

The Effect of Whey Protein addition on the Physical Properties of Alginate-Based Edible Films from Brown Algae (*Sargassum* sp.) as Halal Edible Film Alternative

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Abstract. Edible film serves to protect food so that it remains fresh and durable, but it can also be consumed together with the food it packs. Edible film is an option to reduce plastic, because it is biodegradable. Edible films can be made from polysaccharides, proteins or fats. One of the polysaccharides that can be used as raw material for edible film is alginate from brown algae (*Sargassum* sp.). Edible film from these algae, more halal guaranteed than edible film from fat material, but Alginate-based edible film is hydrophilic that not good for holding water. Other ingredients need to be added to decrease the hydrophilic properties, including whey protein. In this study, whey protein added on alginate-based edible film to obtain edible film has good properties. The results of this research show the addition of whey protein causes a decrease in the value of water absorption, increase the value of tensile strength, and elongation. This shows that the addition of whey protein can reduce hydrophilic properties and can increase the elasticity of edible alginate films.

Keywords: Alginate, biodegradable, edible film, hydrophilic properties, whey protein.

1 Introduction

Public awareness of health and environment increasingly high encourages people to be more selective in choosing foodstuffs. Good packaging technique is needed to get fresher and more durable food products when its storage. Packaging of food using plastic is still widely used, but the effect of using plastic on the environment is quite large, including the problem of waste produced.

One of the solutions is to solve those problem is technology for producing packaging materials that can increase food value and be environmentally friendly (biodegradable). Besides being environmentally friendly, packaging technology is now also directed at edible packaging. Edible packaging consists of two forms, namely as a coating (edible coating) and in the form of sheets (edible film). Edible coating is used directly on food ingredients as a coating, it can be by spraying or dyeing [1]. Usually edible coating is used to coat fruits, meat, fish, and sausages.

This edible packaging can be made from various ingredients, including polysaccharides, proteins, and fats [2]. Edible packaging from polysaccharides such as chitosan, cellulose, carrageenan, and alginate tends to be hydrophilic, so it is not good at holding water vapor [3]. Edible packaging of lipid material is hydrophobic, which can reduce water vapor permeability. But edible packaging made of lipid material is usually thick and easily brittle. In contrast to

edible films from lipids, edible films from protein materials are transparent and elastic, so edible films from these proteins are preferred [1]. Proteins are widely used in making edible films in gelatin and collagen.

The source of edible film of protein are from gelatine and collagen as raw material, mostly taken from animals that the halal status is not yet clear. In Indonesia's majority Muslim country, this problem is a matter of considerable concern. Because Muslims must pay attention to their food so that it is always halal and thayyib as stated in the Letter of Al Baqarah (2) verse 168 which means: "O people, eat the halal things better than what is on earth"

The obligation to pay attention to halal and thayyib food intake besides being a form of obedience and gratitude to Allah SWT there is also a sign of benefit for human beings themselves. Not only does Allah give instructions in the form of orders and prohibitions, in addition to the benefit of human life both in the world and in the hereafter. Halal food is food that God does not forbid or food that is permissible according to syara', while food which is thayyib (good) is food which results in bringing calm and does not cause harm or harm.

To bridge the problems, it is necessary to make an alternative for making edible packaging from material that is undoubtedly halal, for example, from polysaccharides, namely alginates from brown algae (*Sargassum* sp.). The selection of alginate from brown algae (*Sargassum* sp.) As the raw material for the manufacture of edible packaging was based also on the abundance of brown algae (*Sargassum* sp.) In Indonesian waters [4]. Edible packaging from polysaccharides has a deficiency that is hydrophilic, so it is not good for packaging. To improve the performance of edible packaging from brown algae (*Sargassum* sp.), A modification in the packaging manufacturing process is needed in the form of adding hydrophobic ingredients such as whey protein.

