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Effect of Glycerol on Physical Properties of Biofilms Gembili Starch (Dioscorea Esculenta) - Chitosan

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Abstract. In this present era, the demand of plastics as food packaging materials is increase rapidly. However, unfortunately the most of these products are non-environmentally friendly and non-biodegradable. Biofilm starch-chitosan is one of polymer that has been extensively studied for use as a biodegradable plastic. This study was conducted to determine the physical properties of biofilm that made from starch of *Gembili* (*Dioscorea esculenta*) and chitosan with glycerol plasticizer, with dope method. The results showed that biofilms obtained the biodegradability of 68%, water uptake ranging between 0.15 to 0.25, tensile strength ranging between 26.87 to 35.45 MPa and elongation ranging from 18.15 to 31.23%.

1. Introduction

Generally foodstuffs in are very sensitive and susceptible to quality degradation due to environmental factors, chemistry, biochemistry, and microbiology. One way to prevent it is with proper packaging. Food packaging is a process of packaging food by using appropriate packaging materials that have usefulness and characteristics in order to maintain and protect the food from environmental contamination, and it can also provide an attractive appearance.

Food packaging that is commonly used today are plastic [1][2]. Although it is very affordable in terms of price and flexibilities, non-corrosive, transparent, not easily broken, and can be combined with other packaging materials, plastics can be harmful to health. For use as food packaging (food grade), it is needed a packaging requirement; there is no influence of packaging contamination to packaged product, so that it is safe for human health [3]. The use of plastics in hot foods can lead to the decomposition of the plastic polymers into monomer which then can migrate into foodstuffs packed.

Besides, plastics used today are polymers of petroleum that also have a very stable physicochemical properties and will be decomposed in a span of 200-400 years, even they take up to 1,000 years to be completely decomposed [4] . Burning of plastics is also not a good choice. An imperfectly burnt plastic at a temperature of 800 degrees Celsius will form dioxins that are harmful to health [5]. Thus, the excessive use of plastic will cause environmental pollution.

Bioplastics/biofilm made from natural ingredients is now a viable alternative in the choice of packaging materials and has been widely applied to food products. According [6], Skurtys defines biofilm as a thin layer that can be used as a coating or barrier between food and the environment. Biofilms can protect the food, prevent the contamination of microorganisms; extend the shelf life of products, reduce the risk of the pathogenic bacteria growth on the food surface, reduce the transfer of gas and aroma, prevent the decline in the quality of the food for mass transfer (e.g., moisture, gas, and taste). In addition, the biofilm can hinder the respiration rate, transfer oil, oxygen, and water vapor that are not desirable in food products [7], is able to bind water and inhibits the enzyme system some bacteria, so as to improve the food stability and quality. In addition to protect the foodstuffs packed, biofilm is biodegradable in nature so as to reduce environmental pollution caused by solid waste.

Based on those facts, the development of food packaging made from natural ingredients that are biodegradable, are now increasingly conducted [8]. The materials used to make biofilm are compounds found in plants such as cellulose, starch, and lignin, as well as in animals such as casein, proteins and lipids [9]. Starch is a substance that may or easily degraded into environmentally friendly compounds. According to [10], biodegradable polymers made from natural polysaccharide, mainly starch from tubers, can be produced at low cost and on a large scale and their applications in food packaging are promising because of its flexibility, transparency, nature thermoplastic, and is a material that can be degraded by microorganisms into compounds that are environmentally friendly. Starch tubers also have been proven to extend the shelf life and improve the quality of products. The main mechanisms of film formation on the polysaccharides are the segments disconnection of the polymer and the reestablishment of the polymer chains into the matrix layer or gel that is normally achieved by solvent evaporation thus creating a hydrophilic hydrogen bonds as well as electrolytes and ionic crosslinks [11].

Indonesia as an rich area of plants containing starch, especially tubers producer of starch such as bulbs canna ***(Canna discolor),*** cassava *(Manihot utilissima)*, *uwi (Dioscorea alata)*, *irut (Maranta arundinacea), suweg* or *iles-iles (A. muelleri), gembili (Dioscorea esculenta),* and so on, is so potential to develop biofilm [12]. *Gembili (Dioscorea esculenta)* is a plant that is widely grown in rural areas which generally used as food substitute for rice, snack, even just left alone to grow in the yard. This is because these types of bulbs have a cheap price and many people assume that eating tubers as an inferior foodstuffs. Gembili *(Dioscorea esculenta)* is usually used as a source of carbohydrates after being cooked or baked. The *Dioscorea esculenta's* starch, just like the cassava and other tubers starch, is the material that able to form the polymer matrix [13][14][15], flexible, odorless, colorless, transparent, non-toxic and biodegradable [16][17]. *Dioscorea esculenta* Starch is mainly composed of two homopolymers of D-glucose : amylase, a mostly linear α-D(1, 4')-glucan and branched amylopectin, having the same backbone structure as amylose but with many α-1, 6'-linked branch points (Figure 1).

