Journal of Bio & Geo Material and Energy (BiGME)
Volume 2 No. 2, September 2022
P-ISSN: 2808-1560 E-ISSN: 2808-1404

The Effect of Clove Oil Concentration on Barrier, Mechanical and Anti-Bacterial Properties of Edible Film

A.I. Antoro[1], I. Rahmayani[2]
[1]Technology of Agricultural Product Study Program, Faculty of Agriculture, University of Jambi, Jl Raya Jambi-Ma Bulian, Jambi, Indonesia
[2]Medical Study Program, Medical Faculty, Ganesha University of Education, Udayana Street No.11 Singaraja - Bali

Email: adi.antoro@gmail.com

Article info:

Submitted: Agustus 2022
Accepted: September 2022
Published: September 2022

Abstract :
The purpose of this study was to determine the effect of clove oil concentration on the characteristics of edible film from cassava starch. This research was conducted using a completely randomized design (CRD) with 5 levels of treatment of clove oil concentration (0%, 0.4%, 0.8%, 1.2% and 1.6%) and 4 replications to obtain 20 experimental units. The results showed that the concentration of clove oil affects thickness, transparency, compressive strength and was able to inhibit the growth of *Staphylococcus aureus* bacteria on edible films but has no significant effect on the water vapor transmission rate (WVTR). *Edible film* with the addition of 1.6% concentration produces edible film with the best characteristics, namely 0.175 mm thickness, 7.053 %/mm transparency, 72.70 N/m^2 compressive strength, water vapor transmission rate (WVTR) 21.00 g/m^2.hour, and able to inhibit the growth of *Staphylococcus aureus* bacteria with an inhibitory power of 13.82 mm.

Keywords: Jackfruit Seed, Wheat Flour, Crackers.

1. Introduction

The use of packaging in everyday life is very important to protect products or food ingredients so that they can extend the shelf life of products or food ingredients, as well as being able to prevent existing microbial contamination of health (Gennandios and Waller in Estiningtyas, 2010). Packaging is used to protect food from interactions with the environment that can damage quality and reduce product shelf life (Krochta and de Mulder-Johnson, 1997). A type of packaging that is more environmentally friendly and safe is edible film. Edible film has several advantages, namely that it is cheap, can be broken down by microorganisms and can be eaten (Jaya, 2010).

One alternative packaging that can be developed currently is edible film. Edible film is a thin layer that acts as primary packaging for coating food (coating) which functions as a barrier for mass transfer such as oxygen, light, water vapor and fat, and can also be used as a carrier for food additives (Krochta in Estiningtyas, 2010). The development of edible film for food, apart from providing better product quality and extending shelf life, can also be an environmentally friendly packaging material. Edible film provides an alternative packaging material that does not have an impact on environmental pollution because it uses renewable materials and is cheap (Tharamathan, 2003 in Bourtoom, 2007).

One of the ingredients for making edible film is starch. (Chen et al., 2006). Starch is a type of polysaccharide that is abundantly available in nature, is biodegradable, easy to obtain and cheap. The properties of starch are also suitable for edible coating/film materials because they can form quite strong films.

Edible film has weaknesses, namely its resistance to water and low barrier properties against water vapor (barrier) or in other words it has a high-water vapor transmission rate (Garcia et al. 2011). Tomara's research (2019) The addition of 1.5% clove essential oil to edible film from cassava starch produces a water vapor transmission rate value of 21,319 g/m2.24 hours, the same as Ramadhon's (2018) research using cassava starch 3% produces a water vapor transmission rate value of 53.34 g/m².24 hours. This high rate of water vapor transmission rate is caused by using hydrophilic starch. The high rate of water vapor transmission in edible film can shorten the shelf life, making it less than optimal because water vapor and microbes that enter through the film will damage the food. To improve the physical and functional characteristics of starch films, it is necessary to add biopolymers or other materials, including materials that are hydrophobic or have antimicrobial properties (Chillo et al. 2008). One of the anti-microbial components that can be used is clove oil. Clove oil has biological activity because it contains high levels of eugenol, namely as an antiseptic and analgesic in dental and oral treatment (Khoeriyah et al, 2010), antifungal (Nurdjannah and Hidayat, 1994), and antibacterial (Ayoola et al, 2008).