2 Materials, Tools, and Instrument

The sample used is brown Algae. The material used as an extraction solvent is Na_2CO_3 (Merck®). Other chemicals used in the extraction process are NaOH (99%, Merck), HCl (36%, Merck), Isopropyl Alcohol (technical), CaCl_2 (technical). Additional ingredients used in the manufacture of edible films are glycerol plasticizer, whey protein. Other materials used are commercial aquadest.

The tools used included blender, analytic balance (Ohaus PA213), pH-meter (ATC pH-2011), filter paper (Whatman 40), beaker (250 mL, 500 mL and 1000 mL), measuring cup (10 and 50 mL), volumetric flask (25 and 50 mL), stative and clamp, volume pipette (10 mL), drop pipette, spatula, stirring rod and funnel, filler, 100 ° C thermometer, pH meter, oven, furnace, tweezers, cup, desiccator.

Instrumentation used included identification of compounds and components contained in the sample with FTIR spectrophotometer (Agilent 680), to determine the mechanical characteristics of each sample edible film with a Testometric tensile tester (M350-10AT), as well as to see the surface pores of the film that is by SEM (Scanning Electron Microscope). The materials used as extraction solvents were ethanol (70%, CV. Brataco). The chemicals used in titration are NaOH (99%, Merck), and HCl (36%, Merck). Other materials used are commercial aquadest.

3 Produce

3.1 Sample Preparation

The sticky dirt from the dried Algae leaves is cleaned with running water, then the sample is soaked using 1% HCl with a ratio of 1:30 (algae: HCl) (w / v) for 1 hour. Sample with neutral pH is obtained by washing sample using distilled water. Next, sample was soaked again with 2% NaOH for 30 minutes. Then extracting algae to get Na-alginate.

After that, it extracted using 2% Na₂CO₃ with a ratio of 1:30 (w / v) at a temperature of 60-70 °C for 2 hours. Then, it filtered. Subsequently the obtained filtrate was bleached with 10% H₂O₂ for 30 minutes while stirring regularly. The filtrate added by 15% HCl to pH 2-3 to produced alginate acid. Furthermore, the product is converted to Na-alginate by adding 10% NaOH to neutral pH.

The formed Na-alginate was then separated by adding 1: 2 (v / v) isopropyl alcohol. After that, filtering and milling are carried out to do the drying process using the oven for approximately 24 hours until the moisture content is 12%. The next process is grinding by using a grinder to obtain Na-alginate powder and then analyse its physical and chemical properties to get % yield, moisture content, ash content, and characterization using FTIR. Na-alginate which was analyzed using FTIR was then modified to Ca-alginate by the addition of CaCl₂.

3.2 Modification of Na-alginate to Ca-alginate

1 gram of Na-alginate is put into a 100 mL beaker (5 variations are prepared). Then Na-alginate dissolved in 20 mL of distilled water slowly so as not to form agglomerate. Then, the solution is stirred with a magnetic stirrer until it is homogeneous. After that, a CaCl₂ solution with a concentration of 0.05 M was added slowly with a ratio of 1:10 (v/v). Then stirring again until a homogeneous gel is formed.

3.3 Modification of Ca-alginate to Edible Film

At this stage the modified Na-alginate into Ca-alginate was added by a biopolymer namely whey protein. Ca-alginate solution which has been dissolved evenly then added 1 mL of glycerol, then stirred again until the solution is homogeneous. After that, variations in whey protein concentration were added. The variations in whey protein concentrations given were 5%, 7%, 9%, 11% and non-whey protein Ca-alginate. After that the solution is stirred again so that what is formed is an edible film solution that is ready to be printed.