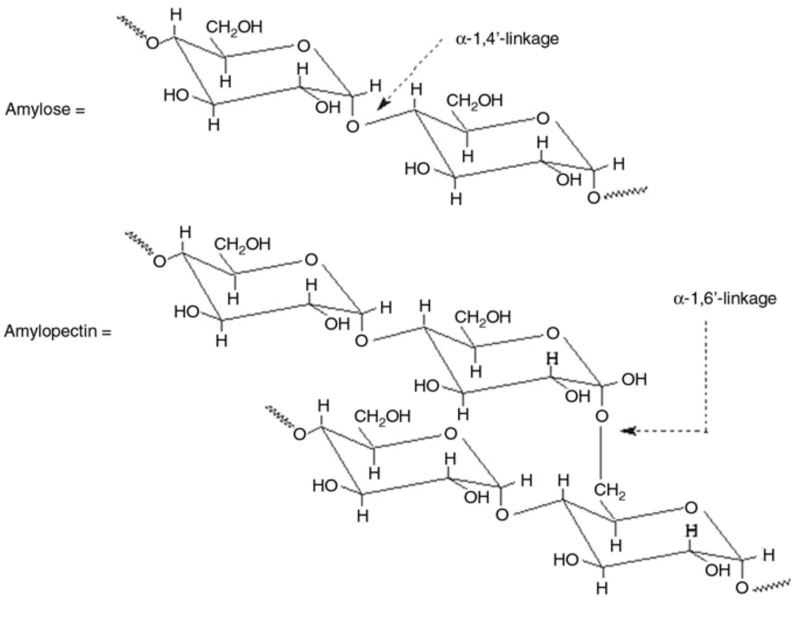

Figure 1. Molecular structure of starch [13]

Some studies that have been conducted related to the manufacture of biofilm with Dioscorea esculenta starch as basic material, among others, 1) The success of [18] who have made a biofilm with a composition of cassava starch – chitosan – poly lactate acid – glycerol with very satisfactory results, because in terms of tensile strength and its elongation percentage have met the criteria of the Indonesian National Standard for plastic; 2) Biodegradable plastics manufacture with the composition of Dioscorea esculenta gembili starch –glycerol and Dioscorea esculenta gembili starch – CMC – glycerol [19], Dioscorea esculenta gembili starch – chitosan – PVA [20]. However, the results of [19], [4], and [21] researchers found that the use of starch as biofilm manufacture's raw material still has some weaknesses including the low tensile strength and elasticity. Plastics made from starch are less resistant to water (less hydrophobic/hydrophilic) and has a low tensile strength and Young's modulus [22]. Biofilms of cassava starch produced without *plasticizer* also showed a lower mechanical strength and brittle with low water content [23]. The low stability of the film will shorten the shelf life due to moisture and microbes that enter through the film would damage the food. A good biofilm must also be resistant to water, so that the hydrophilic nature of the biofilm must be minimized. One way to reduce the hydrophilic nature of the biofilm is to mix the starch with other biopolymers which are hydrophobic, such as cellulose, chitosan, and protein. Chitosan is used as biopolymer mixer to improve the mechanical properties because it can form hydrogen bonds between chains with amylose and amylopectin. The mechanical properties, water barrier properties, and miscibility of biodegradable blend films are affected by the ratio of starch and chitosan [24].

Chitosan as a food additive naturally has attracted attention because of its non-toxic, antibacterial, antioxidant, film-forming, biocompatibility and biodegradability nature [25]. Chitosan has good properties to be molded into plastic and has a micro-bacterial resistant property [26]. Chitosan is also easily degraded and easily combined with other materials [27]. Chitosan film also has a medium water vapor permeability value and can be used to increase the shelf life of fresh produce and food with high water activation. The produced chitosan film is rigid, can be elongated, flexible and not easily torn.

Several studies have shown that the addition of chitosan to the solution of starch will increase the great number of tensile strength, although the elongation value gets smaller with the increasing concentrations of chitosan. This decrease was due to the decline in bond distance between inter-molecular [28], [29] and [30] suggests the addition of chitosan to produce excellent mechanical properties, i.e. between 1% up to 2%.

The high quality biofilms are in the form of a transparent membrane so that the coated product can be seen. The mechanical characteristics of biofilm are tensile strength, puncture strength, percent elongation and elasticity *(elastic modulus/young modulus)* [6]. A good biofilm has a good elasticity and flexibility, low fragility, strong, and does not crack during handling and storage [31] . To produce the mechanical properties (especially elastic properties), can be made by mixing starch with plasticizer [32]. Generally, the presence of plasticizers in a greater proportion will create percentage value of a film elongation increases greater.