Clove oil has biological activities, including antibacterial, antifungal, insect eradicating and antioxidant properties, and is traditionally used as a flavor and antibacterial ingredient in food. (Suryanto, 2012). Clove oil is a natural antibacterial ingredient that is abundant, easy to obtain and is considered to have antibacterial capabilities (Kristijanto et al, 2010).

According to Maizura et al. (2007) adding lemongrass essential oil at a low concentration of up to 0.3% could not affect the value of the water vapor transmission rate. According to research by Harianto (2017), adding palm essential oil to edible film from cassava starch with a concentration of 0.7% produces a transmission rate value of 6.47 g/m2.24 hours. This research tries to make edible film from cassava starch with the addition of clove oil to get the best characteristics.

2. Research Methods

Material

The materials used in this research were cassava starch obtained from previous research, glycerol, distilled water and clove oil. The materials used for analysis are magnesium nitrate ($Mg(NO_3)_2$), calcium chloride ($CaCl_2$), sodium chloride (NaCl), NA media, NB media, Escherichia coli and Staphylococcus aureus bacterial cultures. The tools used in the research were digital scales, glass funnels, glass beakers, measuring cups, magnetic stirrers, hot plate stirrer timers, glass plates, and electric ovens.

Research Design

This research used a Completely Randomized Design (CRD) with treatment use of clove oil concentration consisting of 5 levels of treatment (0, 0.4%, 0.8%, 1.2%, and 1.6%) and 4 repetitions.

Edible Film Preparation

The formula for edible films is presented in Table 1. Starch weighing 4.5 grams was dissolved with distilled water in a beaker and stirred until dissolved without heating for 10 minutes, then heated on a hot plate while stirring for 10 minutes and 3 grams of glycerol added while stirring and heated to a temperature reaching 80°C for 10 minutes. Next, add clove oil according to the specified concentration while stirring until the solution is homogeneous. 25 grams of the homogenized edible film solution is then poured into a petri dish, dried in an oven at 50°C for 24 hours and peeled off. Edible films were equilibrated in a desiccator with RH 52% using a saturated $Mg(NO_3)_2$ solution for at least 48 hours before analysis.

Table 1. The formula of edible film production

Composition	Clove oil concentration (%)				
	0	0.4	0.8	1.2	1.6
Cassava starch (g)	4.5	4.5	4.5	4.5	4.5
Glycerol (g)	3.0	3.0	3.0	3.0	3.0
Clove oil (g)	0	0.6	1.2	1.8	2.4
Distilled water (g)	142.5	141.9	141.3	140.7	140.1
Total (g)	150	150	150	150	150

Film Thickness

The thickness of the edible film was measured using a micrometer screw at five different places and chosen randomly. The results were averaged and expressed in mm as the film thickness.

2.4.2 Transparency (Pineroz-Hernandez et al., 2017)

The film was first cut into a square (50x10mm), then placed in the spectrophotometer cell. Then the % Transmittance was measured with a UV-Vis spectrophotometer at a wavelength of 600 nm. The transparency was calculated using equation below.

$$\text{Transparency} = \frac{\text{T600}}{x}$$

Where x is film thickness in mm

Water Vapor Transmission Rate (WVTR)

WVTR was measured using a method described by Pineros-Hernandez et al (2017). A cell containing calcium chloride was placed in a desiccator containing saturated sodium chloride solution and plotted as a function of time.

WVTR = straight line gradient : cell surface area

Compressive Strength

Compressive strength was measured with a Brookfield brand LFRA Texture Analyzer. TA 7 60 mm was the type of probe that was used for the measurement. The LFRA Texture Analyzer tool was set to 2 g trigger, 2 mm distance, and speed 2 mm/s. The probe was installed in place and the "start" button was pressed to start pressing the edible film. A sample of edible film that has been cut to a size of 5 x 2 cm is placed under the probe and the probe will press the film until the magnitude of the probe force used appears on the screen.

Anti-Bacterial Property

Media Preparation

Nutrient Agar (NA) and Nutrient Broth (NB) media are made based on the manufacturing process stated on the media packaging by dissolving 10 grams of NA with 500 ml of distilled water and 1 gram of NB with 150 ml of distilled water in a glass beaker. Then heated at 60ºC until homogeneous. The media that has been made is put into an Erlenmeyer, then the mouth of the Erlenmeyer is immediately covered with sufficient cotton to prevent contamination. Next, the Erlenmeyer is wrapped in coffee paper then placed in an autoclave and sterilized at 121ºC for 15 minutes.

