JTEP JURNAL KETEKNIKAN PERTANIAN,
Vol. 11 No. 2, p 153-164, 2023
P-ISSN 2407-0475 E-ISSN 2338-8439

Available online:
http://journal.ipb.ac.id/index.php/jtep
DOI: 10.19028/jtep.011.2.153-164

Physicochemical Properties and Microbial Inhibition on Biofilms Cassava Starch with Green Cayenne Pepper Leaf Extract (*Capsicum Frutescens L*)

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Article Info

Submitted: 16 March 2023
Accepted: 25 August 2023

Keywords:
biofilm; cassava starch; green cayenne pepper leaf; microbial inhibition; packaging

Abstract

Biofilm is an alternative packaging for food products in the form of thin layers made from natural materials that are safe to use and for consumption. One of the materials used in the manufacture of biofilms is starch. However, starch-based biofilms have a weakness, namely their low water resistance. The addition of green cayenne pepper (GCP) leaf extract is thought to improve the physicochemical properties as well as the potential to become antimicrobial for biofilms. The purpose of this study was to determine the effect of the addition of GCP leaf extract on the physicochemical and antimicrobial properties of cassava starch biofilm. The GCP leaf extract formulations used in this study were F0 (control), F1 (1 %), F2 (1.5 %, F3 (2 %), F4 (2.5 %) and F5 (3 %) with 3 repetitions each. The results of this study indicate that the addition of green cayenne leaf extract has a significant effect on physical and chemical properties of GCP biofilm. The microbial inhibition zone on F5 higher than F0 and F1.

Doi: https://doi.org/10.19028/jtep.011.2.153-164

1. Introduction

Packaging is an important element in food products that functions to protect food from damage, prevent contamination and extend the shelf life of packaged products. Packaging that is commonly used is synthetic plastic with ingredients that are difficult to decompose, so that the waste becomes a source of environmental pollution (Bhowmik et al., 2022). Biofilm is present as packaging which is expected to be a solution to environmental problems and it made from natural ingredients so they are safe to use and consume (Mulyono et al., 2015). The constituent components of biofilms include hydrocolloids, lipids, and composites. Protein and polysaccharides, one of which being starch, are the primary components of hydrocolloids. Because of its plastic-like qualities, transparent, odorless, and tasteless properties, and strong barrier ability and moisture resistance, starch has the potential to be employed as a material for producing biofilms. Cassava starch is a polysaccharide that is widely used as a biofilm material due to its good properties (Chollakup et al., 2020; Matheus et al., 2023; Mulyono et al., 2015).

Cassava starch has 90% starch content and is composed of 17% amylose and 83% amylopectin. Amylose affects the cohesiveness of the stabilizer component produced by amylopectin. The use of cassava starch can make it easier for the constituent substances to dissolve completely with the solvent used. In addition, the advantages of biofilms made from cassava starch will produce strong and flexible film because they contain high amylose (Maran et al., 2013; Piñeros-Hernandez et al., 2017). Its advantage over plastic packaging is that it can add functional substances to materials so that it can maintain or improve the quality of the packaged product. Opaque films such as starch-based films are able to protect packaged materials from light damage (Pérez-Vergara et al., 2020).

Biofilm is an environmentally friendly packaging that can extend the shelf life of food products. However, the hydrophilic nature of starch can affect the stability and mechanical properties of the film, so starch-based products have weaknesses, including low water resistance and barrier properties against water vapor (Feng et al., 2016; Luchese et al., 2018). This can result in the growth of bacteria that can damage and affect the shelf life of packaged products. Therefore, it is necessary to add antimicrobial agents. One of them is the addition of cayenne pepper leaf extract which contains antimicrobial compounds (Marini et al., 2015; Omolo et al., 2014).

Cayenne pepper leaf extract is an active ingredient added to biofilm production as a parameter for measuring antimicrobial active compounds. Capsaicin, dihydrocapsaicin, and flavanoids are antibacterial chemicals found in cayenne pepper (Capsicum frutescens L). Both have antimicrobial mechanisms by interfering with the synthesis of cell membranes and bacterial cell walls. Likewise on the leaves, cayenne pepper leaves have antimicrobial properties (Marini et al., 2015; Valková et al., 2021).

According to (Feng et al., 2016), the addition of extracts can affect the thickness. Furthermore, thickness is a physical property that is influenced by the transmission rate of water vapor, gases, and volatile chemicals, as well as other physical qualities such as tensile strength and elongation. Based on the explanation above, a study was conducted on the effect of adding green chili leaf extract on the physicochemical properties and microbial inhibition of cassava starch-based biofilms. This study used test parameters, namely thickness, tensile strength, elongation, solubility, water vapor permeability (WVP), moisture content, and microbial inhibition.