Plasticizer is a non-volatile material, has a high boilers which when added to other materials will change the physical properties of the material. The addition of plasticizer can increase the strength of intermolecular, and increase the flexibility of biofilm. Plasticizer most commonly used are polyol (propylene glycol, glycerol, sorbitol, polyethylene glycol), oligosaccharides (sucrose), and polyvinyl alcohol (PVA) [33]. Glycerol is an effective plasticizer because it has the ability to reduce hydrogen bonds to the intra-molecular bonds [11]. Glycerol can provide more elastic properties against plastic when compared to other plasticizers, such as sorbitol. Glycerol includes the commonly found compounds in nature and is relatively inexpensive. In addition, glycerol is environmentally friendly, since they are easily degraded by microorganisms. However, the addition of plasticizers that exceed a certain amount will produce the film with a lower tensile strength.

Based on the facts described above, thus refer to the results that have been obtained by [18], it has carried out research on the manufacture and analysis of physical properties of biofilm from Dioscorea esculenta gembili starch – chitosan plasticizers glycerol without poly lactate acid. Measured physical properties include tensile strength, elongation, water uptake and biodegradability.

2. Research methods

The materials used in this study is gembili starch (*Dioscorea esculenta*) and chitosan from Vannamei shrimp shells manufactured in FMIPA chemical laboratories by [20], while the plasticizers studied were glycerol ($C_3H_8O_3$, MW = 92 g / gmol). The biofilm manufacturing technique is modify the technique of [14][15], starting with dope manufacturing process of making a mixture consisting of 3% Dioscorea esculenta starch and 2% Chitosan. Then to the mixture was added varies glycerol with the concentration of 0.1; 0.2; 0.3; 0.4; 0.5% then all the mixture is stirred for 15 minutes using a magnetic stirrer in a manner heated over a hot plate at a temperature of 70-80 $^{\circ}$C until homogeneous. After the mixture is poured into the mold which has been sterilized beforehand to form the sheet with a still quite high water content and there are air bubbles so that the biofilm should be allowed to stand until the bubbles are gone for 24 hours in the air, and then dried in an oven with a temperature of 60 $^{\circ}$C. After 2 hours, the biofilm is released from the mold and are ready to test its physical properties. For testing the tensile strength and % elongation was performed with a tensile strength test of Pearson Panke Equipment LTD brand in the Laboratory of Mechanical Engineering Materials, Faculty of Engineering Gajahmada University, while another test is carried out in the laboratory of Chemistry, Semarang State University.

2.1 Thickness test (mm)
The film thickness was measured using a micrometer (micrometer digimetic mitutoya). Film placed between jaw micrometer measured randomly at 5-7 different places. The average value of the measurement results is calculated [36].

2.2 Tensile Strength Test and % Elongation
Tensile strength and % elongation are the mechanical properties related to of the chemical structure of the biofilm. Tensile strength indicates the size of biofilm resistance that is the maximum strain acceptable by samples, while % elongation is the maximum length change experienced by the plastic during the tensile strength test that when the sample was torn. Tensile strength test is conducted by using the ASTM standard (American Standard Testing Method) D638. The formula of tensile strength and % elongation are as follows:

Tensile Strength = $\frac{F}{A}$, with F = tensile force (kg), and A = area of the base sample (cm ^{2})

% Elongation = $\frac{L1-L0}{L0}\ x\ 100$ %, with L1 = length after breaking and L0 = initial length.

2.3 Biodegradability test
Biodegradability test is conducted based on [21] research, biofilm cut to size 2 x 6 cm, after being weighed, the sample is inserted into a bacterial culture EM4. After settling for a week, samples were dried in the desiccator and weighed using the Ohaus Adventurer analytical scale ± 0, 0001 grams to obtain a constant weight.
The biofilm weight lost is calculated by formula:

$$\% \text{ W} = \frac{(W1-W2)}{W1} \text{ x } 100\%$$

2.3 Water resistance Test
Water resistance Test of biofilm is conducted as it has been conducted by [37], biofilm pieces measuring 1 x 1 cm is weighed (W_o) put into 10 ml of distilled water in a glass beaker at room temperature. After 1 minute pieces of biofilm were taken and removed the water contained in the plastic surface and then weighed (W). Then the sample is re-immersed into a glass beaker, lifted and weighed every 1 minute, to obtain a constant weight. Water that is absorbed is calculated by formula:

$$\text{Water absorbed } \% = \frac{W-Wo}{Wo} \text{ x } 100\%$$

3. Results and Discussion

Biofilm of Dioscorea esculenta starch – chitosan – glycerol obtained is in the sheet–shaped translucent, transparent, elastic, has a thickness of 0.11 to 0.12 mm as shown in Table 1.

Table 1. Biofilm Dioscorea esculenta starch – glycerol – chitosan thickness

No.	Dioscorea esculenta starch concentration (%)	Chitosan concentration (%)	Glycerol concentration (%)	Average thickness (mm)
1	3	2	0.1	0.11
2	3	2	0.2	0.12
3	3	2	0.3	0.12
4	3	2	0.4	0.12
5	3	2	0.5	0.11

Biofilm formation of starch - chitosan with plasticizer glycerol, starting from chitosan were added to a solution of cellulose that will occur interaction between starch with chitosan. NH_2 group of chitosan through hydrogen bonding and dipole - dipole interaction with -OH groups on starch - chitosan molecule. At the time of the addition of glycerol will also lead to an interaction between the starch - chitosan glycerol through hydrogen bonding and dipole- dipole bonds. This interaction hypothesis is illustrated in Figure 2 and Figure 3.