3.4 Mechanical Test

Before testing the mechanical properties of the edible film first, using a micrometer. Measurements were made at five different places and the value to be reported was an average of five measurements with almost the same thickness. After that the mechanical properties are tested. Testing of mechanical properties includes: 1. Tensile Strength 2. Elongation 3. Elasticity. The testing process for tensile strength will be carried out using the tool Tensile Tester type M350-10AT at Balai Besar Tekstil Bandung. This test is done by means of the tip of the sample clamped with a tensile testing machine. Next, record the thickness and beginning of the sample length. The start button on the computer is pressed and the tool will pull the sample at a speed

of 100mm / minute until the sample breaks. The value of tensile strength is obtained from the results of the maximum stress distribution with the width of the cross section. The maximum voltage is obtained from the sample voltage when breaking up. The cross-sectional area is obtained from the results of multiplying the initial length of the sample with the initial thickness of the sample. This tensile strength test was carried out on three samples of edible film which were then averaged. The tensile strength of edible film is calculated by the equation:

$$\tau = \frac{F_{max}}{A} \quad (1)$$

Description:

T = Tensile strength (MPa)

F_{max} = Maximum voltage (N)

A = Cross-sectional area (mm²)

Then to measure the extension will be carried out in the same way as the test tensile strength. Extension is expressed as a percentage, calculated by:

$$\text{Elongation (\%)} = \frac{\text{stretch at break (mm)}}{\text{initial length (mm)}} \times 100\% \quad (2)$$

While for elasticity value obtained from the comparison of tensile strength and elongation.

3.4 Water Resilience Test with Water Absorption Test

This water resistance procedure will refer to the method carried out, namely the initial weight of the sample to be tested (W_o). Edible film samples to be tested are inserted into containers containing distilled water for 10 seconds. Then the sample is removed from the container containing aquadest and the water found on the plastic surface is removed with paper tissue after which weighing is done. The sample was put back into a container containing distilled water for 10 seconds. Then the sample is removed from the container and weighed again until the final sample weight is constant. Then the water absorbed by the sample is calculated through the equation

$$\text{Water Absorption (\%)} = \frac{W - W_o}{W_o} \times 100\% \quad (3)$$

Description:

W = wet edible film weight

W_o = dry edible film weight

4 Result And Discussion

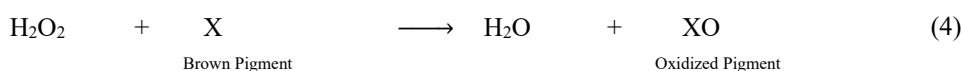
4.1 Isolation of Na-alginate from Brown Algae (*Sargassum* sp.)

The process of making Na-alginate is carried out by extracting from dried brown algae that has been sorted. This extraction aims to release alginate from cellulose and separate it from other components so that pure Na-alginate is obtained. The obtained Na-alginate will be used at a later stage, namely as a raw material for edible film.

In making this Na-alginate, dried brown algae soaked using 1% HCl. This immersion aims to hydrolyse cellulose found on the cell wall so that it will facilitate the subsequent extraction process. Cellulose is a fiber that is not soluble in water so HCl is used as a strong acid to hydrolyse it. In addition, soaking brown algae in an acidic solution aims to dissolve salts, laminarin, mannitol and as a solvent impurity particles attached to the algae. Then washed with aqua destillation and soaked with 0.5% NaOH solution which served to neutralize the acidic conditions.

After that brown algae extracted using a base solution which functions to separate cellulose from alginate. Alkaline solutions can be able to degrade alginate acid by cutting the polymer chain into oligosaccharides by further degrading to 4 deoxy 5 ketouronic acid. In this study the base solution used was Na_2CO_3 . Na_2CO_3 is an alkaline salt that is widely used for alginate extraction because it can dissolve more. Besides the use of Na_2CO_3 in this extraction is to break the pectin in the brown algae cell wall. In addition to cellulose and hemicellulose, the cell walls of brown algae are composed of pectin. Pectin is composed of sugar and acid units. This pectin compound functions as an adhesive between one cell wall and another. This compound is unstable in an alkaline condition. For this reason, Na_2CO_3 is used to dissolve the alginate contained in the cell wall, because alginate dissolves well in alkaline solutions.