2. Research Methods

2.1. Materials and equipments

The equipments used in this study included a hot plate stirrer (Thermo Scientific Cimarec, USA), cabinet dryer, oven (Memmert, Germany), shaking water bath (SWB 30), universal testing machine (Zwick Roell), and vortex (Gemmy WM-300, Taiwan). The materials used in this study were cassava starch (pak TANI gunung, Indonesia), green cayenne pepper (GCP) leaves obtained from cayenne pepper plantations in the Kretek area, Bantul, Yogyakarta Special Region, glycerol (Gamma Scientific Biolab, Indonesia), 1% acetic acid (Gamma Scientific Biolab, Indonesia), test

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DOI: 10.19028/jtep.011.2.153-164

microbes (*Esscherichia coli* ATCC 25922 and *Staphylococcus Aereus* ATCC 23235), and Nutrient Agar media (Himedia, USA).

2.2. Extraction of GCP leaves

Making GCP leaf extract use the (Wijaya et al., 2018) method with modifications. The GCP leaves were dried at 50°C for 12 hours, then the GCP leaf powder was sifted using an 80-mesh sieve. As much as 10% of GCP leaf powder is dissolved in 100 ml of 70% ethanol. Then, it was macerated at room temperature for 24 hours. Next, the filtering process is carried out using filter paper. The solvent evaporation process was carried out using a water bath for 1 hour at 50 °C to obtain GCP extract.

2.3. Biofilm Production

The production of biofilm based on the research by (Muin et al., 2017) and (Rahmadhia et al., 2019) with modifications. As much as 7% of cassava starch was dissolved in 100 ml of distilled water and stirred until completely dissolved. The solution was heated in a hot plate stirrer at 70°C for 10 minutes. Then, 1.5% of glycerol and 1% of acetic acid were added. Reheating was carried out for 20 minutes until gelatinization occurred. After that, the GCP leaf extract (1; 1.5; 2; 2.5; 3% v/v) was added to the film solution. Then the biofilm solution was poured onto a 15 x 20 cm glass plate and dried at 50°C for 15 hours.

2.4. Thickness Analysis of GCP Biofilm

The biofilm thickness analysis carried out in this study referred to the method of (Rahmadhia et al., 2023). Biofilm thickness was measured using a screw micrometer with an accuracy of 0.01 mm. Measurements were made at 5 different points on the biofilm. Then to determine the results of the thickness analysis, the average value is calculated.

2.5. Tensile Strength and Elongation Analysis of GCP Biofilm

The tensile strength and elongation of the samples were measured using a universal testing equipment in accordance with the ASTM D 882 standard procedure (ASTM D 882-02, 2002). The samples were trimmed to a dimension of 50 mm in length and 5 mm in breadth. The specimen was positioned using the upper and lower holding mechanisms. Subsequently, the sample was secured by rotating the handwheel to the point where its release became unfeasible. The test speed for the universal testing machine was configured at 10 mm/min prior to initiating the machine. The universal testing machine is designed to exert tension on the tested material until it reaches its breaking point. The resulting data is then presented on the screen, indicating the tensile strength (MPa) and the percentage of elongation.

2.6. Solubility Analysis of GCP Biofilm

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This biofilm solubility analysis refers to the method of (Rahmadhia et al., 2022). Biofilm samples were cut to a size of 2 x 2 cm. Then, the samples were dried using an oven at 105°C until the weight was constant. After that it is dried in a desiccator. The biofilm samples were then immersed for 24 hours in 50 ml of distilled water. After 24 hours, it is then dried again in the oven at 105°C until the weight is constant. After drying, the samples were dried in a desiccator and weighed. Solubility analysis calculation formula, as follows:

$$\%\ water\ solubility = \frac{m_1 - m_2}{m_1} \times 100\%$$

Note: m_1 = initial weight (g), m_2 = final weight (g)

2.7. Water Vapor Permeability of GCP Biofilm

The water vapor permeability (WVP) of biofilm was researched using the (Rahmadhia et al., 2023) method with some modifications. The WVP test used a cylindrical cup with a diameter of 4 cm and was filled with 2 g of silica gel, then the surface of the cup was covered using a biofilm sample that had been cut to a size of 3 x 3 cm. Then the sample is weighed as the initial weight. Then the cup is put into a desiccator containing 40% NaCl solution (RH = 75%) at 25°C and tightly closed. The cup weight was observed every 12 hours for 5 days. The weight of the cup from each weighing was made into a linear regression equation, so that the increase in the slope of the cup weight (g/day) was divided by the surface area of the film being tested (m^2). The water vapor permeability analysis calculation formula is as follows:

$$WVP = \frac{WVTR}{\Delta P}$$

Note: WVTR = water vapor transmission rate (g/m^2), ΔP= pressure difference (KPa)

2.8. Moisture Content Analysis of GCP Biofilm

Analysis of the water content of GCP biofilm used the thermogravimetric method (AOAC, 2005). Analysis was performed with three repetitions.

