

Characterization of Edible Film from Starch of Taro (*Colocasia esculenta* (L.) Schott) with Addition of Chitosan on Dodol Substituted Seaweed (*Eucheuma cottonii* L.)

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Abstract. Edible film is a thin layer to protect food which it can be consumed. One of the main components to make edible film is starch, which could be derived from taro tubers. Taro tubers contain high starch content about 70-80% which consists of 5.55% of amylose and 74.45% of amylopectin with yield reach to 28.7%. However, the weakness of starch-based edible film are low resistance to water and low barrier properties to water vapor. One of the recommended hydrophobic biopolymers to improve the film characteristics of starch as well having antimicrobial activity is chitosan. The objectives of this research are to know the interaction and influence of starch concentration from taro tuber and concentration of chitosan on physical and mechanical character of edible film, including thickness, density, transparency, tensile, elongation and water vapor transmission eate (WVTR). The best treatment from first objective will apply on dodol (food made by glutinous rice) substituted by seaweed to know the effect of its application on dodol shelf life (texture analysis, weight loss and microbiology). Therefore this research was conducted in 2 stages and 2 experimental design methods. The first stage was the process of making edible film using Randomized Block Design method and the second stage was the application of edible film using Random Design Complete method. The results of physical and mechanical analysis of edible film based on its effect on the storage of dodol showed that the best treatment was obtained of P3K3 treatment (6% of Taro starch: 3% of Chitosan), P3K1 (6% of Taro starch: 1% of Chitosan) and P3K2 (6% Taro starch: 2% of Chitosan) . The application of 3 types of edible films based on the best treatment of dodol substitute of seaweed has significant effects on the value of weight loss, texture and total plate count during the storage period. Effectiveness of edible film in inhibiting damage to dodol was obtained of P3K3 treatment (6% of Taro starch: 3% of Chitosan) with WVTR value equal to 0.189 g / cm² hour, 0.170 N / mm² of tensile strength, 0.088% of elongation, 0.160 mm of thickness, 0.151 of density and 0.000 mm⁻¹of transparency.

Keywords: Edible Film, Starch Talas, Chitosan, Dodol

Introduction

Taro tubers has potent as a source of carbohydrates about 70-80 which consists of 5.55% of amylose and 74.45% of amylopectin with a yield of 28.7% (Sharif and Estiasih, 2013). Its high starch causes taro potenti as a biodegradable packaging material like edible film. The advantages of edible films are can be consumed directly with packaged products, do not pollute the environment, improve the organoleptic properties of packaged products, as flavors, color, antimicrobial agents, and antioxidants. Currently the use of synthetic polymers such as plastics has an important role in the economy of society either modern or traditional industries, as in the dodol industry. Dodol substituted of seaweed

(*Euchema cottonii*) as one of diversification of seaweed processing products usually use plastic for its packaging. The big problem using plastic as packaging material is its waste. Thus, it need to convert the type of packaging material from plastic to edible film.

However, edible films developed based on starch have weaknesses such as low resistance to water and low barrier properties to water vapor due to the hydrophilic of starch which can affect its stability and mechanical properties (Garcia et al., 2011). One of the recommended hydrophobic biopolymers to improve the characteristics of starch-based film as well having antimicrobial activity is chitosan. (Chillo et al., 2008).

Method and Material

This research was conducted at Food Science and Technology Laboratory, Faculty of Agriculture and Animal Husbandry, University of Muhammadiyah Malang and Laboratory of Food Research Institute of Beans and Tubers, Kendalpayak, Malang. This study was conducted from February to July 2017.

