WVP values of 0.179 mm, 0.318 Mpa, 0.057%, 10.096%, and 3.747 g/m².d, 8.586 g/m.d, respectively. The surface of the edible film still had some degree of porosity and texture, according to the morphology.</p>
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INTRODUCTION

Packaging is one of the basic features of modern product distribution: Apart from preventing damage, it helps to maintain the product quality during its life [1]. There are so many different types of materials used. However, plastic is by far the most widespread and easily accessible, with its

excellent strength, flexibility, durability, and resistance to the environment and corrosion. While it has utility, the plastic we rely upon so heavily has led to a dire waste crisis. The percentage of plastic as part of the National Waste in Indonesia will be around 17% in 2021 (MoEF, 2022) [2]. As plastic is non-biodegradable, it accumulates in the

environment for several decades, taking about 10–20 years to decompose naturally [3]. The long degradation phase leads to environmental pollution, a biodegradable ecosystem, and the propagation of global warming, therefore prompting sustainable alternatives [4].

The increasing environmental threat of plastic waste has led to a focus of research into biodegradable packing materials. One of the innovative solutions to this issue is the use of edible films. These coats consist of ultra-thin, edible layers that act as a sustainable replacement for fossil-based plastics due to their ability to provide similar functionalities but in an environmentally friendly and non-toxic for human intake [5]. Recent years have witnessed rapid developments in this area, resulting in notable changes in edible film structure and properties and progress towards bioactive packaging. This packaging type contains bioactive compounds with antimicrobial, antifungal, or antioxidative properties, which can improve the deterioration and safety of the packaged products while simultaneously decreasing the ecological impact of packaging materials.

Green material science: The switch from traditional plastics to more bioactive edible films is revolutionary. By integrating natural and biodegradable components, these films also help with food safety and shelf-life extension, thus addressing environmental concerns. Bioactive packaging is deliberately key to global sustainability movements, ensuring that functional effectiveness meets ecological responsibility at every level—in product and

practice. This novel idea emphasizes the significance of decomposable packaging in decreasing the negative consequences of plastic contamination, increasing garbage disposal, and establishing sustainable consumer behavior.

Bioactive packaging presents a new method for food preservation in which bioactive compounds can be introduced into edible films. These compounds demonstrate antibacterial, antifungal, and antioxidant properties, allowing them to protect the packaged products effectively and, in turn, considerably promoting durability and quality [6]. Chitosan, a natural polymer with film-forming ability and intrinsic antimicrobial activities [7], is one of the important materials used to develop these films. To ensure that chitosan-based films can fulfill different requirements in the packaging field, plasticizers, such as glycerol, are periodically incorporated to reduce the stiffness of the polymer matrix and the subsequent development of flexible films [8]. In addition to these adjustments, scientists are investigating using natural extracts and essential oils to improve the films' quality and expand their antimicrobial and antioxidant properties.

Among the natural additives studied, velvet bean (*Mucuna pruriens*) has shown potential due to its high antioxidative and antibacterial effects. With an IC₅₀ value of 6.76 ppm, velvet bean ethanol extract has demonstrated significant antioxidative activity, exhibiting high efficacy in preventing oxidative degradation [11]. In addition, phytochemical analysis of the extract indicates the presence of flavonoids and

tannins, agents with antimicrobial activity. Due to these characteristics, velvet bean extract can be considered a natural barrier to microbial contamination and an environmentally friendly alternative to synthetic antimicrobial agents [12].

Another topic of recent interest in bioactive film is essential oils that provide functional and sensory benefits. In addition to providing antimicrobial properties to the films, these oils also help prevent water evaporation from the food surfaces, which helps delay degradation [11]. Derived from *Cinnamomum burmanii*, cinnamon essential oil stands out for its strong antibacterial and antifungal effects. Cinnamon oil comprises bioactive compounds, including eugenol and cinnamaldehyde, demonstrating strong efficacy against the following pathogens: *Candida albicans*, *Bacillus aureus*, and *Escherichia coli*. Rizki & Panjaitan found Promega™ to have inhibition zones as high as any other natural antimicrobial, therefore rightly describing it as a strong natural antimicrobial agent [14].