2.9. Microbial Inhibition Analysis of GCP Biofilm

This analysis was conducted to determine the ability of GCP leaf extract to inhibit bacterial growth in biofilm samples. Analysis of the bacterial inhibition of biofilm samples refers to the method of (Chollakup et al., 2020) using the diffusion method. The diffusion method was used to determine the sensitivity of the test microbe to antimicrobial agents. This method is carried out by attaching paper discs containing extracts to the agar media which has been inoculated with bacteria.

3. Results and Discussion

3.1. Thickness of GCP Biofilm

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The thickness of GCP biofilm showed that the average value of biofilm thickness ranged from 0.17 to 0.26 mm (Table 1). Based on the average value of biofilm thickness, this study complied with the (Japanese Industrial Standars, 1975), which is a maximum of 0.25 mm. The value of biofilm thickness tends to increase with increasing concentration of green chili leaf extract, thereby increasing the total solids and polymers that make up the biofilm matrix. The higher the concentration of solids, the thicker the resulting biofilm (Chen et al., 2021). The higher the addition of green chili leaf extract, the viscosity will also increase so that the thickness of the biofilm will also increase. The viscosity of the biofilm after heating is a control that affects the thickness of the biofilm (Fatnasari et al., 2018; Kusumawati & Putri, 2013).

Table 1. Physical properties of Green Cayenne Pepper biofilm.

Formu-lation	Thickness (mm)	Tensile Strenght (MPa)	Elongation (%)	Solubility (%)	WVP (g.m/m^2.s.kPa × 10^{-9})
F0	0.177 ± 0.001^a	2.25 ± 0.12^a	81.90 ± 2.51^f	23.10 ± 0.71^a	9.23 ± 0.58^c
F1	0.187 ± 0.003^b	3.60 ± 0.26^b	73.20 ± 1.77^e	24.20 ± 0.18^b	8.75 ± 0.13^c
F2	0.203 ± 0.007^c	3.98 ± 0.12^c	65.30 ± 2.24^d	24.80 ± 0.30^b	6.20 ± 0.88^b
F3	0.228 ± 0.003^d	4.69 ± 0.41^d	58.80 ± 0.99^c	25.80 ± 0.25	5.54 ± 1.11^a
F4	0.247 ± 0.003^e	5.22 ± 0.03^e	53.70 ± 2.43^b	26.70 ± 0.35^d	5.26 ± 0.34^a
F5	0.265 ± 0.003^f	5.87 ± 0.01^f	44.90 ± 1.24^a	27.60 ± 0.19^e	5.20 ± 3.53 ^{a}
JIS	max 0.25	min 0.392	< 10% very bad >50% very good	-	-

JIS: Japanese Industrial Standars, WVP: Water Vapour Permeability
The different superscripted letters show a significant difference ($p < 0.05$) in the same column. F0 = control, F1 = biofilm with 1% extract, F2 = biofilm with 1.5% extract, F3 = biofilm with 2% extract, F4 = biofilm with 2.5% extract, and F5 = biofilm with 3% extract.

3.2. Tensile Strength and Elongation of GCP Biofilm

The tensile strength of the GCP biofilm showed that the average value ranged from 2.25 to 5.87 MPa (Table 1). Based on (Japanese Industrial Standars, 1975), the average value of the tensile strength of biofilms is at least 0.392 MPa, so, the average value of the tensile strength of biofilms in this study meets the standards. In this research found that the tensile strength value exhibits a positive correlation with the concentration of green chili leaf extract. This relationship can be attributed to the higher total dissolved solids resulting from the addition of green chili leaf extract concentration (Laorenza & Harnkarnsujarit, 2023). Additionally, the study suggests that the tensile strength value is directly proportional to the thickness of the material. The strength required to disrupt a biofilm increases proportionally with its thickness. Consequently, the reduction of intermolecular interactions among the polymer chains leads to an increase in film thickness and enhancement of tensile strength (Chollakup et al., 2020; Huang et al., 2021). According to (Maran et al., 2013), the use of cassava starch in this study will provide a solid structural film matrix,

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DOI: 10.19028/jtep.011.2.153-164

resulting in high tensile strength values. The value of the tensile strength of the biofilm is shown in Table 1.