The materials used in this research-taro tuber, salt, glutinous rice flour, rice flour, cooking oil, milk, sugar and seaweed- were obtained from Pasar Besar Kota Malang, while chitosan, aquades, glycerol, acetic acid 2%, NaCl was purchased from the Makmur Sejati chemical store. Research was divided into 2 types, namely preliminary research and main research. Preliminary research were starch extraction from taro tuber and analysed it. While the main research done with 2 stages and 2 methods of experimental design. The first stage was the process of making edible film using Randomized Block Design method with 2 factors and 3 replications. The first factor was the concentration of taro starch (P) consisting of 3 levels i.e, P1 = 2%, P2 = 4%, and P3 = 6% w/v. The second factor was chitosan concentration (K) consisting of 4 levels i.e, K0 = 0%, K1 = 1%, K2 = 2% and K3 = 3% w/v. The results of characteristic analysis continued with the test using De Garmo method to find out the best treatment combination. The second stage was the process of applying the best treatment of edible film. The research method used was Completely Randomized Design with 6 repetitions. The treatment used was 3 types of edible film with the best value and a control. Furthermore, dodol was stored and observed in storage periods 0, 2, 4 and 6 days.

Result and Discussion

Raw Material Analysis

The results of raw material analysis could be seen in Table 1. Based on the results of the test (Table 1) it can be seen that the value of starch from taro was 53.40%wb (wet based) and 58.57%db (dry based). While the water content on taro tuber starch was 8.83%; amylose of 20.04%wb (wet based) and 21.98%db (dry based); amylopectin of 33.36%wb (wet based) and 36.59%db (dry based). The yield obtained was 21.34%. Factors influencing the analysis of taro starch i.e species / clones, optimum harvested tuber age, weather conditions at harvest, starch

purity level when processed, genetic factors, growing environment and starch processing methods (Ginting et al, 2005).

Table 1. Analysis Results of Taro Starch

Sampel	Yield	Water content	Starch		Amylose		Amylopectin	
	%	%	(%wb)	(%db)	(%wb)	(%db)	(%wb)	(%db)
Taro Starch (Type of "Bentul")	21.34	8.83	53.40	58.57	20.04	21.98	33.36	36.59

Edible Film Characteristics Analysis

Results Thickness

Table 2. Average Value of Edible Film Thickness (mm) by Treatment of Concentration of Taro Starch and Chitosan

Treatments		Thickness (mm)
Concentration of Taro Starch		
P1	2%	0.08 a
P2	4%	0.10 b
P3	6%	0.11 b
Concentration of Chitosan		
K0	0% (Without Chitosan)	0.07 a
K1	1%	0.09 b
K2	2 %	0.11 b
K3	3%	0.10 b

Description: The number followed by the same letter shows no significant difference in Duncan test $\alpha = 5\%$

Table 2. showed the lowest value of thickness was P1 (2% of Taro starch) while the highest was P3 (6% of Taro starch) with 0.08 and 0.11 mm, respectively. This value was not significantly different from P2 (4% concentration) indicated by the notation of letter b. The higher the starch concentration, the thickness value of the edible film would also increase. Moreover, the addition of chitosan also gave obviously effect on the thickness of edible film. The lowest value was K0 (without chitosan) while the highest value was K2 (2% of chitosan) with 0.07 and 0.11 mm, respectively. In principle, the more components are added, the thickness value increases. This is supported by the opinion of Nugroho et al. (2013) which states that the increase in the amount of solids in solution causes the polymers that make up the edible film matrix. In addition to the total solids in the solution, other things that affect the edible thickness are the viscosity and the constituent polymer content.

Density

This density or density defined as the weight per unit volume of material.