This study aims to develop a bioactive edible film based on chitosan, velvet bean ethanol extract, and cinnamon essential oil. The study sets out to develop packaging using these complex carbohydrates and other polymers, fulfilling functional food preservation requirements while responding to environmental and health challenges. These bioactive films can potentially interact with harmful bacteria, such as *Escherichia coli* and *Staphylococcus aureus*, offering a unique and eco-friendly approach to dependable food packaging. This method responds to the increasing

demand for where it is needed effective and sustainable materials, significantly revealing high functionality by combining natural products with cutting-edge materials for packaging.

This research aimed to develop a novel edible bioactive packaging material based on chitosan as a film-forming agent and incorporated with velvet bean ethanol extract and cinnamon essential oil. This study focused on solving the major concerns of food packaging by implementing proper antibacterial protection and sustainability. Chitosan, which has excellent film-forming capabilities and intrinsic anti-pathogenic properties, became the basis of a promising material further doped with a mixture of velvet bean extract and cinnamon essential oil. Velvet bean extract was used as a natural barrier against microbial contamination because of its high antioxidative activity and because it contains phenolic compounds like flavonoids and tannins. Cinnamon essential oil increases the film's activity against foodborne pathogens, such as *Escherichia coli* and *Staphylococcus aureus*, which are known to pose food safety concerns and shorten food shelf life due to spoilage.

This study aimed to develop a biodegradable and multi-functional packaging material that complies with the global sustainability agenda by incorporating the aforementioned natural resources. The study highlighted the developed bioactive film's antibacterial activity and its ability to minimize dependence on conventional plastic packaging, whose environmental hazards are considerable. Developing eco-friendly solutions such as water-activated

adhesive food packaging emphasizes the continuation of using natural resources. It is a functional solution for safe, sustainable, and effective food packaging materials.

METHODS

1. Isolation of Cinnamon Essential Oil

The bark was sorted, washed, dried, powdered, and then placed in an apparatus for steam distillation. This distillation was performed until the resulting liquid had stopped dripping. The essential oil contained water and was separated using a separatory funnel. Anhydrous Na₂SO₄ was introduced into the upper part to absorb the remaining water in the oil, and then the oil was filtered by filter paper [15], [16].

2. Ethanol Extraction of Velvet Bean

On rolling, the velvet bean was soaked in water (3 x 24 hours), in which the skins were peeled, and the water was changed every 6 hours. The velvet bean was subsequently washed and drained before blending with a solvent (70% ethanol) to form a velvet bean porridge. For 6 hours, velvet bean porridge was macerated, and after that, it was filtered and filtrated in a rotary evaporator (50-60°C); a solid product was obtained and dried in an oven (at 50°C for 10 hours) to obtain an extract, generally recognized as velvet bean ethanol extract in this study (V) [17], [11].

3. Formulation of Edible Film Based on Chitosan-Velvet Bean Ethanol Extract (CVE)

This study referenced the study [19] for formulating the basic edible film.

Table 1. Formulation of basic edible film (E)

Material	Mass (gram)
Chitosan	3.00
1% CH ₃ COOH	105.00
Glycerol	1.26
Total	109.26

Table 2. Formulation of edible film based on chitosan-velvet bean ethanol extract (CVE)

Sample Code	Material (gram)		
	E	V	Total
CVE 0	109.26	0	109.26
CVE 10	98.33	10.93	109.26
CVE 20	87.41	21.85	109.26
CVE 30	76.48	32.78	109.26
CVE 40	76.48	43.70	109.26
CVE 50	54.63	54.63	109.26

Notes:

- E = Basic Edible Film
- V = Velvet Bean Ethanol Extract
- CVE = Edible film based on Chitosan-Velvet Bean Ethanol Extract

All substances from [Table 1](#) were mixed until homogeny, then the ethanol extract of the velvet bean was added according to the formulations described in [Table 2](#) and mixed again for 55°C; the edible solution was poured into a 10 x 10 cm mold and baked in the oven at 60°C for about 24 hours. After drying, extract the edible film from the mold and dry it in an oven at a specified temperature. Moreover, the antibacterial activity test was performed to find the velvet bean ethanol extract's best concentration (optimal formula).