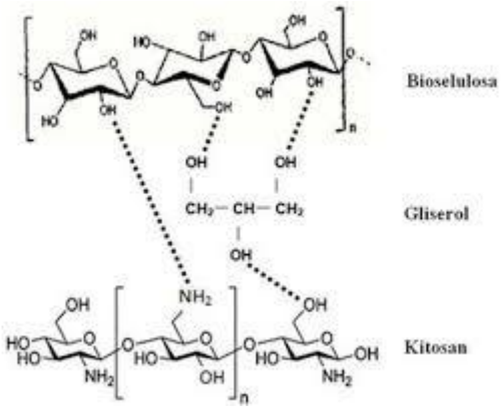

Figure 2. The interaction between the starch-glycerol-chitosan [38]

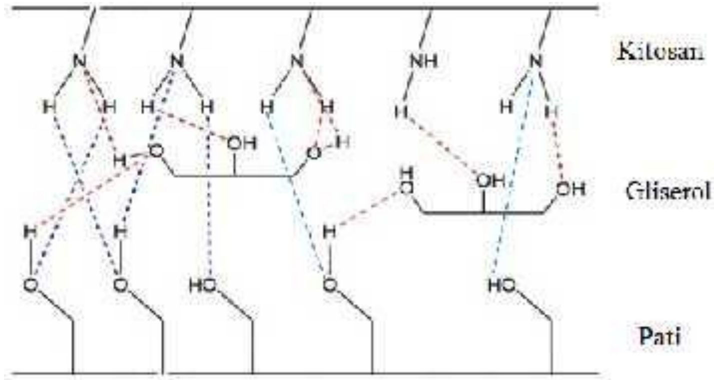

Figure 3 The Hydrogen interaction between the starch-glycerol-chitosan [29]

3.1 Tensile Strength Test Result and Percentage of Elongation (% Elongation)

The objective of tensile strength test is to obtain the appropriate packaging material standards. Tensile strength is influenced by the composition of the biofilm and the degree of homogeneity of the mixture of biofilm. Data results of tensile strength and percent elongation are presented in Table 2.

Table 2. Results of biofilm tensile strength and percentage elongation

No.	Dioscorea esculenta starch concentration (%)	Chitosan concentration (%)	Glycerol concentration (%)	Tensile Strength (Mpa)	Elongation (%)
1	3	2	0.1	35.45	16.15
2	3	2	0.2	33.24	18.80
3	3	2	0.3	32.59	19.26
4	3	2	0.4	32.21	31.23
5	3	2	0.5	26.87	20,21

According to Table 2 it appears that generally with the increased concentration of glycerol, the tensile strength values will decrease. This is consistent with the findings of [39] that the plasticizer in a great amount leads to elastic materials thus lowering the material tensile strength. Glycerol is a small hydrophilic molecule that can easily fit between the chains of molecules and crosslinking will occur between chitosan so that the plastic is not rigid. A decrease in tensile strength, the possibility also can result from the addition of glycerol plasticizer will lower the hydrogen bonds that will enhance the intermolecular distance in the biofilm that will increase the flexibility of plastic. With increased flexibility, the tensile strength of the biofilm will lower.

In the early stages of decline is not too large, this is likely due to the amount of glycerol added is still small, so the biofilm still has great tensile strength. A decrease in tensile strength in the higher addition of plasticizers also can result from less homogenous mixing process, the distribution of the molecular components of the biofilm was uneven, so that the resulting material has a low tensile strength [40].

Judging from percentage of elongation, it appears that with increasing concentration of glycerol, % elongation was also increased through the addition of glycerol 0.4%. These results are consistent with the results obtained by [32], which indicated that the presence of plasticizers in a greater proportion will greatly increase the value of a film percentage. Likewise, [41] obtained the results that the addition of the high rate sorbitol and glycerol plasticizer will produce a biodegradable plastic from caladium tuber (Colocasia esculenta) starch with a lower tensile strength value, while the higher concentration of plasticizer the higher the percentage of elongation.

The encrease of elongation of starch - chitosan - glycerol in order of increasing concentration of glycerol caused by the interaction force that is strong enough glycerol with the polymer so that the starch - chitosan glycerol molecule diffuses into the polymer chain. In this case glycerol molecule will be among the polymer chains (between starch polymer and chitosan) so that the distance between chitosan and starch will increase. This makes hydrogen bonds between the chitosan - starch is reduced and replaced into hydrogen interaction between chitosan - glycerol and glycerol - starch in accordance with the illustration in Figure 2 and 3, thus biofilm will be more elastic so that elongation tends to increase. However, if the amount of plasticizer exceeds this limit, there will be excessive plasticizer so that a plasticizer is not efficient anymore [38].