The filtration process is then carried out to separate dissolved Na-alginate with cellulose, pectin and other impurities contained in the extracted paste. Then the filtrate was blanched using 10% H_2O_2 , this process aims to dissolve the dyes contained in crude alginate solution, namely phenolic compounds contained in alginate polymer bonds so that a clearer solution can be obtained. The reaction equation for the bleaching process using H_2O_2 is as follows:



Then the blanched filtrate is added with 10% HCl which serves to bind alginate to alginic acid. Furthermore, for the formation of alginate acid Na-alginate that has been formed added with NaOH solution. The addition of this alkaline solution aims to obtain alginate in a more stable form, namely Na-alginate. In this process there is an exchange of H^+ ions with Na^+ ions. Na-alginate purification is then carried out using Isopropyl alcohol which aims to attract Na-alginate which is contained in Na-alginate solution so that the sediment can be separated. Furthermore, the sediments obtained were dried using an oven at 60 °C for 48 hours to obtain dry powder.



Figure 1. Extract of Na-Alginate Powder

Na-alginate powder obtained in this study has a golden yellow physique. The golden yellow colour produced is obtained from the bleaching process. The bleaching process is done by adding H_2O_2 to the extracted filtrate. Blanching with H_2O_2 compounds should result in degradation of physical colour to white. This is because Na-alginate which has a food grade quality must be free of cellulose and the colour has been bleached to be bright or white. But besides the grade, there is also something called Industrial grade that still allows for some parts of cellulose, with colours from brownish to white. Brown algae and Habitat types will affect the use of H_2O_2 used in the bleaching process. This can be caused by the pigment content of different brown algae. Na-alginate powder obtained from extraction can be seen in **Figure 1**.

4.2 Characterization of Edible films

The formation of edible film is done by determining the optimum composition of whey protein and Na-alginate which is used by comparing the composition of the two ingredients. The preparation process for making edible film is done by converting Na-alginate to Ca-alginate first with the addition of $CaCl_2$. The addition of $CaCl_2$ serves to convert Na-alginate to Ca-alginate. Alginate can form a stable gel with the addition of divalent or trivalent metal ions. $CaCl_2$ will donate Ca^{2+} Ion which is used in the formation of Na-alginate edible film. The exchange of Na^+ ions in Na-alginate with Ca^{2+} ions forms Ca-alginate. Ca-alginate obtained in the form of a gel that is not soluble in water. In addition, the addition of $CaCl_2$ caused crosslinking due to the presence of chelate complexes between Ca^{2+} ions and carboxylic anions. The formation of chelate complexes results in Ca-alginate produced being more hydrophobic than Na-alginate because it is less likely to form hydrogen bonds with water. Then the formed Ca-alginate is added with glycerol which functions as a plasticizer. Addition of glycerol can cause elastic or flexible properties of edible film because of the decrease in intermolecular attraction so that the edible film produced will not be rigid. Based on data from the Material Safety Data Sheet (MSDS) the maximum concentration of glycerol which is allowed to be added to food ingredients is 10 mg / m³. Addition of glycerol above the maximum concentration can cause edible films to be easily damaged. The equation of the reaction between Ca-alginate after the addition of glycerol can be seen in **Figure 2**.