4. Formulation of Edible Film Based on Chitosan-Velvet Bean Ethanol Extract (CVE) with the addition of Cinnamon Essential Oil (C)

Based on the formulation in [Table 3](#), the cinnamon essential oil was incorporated into the most optimum CVE formula after the most optimum formula of velvet bean ethanol extract had been determined, and then, carried out an antibacterial activity test was

used to find the minimum cinnamon essential oil addition formula.

Table 3. Formulation of edible film based on chitosan-velvet bean ethanol extract (CVE) with the addition of Cinnamon Essential Oil (C) (herein after referred to as CVCE)

Sample Code	Material (%)		
	CVE	C	Total
CVCE 0	100	0	100
CVCE 1	99	1	100
CVCE 2	98	2	100
CVCE 3	97	3	100
CVCE 4	96	4	100
CVCE 5	95	5	100

Notes:

CVE	=	Edible Film based on Chitosan-Velvet Bean Ethanol Extract
C	=	Cinnamon Essential Oil
CVCE	=	Edible Film based on Chitosan-Velvet Bean Ethanol Extract with the addition of Cinnamon Essential Oil

5. Testing the Antibacterial Activities against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923

Antimicrobial activities of CVE 0, CVE 10, CVE 20, CVE 30, CVE 40, CVE 50, CVCE 0, CVCE 1, CVCE 2, CVCE 3, CVCE 4, and CVCE 5 against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 was conducted through the pitting method as follows:

A medium was prepared by dissolving 5.7 grams of Muller Hinton Agar (MHA) in distilled water in a 150 mL Erlenmeyer flask and heating until a homogenous consistency was reached. The medium was then autoclaved at 121°C for 15 minutes and allowed to cool at 45°C; then, it was poured into a 25 mL petri dish and solidified.

Suspension of test bacteria, *Escherichia coli*, and *Staphylococcus aureus*, was prepared by aseptically picking a loop full of colonies from the respective medium (solid NA) into the test tube with 5 mL of

physiological NaCl. In the next steps, once the medium and suspension solution were prepared, a cotton swab spread the suspension solution across the surface of the medium MHA. In the medium, wells were created using a cork borer (6 mm) at seven places. 100 µL of test sample solution, positive and negative control, were added to each well. Antibacterial activity testing using CVE and CVCE edible film solutions; that is, The test was performed as the edible film solution before solidifying into the film.

Petri dishes were subsequently incubated at 37°C for 24 hours, and the diameter of the inhibition zone was measured using a caliper. *Vancomycin* was the positive control for *Staphylococcus aureus* ATCC 25923, *Chloramphenicol* for ATCC 25922, and sterile distilled water was based on the fact that this neutral compound would not affect bacterial growth [16].

6. Characterization of the most optimum CVCE

The best quality edible film was determined through physical property tests, including thickness, tensile strength, elongation, solubility, WVTR, and WVP. The surface morphological analysis of the film was performed using a SEM.

7. Statistical Analysis

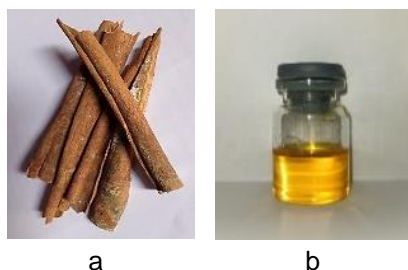
The One-Way ANOVA test analyzed statistical analysis. The data from the antibacterial activity test to determine the effects of different treatments were analyzed using Duncan's Multiple Range Test (DMRT) at a 5% significance level. Data from the antibacterial activity test were analyzed to

see how different treatments were affected at eight repetitions by utilizing the One-Way Anova test followed by Duncan's Multiple Range Test (DMRT) test at a significance level of 5% as a test to compare the means between treatments and determine the mean values that are significantly different.