The addition of glycerol plasticizer more than 0.4 % showing the results of elongation tends to decrease. This occurs because the addition of glycerol has crossed the line so that the excess plasticizer molecules are in a separate phase out phase of starch and chitosan. The situation is causing a decrease in the intermolecular force between the chain declined. From this analysis, it is known that the addition of glycerol is the most effective way to improve the elongation is not more than 0.4 %. It is also in accordance with the opinion [42], that the structure of the biofilm is a matrix formed by the interaction such as hydrophobic bonds, hydrogen and disulfide. Bio film formed by glycerol concentration levels higher will increase the interaction between the ties that ultimately affect the properties of biofilms by bonding the protein molecules which is getting stronger.

If conducted a mechanical properties comparison between polypropylene plastic with biofilm of Dioscorea esculenta starch – chitosan – glycerol, then as shown in Table 4 shows that the tensile strength of biofilm on all compositions has already fulfill the criteria as packaging plastic that commonly used in the market. Chitosan with a higher molecular weight produces a higher tensile strength compared to low molecular weight [43].

Based on Table 2, the percentage of elongation test results ranges from 16 – 31.23. From the percentage of elongation value, there is only one composition that produces the percentage of elongation according to the polypropylene standard namely composition of starch : chitosan : glycerol = 3 : 2 : 0.4 with a percentage of elongation value of 31.23. These results are only slightly correlated with which stated by [44] that most of the bio plastics chitosan mechanical properties are comparable with medium level of commercial polymer. Chitosan biofilm percentage of elongation ranges from 4 – 118% [45].

Water resistance test results (Hydrophobicity)

Hydrophobicity is a water resistance on biofilm that aims to identify the biofilm resistance to water so it can be used to determine the products or materials in accordance with the packaged. This test was determined by the percentage of polymer weight gain after experiencing film swelling by the presence of water [4]. Water resistance is necessary to know the nature of biofilm produced is already in line with the nature of a synthetic plastic or not, because to maintain the quality of the packaged foodstuffs required packaging plastic is resistant to water. Water resistance of plastics made from polypropylene (PP) is 0.01 or 1% [46].

As an effort to make the biofilms produced has a high water resistance, is by adding chitosan as support material. However, based on tests conducted, it is found that the produced biofilms can not lower the hydrophilic properties of the produced film to the fullest. Water resistance test results are presented in Figure 4.

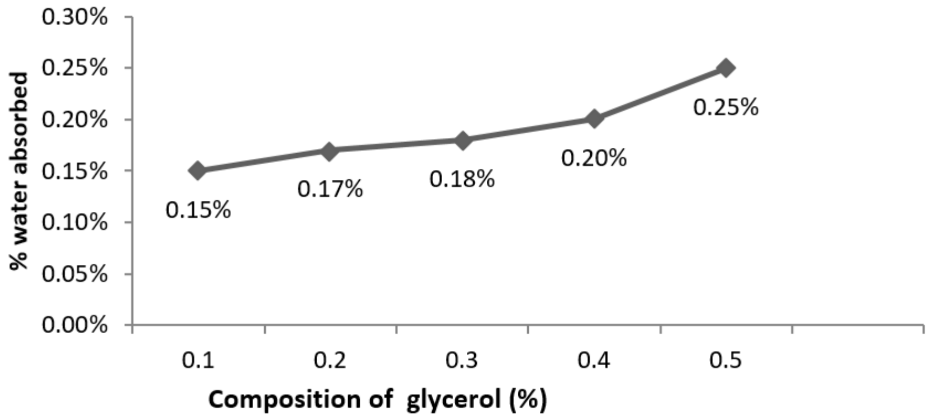

Figure 4 The influence of Dioscorea esculenta starch – chitosan – glycerol comparison against the percentage of water absorbed

From Figure 4, it appears that the percentage of water uptake increased with increasing concentration of glycerol. This could result in their OH groups $^-$ in biofilms that cause biofilm is still hydrophilic [37]. Similarly, the use of hydrophilic plasticizers will also increase its solubility in water. The use of glycerol provide higher solubility compared to sorbitol on starch-based biofilm [24]. The nature of the hydrophilic starch that is able to bind water molecules and form hydrogen bonds between starch and water. Increasing concentrations of glycerol resulted in water suspended in a matrix of starch - chitosan - glycerol increases. Glycerol is hygroscopic and has-OH group is enough to bind with water through hydrogen interaction. This causes bioplastic films have high water absorption as more glycerol was added.

Biodegradability test results

By the standards of the European Union on the biodegradation of plastics, biodegradable plastics should be decomposed into carbon dioxide, water, and humus substance within a maximum of 6 to 9 months. Based on the biodegradability test in this study, obtained the results as presented in Figure 5. In Figure 5 shows the biofilm percentage weight lost value after degraded by bacteria EM4 is directly proportional to the increase in the concentration of glycerol. Similarly, if the terms of the degradation time, the biofilm with constituent components therein which are natural materials are easily degraded is superior to polypropylene and other plastic made from petroleum.