The elongation of CGP biofilm showed that the average value ranged from 44.9 to 81.9% (Table 1). Based on the average value of biofilm elongation, this study met the standards, namely not less than 10% and could be more than 50% (Japanese Industrial Standars, 1975). Increasing the concentration of GCP leaf can fill the intermolecular spaces in the polymer film structure, which can close the intermolecular spaces and produce a less elastic elongation (Chollakup et al., 2020; Maran et al., 2013). The thickness of the film is directly proportional to the elongation at the break of the biofilm. The resulting elongation at break decreases with increasing thickness (Laorenza & Harnkarnsujarit, 2023). The factor that affects the elongation value of biofilms is the addition of the plasticizer used. Inhomogeneous mixing causes incomplete mixing of the plasticizer into the film matrix, or too long drying time and sometimes inconsistent pressure (Zhai et al., 2018).

3.3. Solubility of GCP Biofilm

Solubility in biofilm is the amount of biofilm that dissolves when consumed, as well as when used as packaging. Biofilms with high solubility have lower water resistance and are hydrophilic (Wu et al., 2019). The GCP biofilm solubility showed that the average value ranged from 23.1 to 27.6% (Table 1). The addition of GCP leaf extract will develop a dense biofilm layer that will reduce absorption. The lower absorption value, the better the biofilm produced because it can protect the product from water. Biofilms with high solubility show lower film resistance to water, as well as the hydrophilic properties of the biofilm (Santoso et al., 2018).

The thickness of the biofilm can affect its solubility. As the thickness of the biofilm increases, the proportion of the film that dissolves in water also increases (Alzate et al., 2017). The increase in the quantity of intermolecular contacts between the phenolic compounds present in the extract and the polymers could potentially result in a reduction in the affinity of polysaccharide chains towards water. Consequently, this could lead to an enhancement in the water-related characteristics of the films (Bertolo et al., 2022; Kaya et al., 2018). The results of this study are in line with (Hernández et al., 2023)'s research which states that the addition of active compounds will increase the solubility value of cassava starch-based films.

3.4. Water Vapor Permeability of GCP Biofilm

The results of observations of the biofilm Water Vapor Permeability (WVP) analysis showed that the average value ranged from 5.20 - 9.23 g.m/m^2.s.kPa (Table 1). According to (Alzate et al., 2017), this is due to the increased concentration of the active ingredients. This is because the addition of active ingredients will cause the molecular density to increase, so that the film matrix is maintained. Also, because the polymer solution is getting stronger with increasing concentration of GCP leaf extract and starch used (Ashrafi et al., 2018). The thicker the biofilm, the lower the gas permeability so that it can better protect packaged products. In addition, this study uses starch,

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DOI: 10.19028/jtep.011.2.153-164

which is a hydrophilic material. Hydrocolloid films exhibit reduced resistance to the transmission rate of water vapor owing to their hydrophilic characteristics (Huntrakul & Harnkarnsujarit, 2020). However, they possess the ability to effectively regulate the migration of water vapor and serve as an effective barrier against carbohydrates, lipids, and oxygen. The decrease in water vapor permeability is due to the reduced movement of the polymer chains, which makes the diffusion of water through the biofilm more difficult (Siripatrawan & Vitchayakitti, 2016). Biofilm WVP values are shown in Table 1.

Siripatrawan & Vitchayakitti, 2016, observed a decrease in the WVP values of chitosan-propolis extract films as the extract content increased. This phenomenon can be attributed to the hydrogen/covalent interactions between the chitosan network and polyphenolic compounds, which restrict the availability of hydroxyl groups for the formation of hydrophilic bonds with water. In the (Qin et al., 2019) study, the WVP value decreased as the amount of *L. ruthenicum* anthocyanins extract increased.

3.5. Moisture Content of GCP Biofilm

The moisture content of GCP showed that the mean value ranged from 11.3 to 11.8 % (Table 2). Based on the average value of moisture content, this research biofilm met the SNI 06-3735-1995 edible film quality standard, namely a maximum of 17%. The moisture content of the biofilm decreased as the increasing concentration of GCP leaf extract. This is caused by an increase in the concentration of basic ingredients in the manufacture of biofilms. The polymer bond formed between the starch and the extract used is very influential, the greater the GCP leaf extract used, the stronger the polymer bond will be to reduce the water content in the biofilm (Kumar et al., 2018; Nair et al., 2011).