RESULTS AND DISCUSSION

1. Cinnamon Essential Oil Isolation

The isolation process was carried out by the water vapor distillation method using an initial sample of 3.55 kg of cinnamon bark. The following are the results of the cinnamon essential oil isolation:



Figures 1. Cinnamon (a) and cinnamon essential oil (b)

Table 4. Cinnamon essential oil characteristic test results

Parameter	Outcome
Initial weight (kg)	3.55
Volume (mL)	10.0
Color	Golden Yellow
Form	Liquid
Aroma	Typical scent of cinnamon
Percentage (%)	0.282

The characteristics of cinnamon essential oil obtained in this study are presented in [Table 4](#).

2. Velvet Bean Extraction

The extracted ethyl alcohol was grayish-white with a weight of 143.83 g. As it can dissolve nearly all secondary metabolite

compounds and particular phenolic compounds with antioxidative and bacteriocidal activities, 70% ethanol was the extraction solvent. Furthermore, it can reduce impurities in the extraction solvent, resulting in a better harvest [17], [20].

3. Optimum Anti-Bacterial Activity of CVE

Table 5. CVE inhibition zone

Type of Bacteria	Sample Name	Inhibition Zone (mm)	Category
<i>Escherichia coli</i> ATCC 25922	CVE 0	7.12 ^a ± 0.03	Moderate
	CVE 10	8.00 ^b ± 0.06	Moderate
	CVE 20	9.03 ^c ± 0.08	Moderate
	CVE 30	10.47 ^f ± 0.07	Moderate
	CVE 40	10.12 ^e ± 0.07	Moderate
	CVE 50	9.81 ^d ± 0.08	Moderate
	C+	30.47 ± 0.06	Very Strong
	C-	0.00 ± 0.00	No activity
<i>Staphylococcus aureus</i> ATCC 25923	CVE 0	6.80 ^a ± 0,06	Moderate
	CVE 10	7.68 ^b ± 0,05	Moderate
	CVE 20	8.71 ^c ± 0,04	Moderate
	CVE 30	10.15 ^f ± 0,06	Moderate
	CVE 40	9.80 ^e ± 0,04	Moderate
	CVE 50	9.90 ^d ± 0,04	Moderate
	C+	30.47 ± 0,05	Very Strong
	C-	0,00 ± 0,00	No activity

Notes:

- The data are the average values of three repetitions ± standard deviation
- Different superscripts show significant differences (p<0.05)
- CVE 0 = E 100% + V 0%
- CVE 10 = E 90% + V 10%
- CVE 20 = E 80% + V 20%
- CVE 30 = E 70% + V 30%
- CVE 40 = E 60% + V 40%
- CVE 50 = E 50% + V 50%
- C+ = Positive Control
- C- = Negative Control

Antibacterial test results from CVE ([Table 5](#)) produced varying values until the addition of velvet bean ethanol extract produced a maximum inhibition zone at a concentration. This difference in the inhibition value will also depend on the addition of the compound concentration because not all active compounds behave similarly [21]. The antibacterial activity is influenced by various factors, including the concentration of the extract, the content of antibacterial compounds present, and the type of bacteria

being inhibited. The potency difference of antibacterial inhibition is caused by different concentrations of velvet bean ethanol extract used, affecting the content of active substances, which has an antibacterial effect [21]. This means that the higher concentration of velvet bean ethanol extract would be able to dissolve more of the antibacterial compounds, so the antibacterial activity or inhibition power would be greater. L-Dopa are active compounds that act merely as a monolith to protect microorganisms. Flavonoids can bind to extracellular proteins and form complexes, which destroys the integrity of bacterial cell membranes, thus damaging the permeability of bacterial cell walls.

The largest inhibition zone was obtained with a chitosan-based edible film containing 30% velvet bean ethanol extract (CVE 30) 10.47 mm and 10.15 mm (both moderately) in *Escherichia coli* ATCC 25922 (moderately) and *Staphylococcus aureus* ATCC 25923, respectively. So, the sample CVE 30 is the more optimum formula.

4. Optimum Anti-Bacterial Activity of CVCE

Cinnamon essential oil (C) has eugenol compounds and serves as an antimicrobial, and based on the data in Table 6, the CVCE inhibition zone significantly increased from moderate to strong levels after adding cinnamon essential oil (C). As a phenolic compound, eugenol inhibits the biosynthesis of bacterial cell walls, thereby preventing the co-transport of compounds and ions, including nutrients, into the bacterial cells, resulting in bacterial cell death [22].