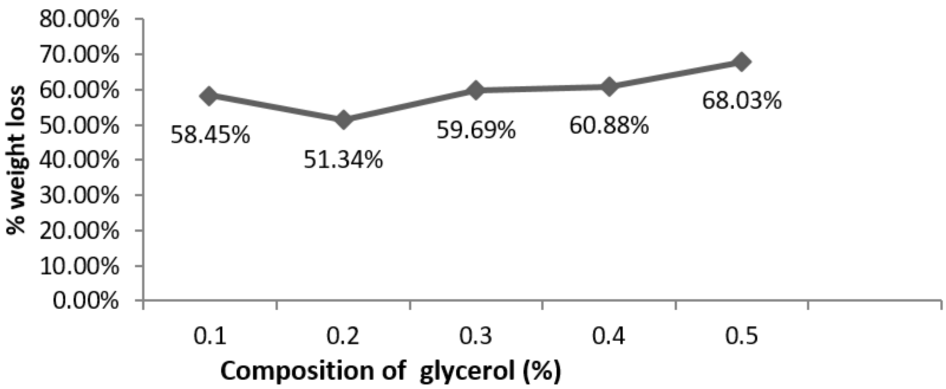

Figure 5. The influence of Dioscorea esculenta starch – chitosan – glycerol comparison against the percentage of weight loss

The results showed that the biofilm made from Dioscorea esculenta starch– chitosan–glycerol is not endangering the health and friendly to the environment. This result is also supported by the results of the FTIR spectra show the emergence of spectra at the carboxyl OH absorption area in the range of 3448.72 cm^{-1}, NH secondary amine absorption area 3942.5 cm^{-1}, CH alkanes in the catchment area 2931.8 cm^{-1} and there is also a carbonyl functional group (C = O) in the catchment area and esters 1635.64 cm^{-1} (CO) in the catchment area 1049.28 cm^{-1}. This group is an organic group and a polar functional group so that the material biofilm produced is an environmentally friendly material because it can be degraded by nature (land). In addition, the results showed that biofilm of Dioscorea esculenta starch – chitosan – glycerol is already much better than biofilm of Dioscorea esculenta starch – CMC – glycerol and biofilm of Dioscorea esculenta starch – glycerol [19] with a ratio as in Table 7.

Table 7. Mechanical Properties Comparison of Plastics PP with biofilm of starch – chitosan - glycerol, starch – glycerol and starch – CMC – glycerol

No.	Mechanical Properties	Polypropylene (PP) *	Biofilm of		
			starch-chitosan-glycerol **	Starch-glycerol ***	Starch-CMC-glycerol ****
1.	Tensile Strength (Mpa)	24.7 – 302	32.21	7.10	12.37
2.	Elongation (%)	21 – 220	31.23	-	2.38
3.	Water absorbed (%)	0.01	0.20	-	95.65

Source : * SNI [46]
** Primary data, Dioscorea esculenta starch – chitosan – glycerol, 2015
*** [19] Data
****[19] Data

However, the results obtained are still lower than the results produced by [18], where the film packaging in the synthesis of composite cassava starch – glycerol 1% – APL 5% – chitosan 1% had relatively better morphological and mechanical characteristics than other formulas. It can be seen from the value of tensile strength, elongation and modulus. Film of cassava starch – chitosan – glycerol – APL of this formula has a tensile strength: 104.42 N/m^2, elongation: 33.80%, and modulus: 309.54 N/m^2.

Conclusion

Based on the results have been obtained biofilm with the best composition of the mixture of Dioscorea esculenta starch – chitosan –glycerol in the ratio of Dioscorea esculenta starch : chitosan 3 : 2 by the addition of plasticizers glycerol 0.4% to the value of tensile strength of 32.21 MPa, elongation 31.23 %, water resistance of 0.20 and a weight reduction of biofilm in solution EM4 for 1 week at 60, 22%. Thus, it can be concluded that the characteristics of the biofilm is a biodegradable film, has a tensile strength and percent elongation that have met the SNI, but it has limitations in terms of resistance to water. The low percentage of elongation is likely to be overcome by the addition of poly lactate acid or other acids. For that they need to do further research in order to obtain biofilm compounds made from starch and other natural materials which are biodegradable, and have characteristics that match the quality of plastics derived from petroleum.

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References

[1] Aryanti, N., 2013. Biopolimer sebagai Plastik Ramah Lingkungan. *Buletin Teknologi Terapan Populer*, 1(1).
[2] Wahyu, M. K., 2009. *Pemanfaatan Pati Dioscorea esculenta Singkong Sebagai Bahan Baku Edible Film.* , Bandung.: Universitas Padjadjaran.