The bacteria that will be used are activated first, by taking the S. aureus or E. Coli bacteria with a loop needle and then streaking them onto media containing NA. The petri dish was wrapped in coffee paper and incubated for 1x24 hours at 37^{o}C. A total of 12 ml of nutrient broth (NB) was put into a test tube. The bacterial culture of S. aureus or E. coli is taken from a colony that has been grown in NA media using a loop needle, then put into a test tube containing NB then incubated for 1x24 hours at 37ºC and the inoculum is ready for use.

Inhibition Zone

A total of 1 ml of the *Escherichia coli* and *Staphylococcus aureus* bacterial culture was taken and poured into a sterile petri dish. Add 14 ml of nutrient agar to the petri dish containing the bacterial culture, then shake it horizontally to form a figure of eight and allow it to solidify. Then a well (hole) with a diameter of 10 mm is made. Edible film (1.6% clove oil concentration) was inserted into the hole, then incubated at 37^{o}C for 24 hours, then the bacterial growth inhibition zone was observed and measured, which was marked by the presence of a clear area indicating the absence of bacterial growth. The test results are expressed in block diameter (mm) which is measured using a caliper.

Data Analysis

The data were analyzed using the Analysis of Variance (ANOVA) and Duncan's New Multiple Range Test (DNMRT) when necessary using SPSS software.

3. Result and Discussion

Film Thickness

The increase in clove oil concentration significantly increased film thickness (Table 2). The increase in the concentration of clove oil caused an increase in the total solids in edible film. In line with Pramadita (2012) that increasing the concentration of cinnamon essential oil affects the thickness of the edible film which causes the total solids to increase. The results of research on the thickness of cassava starch edible film with the addition of clove oil produced an average value of between 0.137 mm - 0.175 mm. This research produces a higher thickness than previous research that produced edible film with different materials. Warsiki et al, (2009) added that the thickness of edible film is influenced by the type of antimicrobial material used, where betel extract produces a greater thickness of edible film than turmeric and onion extracts.

Table 2. The characteristics of edible film with several concentration of clove oil

Clove Oil (%)	Thickness (mm)	Transparency (%/mm)	WVTR (g/m^2.day)	Compressive Strength (N/m^2)
0	0.13^{a}	13.276^{d}	23.16	88.976^{e}
0.4	0.148^{b}	12.307^{cd}	22.74	86.125^{d}
0.8	0.161^{c}	11.365^{c}	22.37	82.375^{c}
1.2	0.168^{d}	10.210^{b}	21.89	79.340^{b}
1.6	0.175^{e}	7.053^{a}	21.00	72.704^{a}

Note: numbers in the same column followed by the same superscript are not significantly different at the 5% level in the DnMRT

Transparency

The transparency reflects the amount of light transmitted by the edible film. Based on the analysis of variance, the concentration of clove oil had a significant effect ($p<0.05$) on the transparency of edible films (Table 2). As the concentration of clove oil increases, the transparency of cassava starch edible film decreases. The decrease in transparency was caused by the addition of antimicrobial ingredients used, namely clove oil which has a high level of turbidity, thus affecting the transparency value of edible film.

From the data above, the increase in clove oil concentration is inversely proportional to the transparency value. This result is lower than a report by Ulyarti et al (2019) on edible film of yam starch. Yam starch with a concentration of white yam and purple yam starch of 1.33% - 3.33% produces film with a transparency 12.511%/mm - 17.767%/mm for white yam and 12.055 %/mm - 16.399 %/mm for purple yam. The present study produces film with higher transparency than previously reported study using clove oil and yam starch (Ulyarti et al., 2021). The high transparency film in present research may be due to the differences between the characteristics of yam starch and cassava starch. As stated by Warsiki et al. (2009) that the transparency of edible film is greatly influenced by the type or character of the ingredients used in making the edible film, edible film with additional ingredients of betel and turmeric produces greater transparency than onions.