Table 6. CVCE inhibition zone

Type of Bacteria	Sample Name	Inhibition Zone (mm)	Category
<i>Escherichia coli</i> ATCC 25922	CVCE 0	10.47 ^a ± 0.07	Moderate
	CVCE 1	15.50 ^b ± 0.05	Strong
	CVCE 2	17.31 ^d ± 0.08	Strong
	CVCE 3	19.36 ^f ± 0.05	Strong
	CVCE 4	18.05 ^e ± 0.06	Strong
	CVCE 5	16.70 ^c ± 0.03	Strong
	C+	25.89 ± 0.05	Very Strong
	C-	0.00 ± 0.00	No activity
<i>Staphylococcus aureus</i> ATCC 25923	CVCE 0	10.15 ^a ± 0.06	Moderate
	CVCE 1	15.08 ^b ± 0.06	Strong
	CVCE 2	16.89 ^d ± 0.05	Strong
	CVCE 3	18.94 ^f ± 0.05	Strong
	CVCE 4	17.63 ^e ± 0.04	Strong
	CVCE 5	15.48 ^c ± 0.03	Strong
	C+	29.01 ± 0.04	Very Strong
	C-	0.00 ± 0.00	No activity

Note:

- The data are the average values of three repetitions ± standard deviation
- Different superscripts show significant differences (p<0.05)
- CVCE 0 = CVE 30 + 0% C
- CVCE 1 = CVE 30 + 1% C
- CVCE 2 = CVE 30 + 2% C
- CVCE 3 = CVE 30 + 3% C
- CVCE 4 = CVE 30 + 4% C
- CVCE 5 = CVE 30 + 5% C
- C+ = Positive Control
- C- = Negative Control

Cinnamaldehyde derivatives also interrupt bacterial growth by destroying the cytoplasmic membrane. The inhibition activity observed was successful but varied as the highest inhibition zone was recorded at a specific concentration. The difference in the inhibition zone is caused by differences in the concentration of the added cinnamon essential oil [21]. Incorporating 3% cinnamon oil generated the greatest inhibition zone on CVCE. Thus, it can be concluded that, based on ED (Expected Dose), the optimal edible film concentration of cinnamon essential oil was 3%, illustrated by strong antibacterial activities on the *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 had an inhibition zone of 19.36 mm and 18.94 mm, respectively (Figure 2 and Table 2).

5. Characterization of the most optimum CVCE

The physical properties of the most optimum CVCE were determined through

tests of thickness, tensile strength, elongation, solubility, WVTR, and WVP, according to [Table 7](#) below.

Table 7. The physical properties of the most optimum CVCE

Repetition	Thickness (mm)	Tensile strength (Mpa)	Elongation (%)	Solubility (%)	WVTR (g/m ² .day)	WVP (g/m.day)
1	0.179	0.318	0.057	10.096	3.747	8.587
2	0.179	0.317	0.057	10.094	3.747	8.584
3	0.178	0.320	0.057	10.097	3.747	8.586
Average	0.179	0.318	0.057	10.096	3.747	8.586
Std. Deviation	0.001	0.001	0.000	0.001	0.000	0.001

6. Thickness

The thickness influences the application; higher thickness values indicate stiffer and harder films, resulting in a more protected packaged product [23]. Based on [Table 7](#) CVCE 3 thickness complies with the Japanese International Standard [24] requirements because the maximum thickness for a film is not to exceed 0.25 mm. The thickness of cassava starch-based edible films incorporated with cinnamon essential oil was in the range of 0.114-0.176 nm [25]—a thin film makes the product prone to damage. On the contrary, a too-thick film could influence these factors (appearance, texture, and the taste of the packaged product) [26]. However, a thick film can extend the product's shelf life by lowering the rate of water vapor, gas, and other volatile compounds.