[3] Linssen, J., van Willige, R. & Dekker, M., 2003. *Packaging-flavour Interactions. In Ahvenainen, R.*. Novel Food Packaging Techniques. : Woodhead Publishing Limited..
[4] Sanjaya, M. H. I. G. & Puspita, T., 2011. *Pengaruh Penambahan Khitosan dan Plasticizer Gliserol Pada Karakteristik Plastik Biodegradable dari Pati Dioscorea esculenta Limbah Kulit Singkong.,* Surabaya: ITS.
[5] Vedder, T., 2008. *Edible Film.*. [Online] Available at: http://japemethe.port5.com (Accessed 3 july 2015).
[6] Skurtys, O. et al., 2009. *Food Hydrocolloid Edible Films and Coatings.*, Chile, pp. 34: Department of Food Science and Technology, Universidad de Santiago de Chile.
[7] Darmadji, P. & Izumimoto, M., 1994. Effect of chitosan in meat preservation.. *Meat science, 38,* pp. 243-254.
[8] Lu, D. R., Xiao, C. M. & Xu, S. J., 2009. Starch-based completely biodegradable polymer materials.. *eXPRESS Polymer Letters 3(6),* p. 366–375 DOI: 10.3144/expresspolymlett.2009.46.
[9] Steven, M. P., 2007. *Kimia Polimer. (Translater Iis Sopyan).*. Jakarta: PT Pradnya Paramita.
[10] Bonilla, J. et al., 2013. Effect of the incorporation of antioxidants on physicochemical and antioxidant properties of wheat starch–chitosan films. *Journal of Food Engineering,* 118(3), pp. 271-278.
[11] Butler, B. et al., 1996. Mechanical and Barier Properties of Edible Chitosan Films as affected by Composition and Storage.. *J. Food Science. 61(5)* , pp. 953-955.
[12] Richana, N. & Sunarti, T. C., 2004. Karakterisasi Sifat Fisikokimia Tepung Umbi dan Tepung Pati Dioscorea esculenta Dari Umbi Ganyong, Suweg, Ubi Kelapa dan Dioscorea esculenta . *Jurnal Pascapanen 1(1),* pp. 29-37.
[13] Bergo, P., Freitas Moraes, I. C. & do Amaral Sobral, P. J., 2012. Effects of moisture content on structural and dielectric properties of cassava starch films. DOI: 10.1002/star.201200023. *Starch. Biosynthesis Nutrition Biomedical,* 64(10), p. 835–839 .
[14] Bergo, P. et al., 2010. Physical properties of gelatin films plasticized with glycerol, studied by spectroscopic methods. *Materials Science Forum* , Volume 636, pp. 753-758.
[15] Bergo, P. et al., 2008. Physical properties of edible films based on cassava starch as affected by the plasticizer concentration. *Packaging Technology and Science,* 21(2), pp. 85-89.
[16] Chiumarelli, M. & Hubinger, M., 2014. Evaluation of edible films and coatings formulated with cassava starch, glycerol, carnauba wax and stearic acid.. *Food Hydrocoll* , Volume 38, pp. 20-27.
[17] Belibi, P. et al., 2014. A Comparative Study of Some Properties of Cassava and Tree Cassava Starch Films. doi:10.1016/j.phpro.2014.07.032. *Physics Procedia. 8th International Conference on Material Sciences, CSM8-ISM5,* Volume 55, pp. 220-226.
[18] Firdaus,F. ; Mulyaningsih , S. ; Anshory, H., 2008. Sintesis Film Kemasan Ramah Lingkungan dari Komposit Pati, Kitosan dan Asam Polilaktat dengan Pemlastik Gliserol. Studi Morfologi dan Karakteristik Mekanik. *Jurnal Logika Vol 5 No 1.*
[19] Hidayat, M. K., Latifah & Sedyawati, S. M. R., 2013. Penggunaan Carboxy Methyl Cellulose dan Gliserol pada Pembuatan Plastik Biodegradable Pati Gembili. *Indonesian Journal of Chemical Science, 2 (3).*
[20] Listyaningsih, D., 2013. *Pembuatan dan Karakterisasi Biofilm Pati gembili-. Kitosan dengan Plasticizer Polivinil alkohol (PVA),* Semarang: Universitas Negeri Semarang.
[21] Wafiroh, S., Ardianto, T. & Agustin, E. T., 2011. *Pembuatan dan Karakterisasi Edible Film dari Komposit Kitosan-Pati Dioscorea esculenta Garut (Maranta Arun Dinaceae L) dengan Pemlatis Asam Laurat.* , Surabaya. : Universitas Airlangga.
[22] Garcia, M., Martino, M. N. & Zaritzky., N. E., 2000. Lipid addition to improve barrier properties of edible film starch based films and coatings. .. *J. Food Sci,,* 65(6), pp. 941-947.
[23] Vicentini, N. M. et al., 2005. Prediction of cassava starch edible film properties by chemometric analysis of infrared spectra.. *Spectroscopy Letters,* Volume 38, p. 749.
[24] Bourtoom, T. & Chinnan, M. S., 2008. Preparation and properties of rice starch-chitosan blend biodegradable film.. *LWT-Food Science and Technology,* Volume 41, p. 1633–1641.
[25] Majeti, N. V. & Kumar, R., 2000. A review of chitin and chitosan applications. *Reactive & Functional Polymers,* Volume 46, pp. 1-27.