WVTR

Water Vapor Transmission Rate (WVTR) is the most important parameter in assessing the quality of edible film. Based on analysis of variance, clove oil concentrations significantly affect WVTR of edible film. Table 2 shows that WVTR in cassava starch edible film decreased as the concentration increased. Clove oil is a hydrophobic material which provides hydrophobic properties to the edible film. The water vapor transmission rate of cassava starch edible film with clove oil concentration ranges from 21.00 g/m^2.day – 23.16 g/m^2.day. These are lower than other studies reported previously. As in research by Tomara (2019), the effect of clove oil concentration on the characteristics of edible film from yam starch (*Dioscorea alata*) produces a water vapor transmission rate between 36.56 g/m^2.24h - 21.31 g/m^2.24h. Furthermore, research conducted by Nuansa (2017) revealed edible film from refined kappa carrageenan with the addition of green betel leaf essential oil (0% - 1%) which produced film with WVTR between 37.92 g/m2.24 hours - 35 .04 g/m2.24hr. Based on Japanesse Industrial Standards (JIS), film as food packaging has a maximum water vapor transmission rate of 10 (g/m^2.24hours) (Saputra et al., 2015). Thickness is directly proportional to the decrease in the water vapor transmission rate of the edible film. The thicker the edible film, the longer path for water vapor to penetrate the edible film, so the WVTR will be lower (Kusumawati, 2013). The WVTR of starch-based edible films is influenced by several factors such as temperature, relative humidity (RH), edible film thickness (Bertuzzi et al., 2007), and the composition of film forming solution.

A good edible film should have a low water vapor transmission rate, because it inhibits the loss of water from the product and inhibits the product from absorbing water from the environment so that the freshness of the product is maintained. Low WVTR also prevents damage by hydrolysis or damage by microorganisms due to the presence of water (Gunawan, 2009).

Compressive Strength

Compressive strength is one of the important mechanical properties of edible film. The concentration of clove oil had a significant effect on the compressive strength of edible film (Table 2). Edible film with a high compressive strength is needed for use as packaging for food products which aims to protect food ingredients during handling, transportation, and marketing (Pitak and Rakshit 2011). As seen in Table 2, the increasing in the concentration of clove oil decreases the compressive strength of the edible film. According to Panjaitan (2019) that the increase in the compressive strength of edible film could be due to the greater total amount of solids used in edible film production. Meanwhile, in this study, clove oil was used. Clove oil added to edible film can result in a decrease in intermolecular interactions between starch molecules, increase the free space between starch chains, and increase polymer mobility. As a result of these influences, the structural integrity of the film matrix decreases, and this results in a decrease in compressive strength.

The value of compressive strength in this study is comparable to previous research conducted by Herawati (2018) within the range 47.27 N/m^2 to 90.80 N/m^2. According to Manuhara (2003), mechanical properties depend on the strength of the material used in making edible film and to form strong molecular bonds. According to Santoso, et al. (2004), Making a composite edible film solution between hydrophobic and hydrophilic materials with added emulsifier can increase stability.

Inhibition Zone

The inhibition zone is the clear zone created if microorganisms do not grow in the specified area where antimicrobial is present. In this study, edible films prepared using 1.6% clove oil were investigated for their capacity for anti-microbial films. The inhibition zone was observed against *Staphylococcus aureus* only but not against *Escherichia coli*. The anti-bacterial capacity against *Staphylococcus aureus* was observed by the presence of a clear zone of 13.82mm diameters. This result is comparable with our previous study using yam starch and 1.5% clove oil (Ulyarti et al., 2021). *Staphylococcus aureus* are gram positive bacteria whose cell walls consist of peptidoglycan and teichoic acid layers and a low lipid content (1-4%) making it easier for antibacterial bioactive ingredients to enter the cells. On the other hand, gram negatives bacteria such as *E coli* have 3 layers (lipoprotein, middle layer, and lipopolysaccharide) and has peptidoglycan with a high lipid content (11-12%). These thick layers in the cell wall prevent the antibacterial active ingredients from entering the cell (Jawetz et al., 2013). The same clove oil was used in the previous study (Ulyarti et al., 2021) which contains 54.9% eugenol and 38.2% triacetin/1,2,3-propanetriol.