Table 2. Moisture content and microbial inhibition zone of Green Cayenne Pepper biofilm.

Formulation	Moisture content (%)	Microbial inhibition zone (mm)	
		E. coli	*S. aureus*
F0	11.80 ± 0.05[c]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
F1	11.80 ± 0.05[c]	-	-
F2	11.70 ± 0.13[b]	-	-
F3	11.60 ± 0.16[b]	5.30 ± 0.38[b]	5.47 ± 0.28[b]
F4	11.50 ± 0.12[b]	-	-
F5	11.30 ± 0.02[a]	9.22 ± 0.29[c]	9.47 ± 0.41[c]

JIS: Japanese Industrial Standars, WVP: Water Vapour Permeability
The different superscripted letters show a significant difference ($p < 0.05$) in the same column.
F0 = control, F1 = biofilm with 1% extract, F2 = biofilm with 1.5% extract, F3 = biofilm with 2% extract, F4 = biofilm with 2.5% extract, and F5 = biofilm with 3% extract.

The use of cassava starch will also increase the amount of polymer and viscosity in the film matrix. This is supported by the statement of (Wang et al., 2013) that the greater the polymer that makes

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DOI: 10.19028/jtep.011.2.153-164

up the film matrix, the lower the amount of water left in the film network and the increase in viscosity will affect the increase in biofilm thickness so that the water content will decrease. The elevated water content is attributed to the incorporation of a film function acting as a humectant, which possesses the ability to attract and retain water molecules. This, in turn, enhances the cohesion of the matrix network by the strengthening of hydrogen bonds, thus leading to an augmentation in the overall water content (Zhai et al., 2017). The value of biofilm moisture content is shown in Table 2.

3.6. Microbial Inhibition *Biofilm*

The results of the observed analysis of biofilm microbial inhibition showed that the mean values for *E. coli* bacteria ranged from 5.20 - 9.22 mm, while the average values for inhibition against *S.aureus* bacteria were 5.47-9.47 mm. Based on this average value, the biofilms in this study were F1 films with a weak inhibition zone, while F5 films with a moderate inhibition zone. The category of inhibition zone ≤ 5 mm is a weak category, 6-10 mm is a medium category, 11-20 mm is a strong category and ≥ 20 mm is a very strong category (Chollakup et al., 2020). The inhibition of biofilm bacteria against *E. coli* and *S. aureus* bacteria increased with increasing concentration of GCP leaf extract. This is due to the difference in the content of the active antibacterial substance contained therein and the rate of diffusion of the antibacterial substance into the agar medium and makes a difference in the size of the inhibition zone formed at each concentration (Noshirvani et al., 2017). This is in accordance with the statement of (Rezaeigolestani et al., 2017) which states that the higher the concentration of antibacterial substances further inhibits bacterial growth. The presence of flavonoid compounds is thought to be responsible for the size of the inhibition zone in biofilms because the antibacterial activity of flavonoids can cause damage to the protein structure found in the bacterial cytoplasmic wall. Capsaicin compounds with antibacterial mechanisms that interfere with cell membrane synthesis, as well as dihydrocapsaicin compounds, are selectively active against bacterial cell walls (Balaguer et al., 2013; Marini et al., 2015). The diameter of the inhibition zone on the *S. aureus* bacterial film was larger than that of *E. coli*, this indicated that a greater concentration was needed to inhibit the growth of *E. coli* than to inhibit *S. aureus*. Because the antimicrobial effect is also influenced by the solvent used in the extraction process. The extracted compound is relatively polar. The polarity of these compounds causes these compounds to more easily penetrate the cell walls of gram-positive bacteria so that the diameter of the inhibition zone of S. aureus is larger than that of E. coli. This is because the majority of the cell walls of gram-negative bacteria consist of more lipid content than gram-positive bacterial cells, the majority of which contain peptidoglycan. So, if a compound that is polar is difficult to pass through the gram-negative cell wall (Oussalah et al., 2004). The value of the inhibition of biofilm microbes is shown in Table 2.

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DOI: 10.19028/jtep.011.2.153-164

4. Conclusion

The addition of green chili leaf extract affected the physicochemical properties and microbial biofilm inhibition of cassava starch. The most optimal formula is biofilm with 3% extract of green cayenne pepper in terms of physical, chemical and microbial inhibition.

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Vol. 11 No. 2, p 153-164, 2023
P-ISSN 2407-0475 E-ISSN 2338-8439
Available online:
http://journal.ipb.ac.id/index.php/jtep
DOI: 10.19028/jtep.011.2.153-164

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