7. Tensile Strength

Tensile strength acts upon the determination of the film if it can carry the load itself before it breaks/tears. Tensile strength

Per JIS 1975, the higher value of the film tensile strength is 0.3 Mpa, which is good [24]. We denote an engineering term for a film defined by tensile strength value, which tells us how tenacious it is [9]. CVCE 3 has tensile strength that meets 1975 JIS, namely 0,303 Mpa. Its thickness influences this. Different natural materials, such as phenolic compounds, can assist in building up a more dense film structure with the part of a reduced –OH group because of the activity of chitosan. CH bond Tensile strength ↑ Chitosan polymer with –OH group has weak hydrogen bonds that interact and form a new, stronger hydrogen bond. On the other hand, the incorporation of very high concentrations of extracts and essential oils could lead to excessive dissolution of phenolic compounds, compromising the intermolecular bonds of the film, which may decrease its tensile strength [27].

8. Elongation (%)

The stretch of a movie shows what it looks like when it tears apart after being taken

from its original size. The higher the elongation value of an edible film, the better its quality because it is more elastic and not easy to tear. According to Krochta & Johnston (1997), the elongation value of the edible film greater than 50% is good, and less than 10% is poor [28]. According to JIS, in 1975, however, the threshold was 70%. According to Table 7, CVCE 3 has a percent elongation lower than the recommended percentages by Krochta & Johnston (1997) and the 1975 JIS. This is due to a covalent linkage that reduces the ratio of the specific elongation of the film caused by the supplementation of essential oils at higher concentrations. Moreover, such chitosan-based films are also prone to damage because incorporating such active ingredients results in an upsurge in the surface matrix of the resulting film, which strengthens the film structure 29.

9. Solubility

The solubility of a film represents its ability to be dissolved in water, digested in the body, and degraded in the environment [30]. A high solubility suggests that the film is readily ingestible with the product it covers. The CVCE 3 solubility obtained from Table 7 is 10.096%, i.e., the sample is soluble. Films from edible cellulose derivatives (e.g., extracts) are usually transparent, flexible, have solubility in water, and tend to provide resistance to air. When treated with essential oil additives, the vulnerability of polymer and hydrogen chain arrangements makes starch more soluble in water. Essential oils are composed of bioactive compounds that contain the -OH group. The -OH group is

increased with increasing concentration of essential oils so that the purity higher increases solubility [31].

10. Water Vapor Transmission Rate (WVTR)

Table 7 CVCE 3 has an average water vapor transmission rate (WVTR) value of 3.747 g/m². Day), thereby satisfying Austria's 1975 JIS (Japanese Industrial Standards), an upper bound of 10 g/m². Day. WVTR stands for water vapor transmission rate, which is the film's ability to resist water vapor transmission over time. Hydrocolloid materials such as chitosan and velvet bean extract can have the disadvantage of tending to absorb water easily. Still, adding glycerol can increase the permeability of the film while increasing the mechanical strength. The high concentration of essential oils also influences the film's thickness, reducing the water vapor transmission rate. Incorporating essential oils (hydrophobic lipids) into hydrophilic film polymers (chitosan-velvet bean ethanol extract) can enhance water vapor transfer. This condition affects the transmission rate of water vapor; the WVTR value is according to the addition of essential oil concentration [32].

11. Water Vapor Permeability (WVP)

The WVP values rely on the amount of respective concentrations on both sides, the film's thickness, and the film's hydrophobic and hydrophilic components. In line with Table 7, the higher concentrations of incorporated essential oils lead to a lower water vapor permeability value. It can be seen that the WVP value derived from CVCE

3 is 8.586 g/m.day and does not meet the WVP physical property test standard because it exceeds the standard of 7 g/m.day. The excessive polarity indicated by the high WVP is explained by the amount of polar groups (-OH) in the film. NOH derives

from incorporating and including active contents like velvet bean ethanol extract and cinnamon essential oil [33], [34]. A film's water vapor permeability rate increases with higher concentrations of essential oils.