Conclusion

The concentration of clove oil has a significant effect on thickness, transparency, and compressive strength, but does not have a significant effect on WVTR. Edible film from cassava starch prepared with 1.6% clove oil performed the best characteristics, namely thickness 0.175 mm, transparency 7.053%/mm, compressive strength 72.70 N/m^2, WVTR 21.00 g/m^2.24hr. and could inhibit the growth of *Staphylococcus aureus* bacteria with diameter inhibition zone of 13.82 mm.

References

ASTM. (1997) : Foaming Agents for Usse in Producing Cellular Concrete Using Preformed Foam, ASTM C 796 – 87.

Ayoola, G.A., F.M. Lawore, T. Adelowotan, I.E.Albinu, E.Adenipekun, H.A.B. Coker, and T.O. Odugbemi, 2008, Chemical Analysis and Antimicrobial Activity of The Essential Oil of *Syzygium aromaticum* (Clove), African Journal of Microbiology Research, 2, pp. 162-166.

Bertuzzi M.A., Vidaurre E.F.C., Armada M. And Gottifredi J.C., 2007. Water Vapor Permebility of *Edible* Starch Based *Films*. J. of Food Engineering. 80: 972-978

Bourtoom, T. 2007. Effect of Some Process Parameters on The Properties of Edible Film Prepared from Starch. Department of Material Product Technology. Songkhala.

Chen, H.L., Cheng, H.C., Liu, Y.J. & Wu, W.T. 2006, Konjac Acts as a Natural Laxative by Increasing Stool Bulk and Improving Colonic Ecology in Healthy Adults, Journal Nutrition, 22:1112-1119.

Chillo, S., S. Flores, M. Mastromatteo, A. Conte, Lý´a Gerschenson, and M.A. del Nobile. 2008. Influence of glycerol and chitosan on tapioca starch-based edible film properties. J. Food Engin. 88: 159–168.

Estiningtyas, H.R. (2010). *Aplikasi Edible film* Maizena *dengan Penambahan Ekstrak Jahe Sebagai Antioksidan Alami Coating Sosis Sapi*. Skripsi. Fakultas Pertanian. Universitas Sebelas Maret. Surakarta.

Garcia, N.L., L. Ribbon, A. Dufresne, M. Aranguren, and S. Goyanes. 2011. Effect of glycerol on the morphology of nanocomposites made from thermoplastic starch and starch nanocrystals. Carbohydrate Polymers 84(1): 203−210.

Gunawan, V. 2009. Formulasi dan Aplikasi Edible Coating Berbasis Pati Sagu dengan Penambahan Vitamin C pada Paprika. Skripsi. Fakultas Pertanian, IPB. Bogor.

Harianto, M. Jusuf Djafar dan Himawan Adinegoro. 2017. *Pengaruh Penambahan Minyak Sawit Terhadap Karakteristik Edible Film Dan Daya Simpan Bumbu Mie Instan.* Teknologi Pengolahan Hasil Perikanan dan Peternakan, LAPTIAB, BPPT.

Herawati, 2018. Pengaruh Penambahan Filtrat Gambir (Uncaria Gambir Roxb)Dan Filtrat Daun Pepaya Sebagai Zat Antimikroba Terhadap Karakteristik Edible Film. Skripsi. THP-FP. Universitas Sriwijaya. Indralaya

Hernandez, E. (1994). Edible coating from lipids and resins. In J.M. Kroctha, E.A. Baldwin, dan Niperos-Carriedo, M.O. (Eds). Edible coating and fi lms to improve food quality. Lancaster/Basel: Technomic Publishing Co., hal 279-300.

Jawetz, Melnick, & Adelberg's. 2013. Medical Microbiology Twenty-Seventh Edition. McGraw- Hill Companies. US

Jaya, D., dan Sulistyawati, E. (2010). Pembuatan *edible film* dari tepung jagung. *Jurnal Eksergi.* 10(2): 5-10.

JIS (Japan Industrial Standard). 2019. General Rules of Plastic Films for Food Packaging. Z 1707. Japanese Standard Association

Khoeriyah et al, 2010, Karakterisasi Senyawa Aktif Anti Bakteri Minyak Atsiri Bunga Cengkeh (*Syzigium arimaticum).* Program Studi Kimia dan Biologi. Fakultas Sains dan Teknologi UIN Syarif Hidayatullah Jakarta.