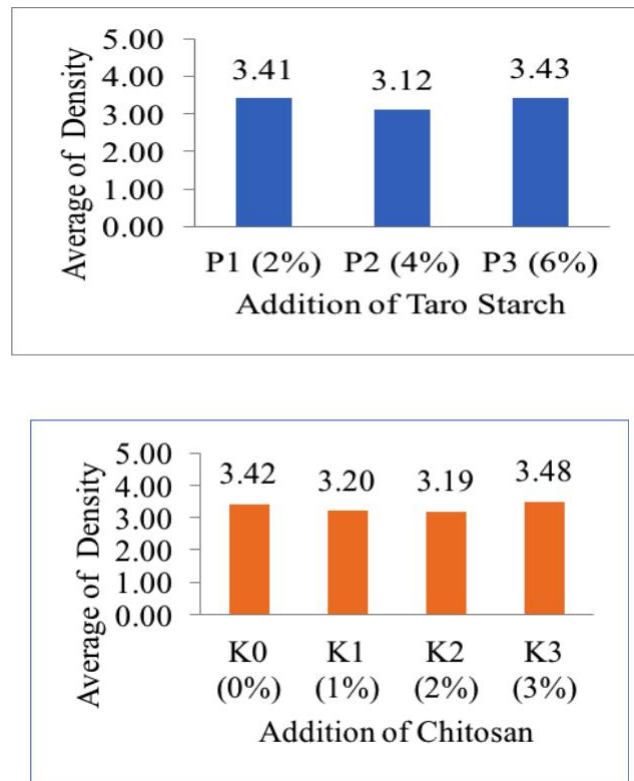


Figure 1. Density Analysis Result in Edible Film (g/cm₃) due to a) Addition of Taro Starch and b) Addition of Chitosan

Based on Table 2 and Figure 1. displayed that the concentration of starch and chitosan affect the thickness but not effect the density of film. The variation of concentration from both treatments did not affect the increase of the distance between the matrix composed edible film.

Transparency

Based on the analysis, resulted that the addition of starch concentration has a significant effect on the transparency value of edible film (Table 3). The highest transparency value was obtained at 6% of starch addition concentration, while the lowest value was 2% of starch with 5.68 and 4.55 mm⁻¹, respectively.

Table 3. Average Value of Edible Film Transparency by Treatment of Concentration of Taro Starch and Chitosan

Treatments		Transparency (mm ⁻¹)
Concentration of Taro Starch		
P1	2%	4.55 a
P2	4%	5.14 ab
P3	6%	5.68 b
Concentration of Chitosan		
K0	0% (without Chitosan)	4.16 a
K1	1%	4.89 ab
K2	2%	5.27 bc
K3	3%	6.17 c

Description: The number followed by the same letter shows no significant difference in Duncan test $\alpha = 5\%$

The higher of transparency value due to higher of concentration of starch addition made more opaque the edible film produced. The transparency value of edible film might be affected by its thickness and components. The thicker the film, the higher the transparency value because more light was absorbed on film shot by a spectrophotometer with a certain wavelength. Wiryawan (2007) states that the transparency of a film will be proportional to the thickness and concentration. Meanwhile, according to Rumapea (2009), fluctuating transparency could be influenced by several factors, including stirring, particle size, and number of components. In addition, another factor suspected to affect color change in edible film was the process of installing each edible film on a less precise cultpure during the test.

Tensile Strength

The average of tensile strength of edible film by treatment of taro tuber starch concentration and chitosan concentration could be seen in Table 4. The value of tensile strength of the film ranges from 0.5 to 2.5 N / mm². The highest value was obtained at P3K3 (6% of Taro Starch + 3% of Chitosan), while the lowest value was P1K1 (2% of Taro Starch + 1% of Chitosan) with 2.50 and 0.50 N/mm², respectively. This indicated that the higher starch concentration and chitosan concentration added to edible film production had an effect on the higher tensile strength value. The higher value of tensile strength was influenced by the addition of starch, thus the matrix formed would more solid, so the power given to attract the film was greater. The higher of tensile strength shows resistance to damage due to stretching and greater pressure, resulting in improved physical quality. The higher concentration of starch, the higher amylose content in the edible film solution, as the higher number of polymers in the matrix formation, the polymer bond strengthens and the tensile strength was also greater (Warkoyo, 2014).