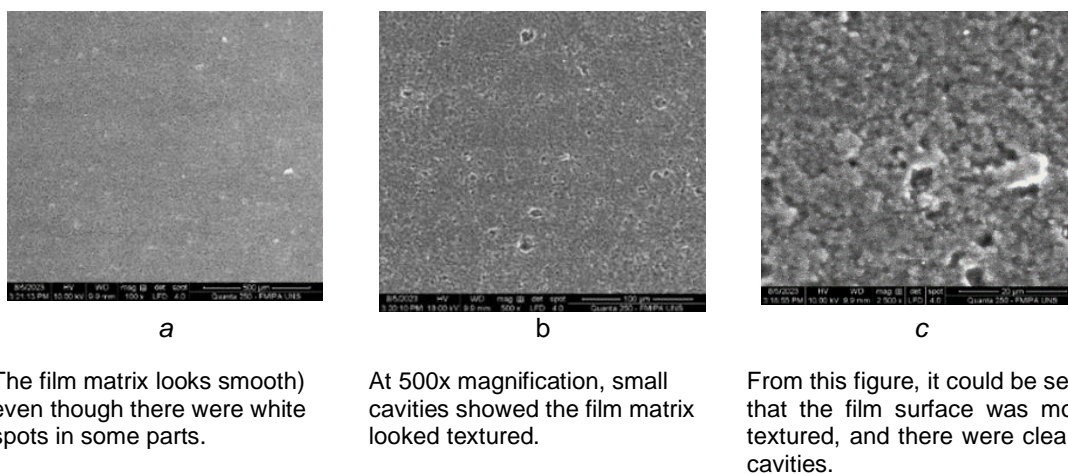


Figure 2. CVCE 3 morphologies (a) CVCE 3 (100x, 500µm); (b) CVCE 3 (500x, 100µm), (c) CVCE 3 (2000x, 20µm)

The surface morphology closely resembles that of cinnamon essential oil-added chitosan-velvet bean ethanol extract-based edible film at magnifications of Images which appear at several (500x, 1,000x, and 10,000x) magnifications of a SEM microscope according to the optical properties as obtained from the most optimum condition of research is as follows. For the concordance analysis, the morphologically CVCE 3 in Figure 2 . features a porous design and hollow, slightly textured surface. This results in a non-homogeneous structure due to the addition of velvet bean ethanol extract in the film phase. As quoted by Yulistiani et al. and some extracts, it can reduce hydrogen bonds between the film [35] and widen the distance between the

molecules present in it, causing the matrix and film surface to turn out to be considerably less tight or hollow [35].

The results of this study showed that the best edible film was obtained from the addition of 30% velvet bean ethanol extract and 3% cinnamon essential oil, based on the antibacterial activity against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 with an inhibition power of 19.36 mm (strong) and 18.94 mm (strong) respectively. The total solids in the film matrix increased with 30% velvet bean ethanol extract and 3% cinnamon essential oil, increasing the film's thickness, tensile strength, and solubility. At the same time, the elongation, WVTR, and WVP decreased.

The thickness of chitosan-based edible film containing 30% velvet bean ethanol extract and 3% cinnamon essential oil was 0.179 mm, tensile strength was 0.318 Mpa, elongation was 0.057%, solubility was 10.096%, and WVTR was 3.747 g/m². d, and WVP of 8.586 g/m². d. From the pores and texture, the morphology of the film's surface was still slightly porous and textured. According to these results, the edible film of chitosan–ethanol of velvet bean–cinnamon essential oil had strong antibacterial activity and could contribute to producing bioactive packaging. This study could also be used as a reference for further research on the utilization of edible film based on chitosan-ethanol extract of velvet bean with cinnamon essential oil as bioactive packaging for various food products and the ease of improving the physical quality and morphology of the film matrix.

CONCLUSION

The study findings revealed that the incorporation of 30% velvet bean ethanol extract and 3% cinnamon essential oil could create the best edible film based on the antibacterial activity against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 with the inhibition zone of 19.36 mm (strong) and 18.94 mm (strong), respectively. The incorporated 30% velvet bean ethanol extract and 3% cinnamon essential oil led to more total solids in the film matrix due to that the film thickness, tensile strength, and solubility of the films have increased, while the elongation, WVTR, and WVP of the films have decreased simultaneously. Edible film containing 30%

velvet bean ethanol extract with 3% cinnamon essential oil had a thickness of 0.179 mm) tensile strength of 0.318 Mpa, an elongation of 0.057%, a solubility of 10.096%, a WVTR of 3.747 g/m². And a WVP of 8.586 g/m. d. The pores and texture showed the film's surface morphology was still slightly porous and textured.

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