Kristijanto AI, Soetjipto H, Purtanto FT. 2010. Ekstak kasar limbah cengkeh (*Syzygium aromaticum L*.) fraksi heksan sebagai larvisida alami terhadap jentik nyamuk demam berdarah (Aedes aegypti Linn.) instar II dan IV. h.207-217

Kusumawati, D.H., dan Widya, D.R.P. 2013. Karakteristik Fisik dan Kimia Edible Film Pati Jagung yang Diinkorporasidengan Perasan Temu Hitam. Jurnal Pangan dan Agroindustri, 1(10): 90-100.

Krochta, J.W., and De Mulder-Johton, C. 1997. Edible And Biodegradable Polymer *Film*: Challenges And Opportunities. *J. Food Tech*. 51 (2): 61-74.

Maizura, M., Fazilah, A., Norziah, M.H. dan Karim, A.A. 2007. Antibacterial activity and mechanical properties of partially hydrolyzed sago starch-alginate edible film containing lemongrass oil. Journal of Food Science 72 (6): C324-C330.

Manuhara, G. J. 2003. Ekstraksi karagenan dari rumput laut Eucheuma sp. untuk pembuatan edible film. Skripsi. Fakultas Teknologi Pertanian Universitas Gajah Mada. Yogyakarta.

Nurdjannah, N. dan T. Hidayat, 1994. Pengaruh cara dan waktu penyulingan terhadap mutu minyak bunga cengkeh. Balai Penelitian Tanaman Rempah dan Obat. Vol. 9 (2).

Pitak N, Rakshit SK. 2011. Physical and antimicrobial properties of banana flour/chitosan biodegradable and self sealing films used for preserving Freshcut vegetables. LWT - Food Science and Technology. 44(10): 2310-2315.

Pineroz-Hernandez D, Medina-Jaramillo, C, Lopez-Cordoba A. (2017). Edible cassava starch films for potential use as active food packaging. Food hydrocolloids 63:488-495.

Pramadita, R.C. dan Sutrisno, A. 2012. Karakterisasi *Edible film* Dari Tepung Porang (*Amorphophallus omcophyllus*) dengan penambahan Minyak Atsiri Kayu Manis (*Cinnamon Burmani*) Sebagai Antibakteri. *Skripsi*. Fakultas Teknologi Pertanian. Universitas Brawijaya. Malang.

Ramadhon, R. 2018. Karakteristik Edible Film Dari Pati Singkong Dengan Penambahan Gelatin. *Skripsi*. Fakultas Teknologi Pertanian Universitas Jambi. Jambi Rodriguez, M., J., Ose's, K. Ziani and J.I Mate. 2006. Combined effect of *plasticizer* and surfactants on the physical properties of starch based *edible film*s. *Food Research International*. 39:840-846.

Santoso, B., D. Saputra, dan Pambayun, R. 2004. Kajian Teknologi Edible Coating dari Pati dan Aplikasinya Untuk Pengemas Primer Lempok Durian. Jurnal Teknol dan Industri Pangan XV (3).

Saputra, E., Kismiyati, H. Pramono, A. A. Abdillah. dan M.A Alamsjah. 2015. An edible film chartceristic of chitosan made from shrimp waste as a plasticizer. Journal of natural sciences research 5 (4) : 118-124.

Suryanto, E. 2012. *Fitokimia Antioksidan*. CV. Putra Media Nusantara. Surabaya.

Ulyarti, E Maryana, I Rahmayani, Nazarudin Nazarudin, Susilawati Susilawati, Aris Doyan. 2019. The characteristics of yam (dioscorea alata) starch edible film. JPPIPA: 5(1).

Ulyarti, Lavlinesia, Surhaini, Siregar N., Tomara A., Lisani, Nazarudin. 2021. Development of Yam-Starch-Based Bioplastics with the Addition of Chitosan and Clove Oil. *Makara Journal of Science*: Vol. 25: Iss. 2, Article 5. DOI: 10.7454/mss.v25i2.1155

Warsiki, E., Sunarti, T.C. dan Martua, R.D. 2009. Pengembangan kemasan antimicrobial (AM) untuk memperpanjang umur simpan produk pangan. Prosiding Seminar Hasil-Hasil Penelitian Institut Pertanian Bogor hal 579-588. Bogor.