Table 4. Mean Tensile Strength (N / mm²) Edible Film Value by Treatment of Concentration of Taro Starch and Chitosan

Treatments		Tensile strength (N/mm ²)
P1K0	2% of Taro Starch + 0% of Chitosan	0.53 a
P1K1	2% of Taro Starch + 1% of Chitosan	0.50 a
P1K2	2% of Taro Starch + 2% of Chitosan	0.95 ab
P1K3	2% of Taro Starch + 3% of Chitosan	1.37 ab
P2K0	4% of Taro Starch + 0% of Chitosan	0.71 ab
P2K1	4% of Taro Starch + 1% of Chitosan	0.81 ab
P2K2	4% of Taro Starch + 2% of Chitosan	1.35 ab
P2K3	4% of Taro Starch + 3% of Chitosan	1.67 bc
P3K0	6% of Taro Starch + 0% of Chitosan	0.57 a
P3K1	6% of Taro Starch + 1% of Chitosan	1.33 ab
P3K2	6% of Taro Starch + 2% of Chitosan	1.75 bc
P3K3	6% of Taro Starch + 3% of Chitosan	2.50 c

Description: The number followed by the same letter shows no significant difference in Duncan test $\alpha = 5\%$

Elongation

The average of edible film elongation by treatment of taro tuber starch concentration difference and chitosan concentration could be seen in Table 5

Table 5. Average Value of Edible Film Elongation by Treatment of Concentration of Taro Starch and Chitosan

Treatments		Elongation (%)
Concentration of Taro Starch		
P1	2%	40.12 a
P2	4%	71.42 b
P3	6%	102.31 c
Concentration of Chitosan		
K0	0% (without Chitosan)	91.67 b
K1	1%	87.47 b
K2	2%	62.78 ab
K3	3%	43.21 a

Description: The number followed by the same letter shows no significant difference in Duncan test $\alpha = 5\%$

Based on Table. 5 displayed that the highest value was obtained at P3 (6% of Taro Starch), while the lowest value was P1 (2% of Taro Starch) with 102,31and 40.12 %, respectively. The higher starch concentration added, the elasticity of edible film increased due to its amylose and amylopectin. On the addition of chitosan treatment, the highest elongation was obtained at K0 and K1(indicated by the notation b), showed that both without and with 1% of chitosan were not significant in tensile strength with 91.67 and 87.47 %, respectively. Meanwhile, the lowest elongation was obtained at K3 (3% of chitosan) of 43.21%. The higher concentration of chitosan given the effect of lower elongation value. This can be influenced by the higher concentration of

chitosan would affect the viscosity of edible film solution before printing. In addition, it was also influenced by the addition of plasticizer, in which the increasing concentration of dissolved chitosan without accompanied by an increase in plasticizer concentration would result in a more rigid edible film resulting in lower elongation values associated with the increasing amount of dissolved solids. Another factor that affects the value of elongation was the relatively thin edible film produced, so easy to break if done withdrawal.

Water Vapor Transmission Rate (WVTR)

The highest of WVTR by taro starch addition was obtained at P1 (2% of Taro starch) equal to $10,03 \times 10^{-5}$ g/cm².h whereas the lowest was obtained at P2(4% of Taro starch) and P3 (6% of Taro starch) marked with a notation with 7.52×10^{-5} and 6.52×10^{-5} g/cm².h, respectively. This showed that the results of P2 and P3 treatments were not significantly different and both were significantly different from P1 treatment. The higher concentration of taro starch had an impact on the decrease of water vapor transmission rate in the film. The lower the transmission rate means the better the film holds the diffusing water vapor into the material. The high value of water vapor transmission was influenced by the ratio of hydrophobic and hydrophilic materials. In addition, according to Praseptiangga (2003) the value of the transmission rate is inversely proportional to the thickness value. The thicker the film, the vapor transmission rate the lower because the thickness is the distance that must be taken by water vapor to diffuse through the film, so the thicker the film, the mileage further and takes a long time