[26] Dutta, P. K., Tripati, S. & Mehrotra, G., 2009. Physicochemical and Bioactivity of Cross-linked Chitosan-PVA Film for Food Packaging Applications.. *Journal of Biogical Macromolrcules. 45,* pp. 72-76.

[27] Viorica, N. S. et al., 2011. Strach/Chitosan Film Forming Hydrogel. *Revue Rumaine de Chimie. 56(8),* pp. 827-832.

[28] Sanjaya, M. H. I. G. & Tyas, P., 2011. *Pengaruh Penambahan Khitosan dan Plasticizer Gliserol Pada Karakteristik Plastik Biodegradable dari Pati Limbah Kulit Singkong. ,* Surabaya: ITS.

[29] Agustin, Y. & Padmawijaya, K. S., 2016. Sintesis bioplastik dari kitosan-pati kulit pisang kepok dengan penambahan zat aditif.. *Jurnal Teknik Kimia,* 10(2), pp. 40-48.

[30] Hartatik, Y., Nuriyah, L. & Iswarin, S. J., 2014. Pengaruh Komposisi Kitosan Terhadap Sifat Mekanik dan Biodegradable Bioplastik. *Physics Student Journal,* 2(1).

[31] Barreto, P., Pires, A. & Soldi, V., 2003. Thermal degradation of edible films based of milk proteins in gelatin in inert atmospher. *Polym. Degrad.Stabil.,* Volume 79, pp. 147-152.

[32] Darni, Y., Chici, A. & Sri Ismiyati, D., 2008. *Sintesa Bioplastik dari Pati Pisang dan Gelatin dengan Plasticizer Gliserol. Prosiding Seminar Nasional Sains dan Teknologi-II.* Lampung, Universitas Lampung.

[33] Tudorachi, N. & Cascaval, C. N., 2000. Testing of Polyvinyl Alcohol and Starch Mixtures as Biodegradable Polymeric Materials.. *Polimer Testing (19),* pp. 785-799.

[34] Kim, K., Ko, C. & Park, H., 2002. Mechanical properties water vapor permeabilities and solubilities og highly carboxymethylated starch-based edible film.. *J.Food Sci,* 67(1), pp. 218-222.

[35] Darni, Y. & Utami, H., 2010. Studi Pembuatan dan Karakteristik Sifat Mekanik dan Hidrofobisitas Bioplastik dari Pati Dioscorea esculenta Sorgum.. *Jurnal Rekayasa Kimia dan Lingkungan, 7(4),* pp. 88-93.

[36] Wardhani, R. A. K., Rudyardjo, D. I. & Supardi, A., n.d. Sintesis dan Karakterisasi Bioselulosa-Kitosan Dengan Penambahan Gliserol Sebagai Plasticizer.

[37] Darni, Y., S.Ismiyati, S. & Marbun, T., 2010. Influence Concentration Of Plasticizer and Formulation of Banana Starch Chitosan To Mechanical property and water uptake of Bioplastic. .. *International Journal of Engineering and Science,* 7(4), pp. 1-8.

[38] Buzarovska, B. et al., 2008 . Potential use of rice straw as filler in eco-composite material.. *Australian Journal Of Crop Science, 1(2),* pp. 37-42.

[39] Ferdiansyah, A., 2015. *Pengaruh komposisi plasticizer dan kitosan terhadap sifat mekanik bioplastik dari pati umbi keladi,* Palembang: Jurusan Teknik Kimia, Politeknik Negeri Sriwijaya.

[40] Gennadios, A., McHugh, T., Weller, C. & J.M., K., 1994. *Edible Coating and Film Based on Protein. In Krochta, J.M., E.A.Baldwin and M.O. Nisperos-Carriedo. 1994. Edible Coatings and Films to Improve Food Quality..* (15), 234-236. ed. Pennsylvania: Technomic Publishing Company, Inc..

[41] Kerch, G. & Korkhov, V., 2011. Effect of storage time and temperature on structure, mechanical and barrier properties of chitosan-based films.. *Springer. Eur Food Res Technol. Volume 232, Issue 1,* pp. 17-22.

[42] Shahidi, F., Arachi, J. & Jeon, Y., 1999. Food Application Of Chitin and Chitosan.. *Trends in Food Science & technology,,* Volume 10, pp. 37-51.

[43] Park, S., Marsh, K. & Rhim, J., 2002. Characteristics of different molecular weight chitosan films affected by the type of organic solvents.. *J. Food Science. 67(1),* pp. 194-197.

[44] Darni, Y., Utami, H. & Asriah, S. N., 2009. *Peningkatan Hidrofobisitas dan Sifat Fisik Plastik Biodegradabel Pati Dioscorea esculenta Tapioka Dengan Penambahan Selulosa Residu Rumput Laut Euchema spinossum. Seminar Hasil Penelitian dan Pengabdian Kepada Masyarakat. ,* Lampung: Universitas Lampung.