Table 6. Average Value of WVTR Edible Film (g/cm².h) by Treatment of Concentration of Taro Starch and Chitosan

Treatments		WVTR (g/cm ² .h)
Concentration of Taro Starch		
P1	2%	10.03 x 10 ⁻⁵ b
P2	4%	7.52 x 10 ⁻⁵ a
P3	6%	6.52 x 10 ⁻⁵ a
Concentration of Chitosan		
K0	0% (without Chitosan)	10.65 x 10 ⁻⁵ c
K1	1%	8.23 x 10 ⁻⁵ b
K2	2%	7.77 x 10 ⁻⁵ b
K3	3%	5.45 x 10 ⁻⁵ a

Description: The number followed by the same letter shows no significant difference in Duncan test $\alpha = 5\%$

Based on the Table 6 showed the WVTR value of the film by addition of chitosan ranged between 10.65×10^{-5} - 5.45×10^{-5} g/cm².h. The the highest of WVTR was obtained by 0% of chitosan while the lowest was obtained by 3% of chitosan. This indicated that the addition of chitosan given obviously significant

in WVTR value. The greater the chitosan concentration added, the lower the vapor transmission rate.

Determination of Best Treatment

Determination of the best treatment used the effectiveness index method (De Garmo et al., 1984). The principle of this method is to compare all parameters measured.

Tabel 7.Result of Best Treatment

Parameters	Value		
	P3K3 (1)	P3K2 (2)	P3K1 (3)
WVTR	0.189	0.179	0.176
Tensile Strength	0.170	0.106	0.070
Elongation	0.088	0.100	0.136
Thickness	0.160	0.170	0.129
Density	0.151	0.092	0.069
Transparancy	0.000	0.038	0.081
Total	0.757	0.686	0.661

Results of Analysis During Storage

Weight loss

Table 8. Average value of weight loss of Dodol substituted of seaweed by coating of edible film during storage period

Treatments	Weight loss (%)		
	2 nd day	4 th day	6 th day
A0 (Control)	0.035c	0.134 b	0.191 b
A1 (Edible film of P3K1)	0.018ab	0.048 a	0.092 a
A2 (Edible film of P3K2)	0.018b	0.050 a	0.105 a
A3 (Edible film of P3K3)	0.016a	0.046 a	0.098 a

Description:

The number followed by the same letter shows no significant difference in LSD test $\alpha = 5\%$

Based on Table 8. Showed the weight loss of dodol substituted seaweed on the 2nd day ranged from 0.016% -0.035%. The lowest value was obtained at A3 treatment of 0.016%. This value was not significant with the treatment of A1 followed by a notation. Meanwhile the highest value was obtained at control treatment. In the 4th day has a higher weight loss value when compared with 2nd day. The weight loss of dodol substituted seaweed on the 4th day ranged from 0.046-0.134%. The average value of weight loss on 6th day increased significantly, ranged from 0.098 to 0.191%. The greater the weight loss value, the greater the water vapor coming out of the material, the worse the packaging material in preventing product damage. Factors that cause increased weight loss during storage were loss of water content and other volatile compounds during storage. In addition, the weight loss correlated positively with the thickness of the edible film.

Texture

Texture is one factor that determines the quality of food products. Based on Table 9. it was known that the average of dodol texture decreased during the storage period showed by getting smaller value.

Table 9. Average value of texture of Dodol substituted of seaweed by coating of edible film during storage period

Treatmens	Texture (mm)							
	0 day		2 nd day		4 th day		6 th day	
A0 (Control)	5,104	a	4,328	a	4,042	a	3,118	a
A1 (Edible film of P3K1)	5,707	ab	6,336	bcd	6,469	bcd	5,631	b
A2 (Edible film of P3K2)	6,467	bc	6,362	cd	6,894	d	6,214	cd
A3 (Edible film of P3K3)	6,958	c	6,532	d	6,762	cd	6,465	d

Description: The number followed by the same letter shows no significant difference in LSD test $\alpha = 5\%$

The harder the sample the greater the force required to puncture the sample. The main factor affecting dodol texture during storage was type of edible film coating. Each type of edible film has different physical characteristics and influences the dodol texture value during the test. Meanwhile, according to Purnomo (1995) other factors affecting the texture of food materials include the ratio of protein-fat content, protein type, processing temperature and water content. While texture changes during storage could be caused the occurrence of retrogradation, enzymatic reactions, and water absorption as well as increased oxidation reactions. The effect of water absorption depends on the level of the product's water content and its texture characteristics, in which water might increase or loss during storage.

Total Plate Count (TPC)

Based on Table 10. it was known that edible coating with different concentration of the material has an effect on TPC dodol substituted of seaweed during storage period. Edible film was able to inhibit microbiological damage to dodol substituted of seaweed. On 0 day, the average value of TPC ranged from $27,17 \times 10^5$ - $100,33 \times 10^5$ CFU/gram and it increased during storage period. Factors that cause increased TPC value was possibility of contamination when the process of applying of edible film. According to Fardiaz (1989), factors affecting the growth of microorganisms include intrinsic factors and extrinsic factors, process factors, and implicit factors. Intrinsic factors include pH, water activity (aw), oxidizing-reduction capability, nutrient content, antimicrobial ingredients, and food structure. Extrinsic factors affecting the growth of microorganisms are storage temperature, humidity, gas pressure (O₂), light and the influence of ultraviolet light.

Although TPC value increased when during period, but it was proven that edible film was able to inhibit the growth of microorganisms during storage. During the storage process, growth and development of mold and various

microbes were inhibited by the presence of chitosan. Chitosan has antimicrobial properties by inhibiting the development of cells that have a negative charge. The concentration of the constituents of the edible film is certainly very influential on the inhibition of growth of microbes.

Table 10. Average value of Total Plate Count of Dodol substituted of seaweed by coating of edible film during storage period

Treatments	TPC (CFU/gram)			
	0 day	2 nd day	4 th day	6 th day
A0 (Control)	100,33x10 ⁵ c	213,50x10 ⁵ b	152,83x10 ⁵ bc	139,33x10 ⁵ b
A1 (Edible film of P3K1)	59,83x10 ⁵ b	29,33x10 ⁵ a	157,00x10 ⁵ c	65,00x10 ⁵ a
A2 (Edible film of P3K2)	28,00x10 ⁵ a	27,50x10 ⁵ a	129,17x10 ⁵ ab	74,50x10 ⁵ a
A3 (Edible film of P3K3)	27,17x10 ⁵ a	30,67x10 ⁵ a	91,33x10 ⁵ a	88,83x10 ⁵ a

Description: The number followed by the same letter shows no significant difference in LSD test $\alpha = 5\%$

Conclusion

The conclusions that can be taken in this research are:

1. There were interaction between the concentration of taro starch and chitosan concentration on the tensile strength character of edible film.
2. The effect of taro starch concentration has a significant effect on thickness, transparency, elongation and WVTR on edible film.
3. Effect of chitosan concentration has a significant effect on thickness, transparency, elongation and WVTR on edible film.
4. The best three treatment which has a significant effect on weight loss, texture, and Total Plate Count elongation were P3K3 (6% of Taro Starch and 3% of Chitosan), P3K2 (6% of Taro Starch and 2% of Chitosan) and P3K1 (6% of Taro Starch and 1% of Chitosan). Meanwhile, best edible film in inhibiting the damage of dodol that is edible film was obtained at P3K3 with 0.189 g / cm².h of WVTR, 0.170 N/mm² of tensile strength, 0.088% of elongation, 0.160 mm of thickness, 0,51 of density, and 0.000 mm⁻¹ of transparency.

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