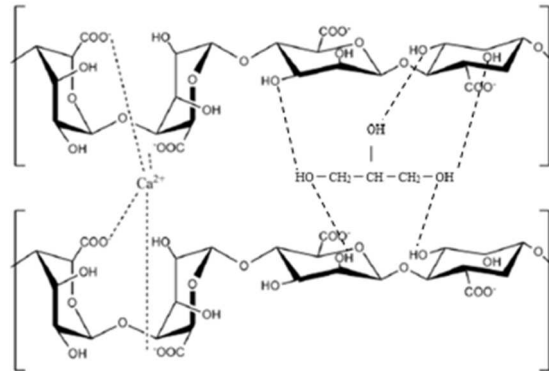


Figure 2. Ca-alginate with glycerol Plasticizer Reaction Equation

After that, whey protein was added as an additional biopolymer. The addition of whey protein aims to improve the characteristics of edible film both physically and mechanically. In addition, the addition of whey protein is expected to reduce the hydrophilic properties of edible films from the addition of glycerol and improve hydrophobic properties. In the process of adding whey protein this heat denaturation is needed because whey protein has a globular structure. This heat denaturation will give rise to and stimulate intramolecular peptide bonds, ionic bonds and hydrogen bonds that have hydrophobic groups which when exposed to these chains will cause the protein to be hydrophobic and constitute the structure of whey edible protein film [5]. The formation of edible film structures requires the role of intermolecular peptide bonds so that later edible films produced by adding whey protein will be difficult to dissolve and the integrity of the film on coated food products will be maintained. The bond formed will make the film transparent and soft, but the absence of plasticizers will make the film brittle. Cross polymerization of whey protein that causes protein gelatinisation in plasticizer materials. Therefore, the addition of biopolymers that have more hydrophobic properties will also help improve the mechanical properties and water resistance of edible films produced [6]. From the results it is known that film formation ability is influenced by amino acid composition, polarity, conditions needed for ionic cross-link reactions between amino and carboxyl groups, hydrogen bonding groups and intramolecular and intermolecular disulfide bonds.

The process of printing edible film was carried out on a glass plate with the same plate length and the same thickness of edible film printing. Then oven is done for 24 hours at a temperature of 60°C. The edible film produced in this study is yellow and has a slightly rough texture as can be seen in **Figure 3**. The morphological form of edible films such as texture and colour greatly influence the appearance of edible films produced. This is because the colour of the edible film is influenced by the colour of the base material used. In addition, the slightly rough texture of edible film can be due to the obtained Na-alginate granules that are not uniform in size, thus causing not homogeneous Na-alginate granules during the stirring process. This has an impact on the texture of the edible film obtained is less attractive.



Figure 3. *Edible film* (1) Ca-alginate+WP5%, (2) Ca-alginate+WP7%, (3) Ca-alginate+WP9%, (4) Ca-alginate+WP11%, (5) Ca-alginate non *Whey*

4.2.1 Thickness of Edible films

The thickness of edible film is a parameter of physical properties that is very important because it affects the function of edible film itself, which is to package or coat the product. Thickness will affect the water and gas vapor transmission rate, tensile strength and elongation of edible film. This thickness testing was carried out at the Physics Laboratory of UIN SGD Bandung. The higher the thickness, the more edible the stiffness of the resulting film. The thickness of edible film is influenced by the mold are, the volume of solution and the total amount of solids in the solution [7].

The results of the study with the influence of the composition of whey and Na-alginate proteins on the thickness of edible film can be seen in **Table 1** below:

Table 1. The Average Value of Thickness of Edible Films with Variations in the Composition of Whey and Na-Alginate Proteins

<i>Edible film</i> Sample	Thickness (mm)
Ca-alginate non <i>Whey</i>	0,014 mm
Ca-alginate + <i>Whey</i> 5%	0,015 mm
Ca-alginate + <i>Whey</i> 7 %	0,015 mm
Ca-alginate + <i>Whey</i> 9 %	0,015 mm
Ca-alginate + <i>Whey</i> 11 %	0,015 mm

Based on **Table 1** the thickness of edible film increases with increasing whey protein composition. The highest thickness value is 0.015 mm. Based on Japanese Industrial Standard the maximum standard for edible film thickness is 0.25 mm so that edible films with variations in whey protein composition have met these standards.

4.2.2 Water Absorption

Water resistance analysis aims to determine the value of the resistance of materials to water. This analysis is one of the important factors in food packaging materials, because the value obtained is closely related to the shelf life of food products and biodegradability of films. The tests carried out are through the absorption of edible film on water (water uptake). The types of edible film constituents, the concentration of plasticizers and additional biopolymers used will influence the absorption properties of edible film on water. Testing of water resistance was carried out at the Chemical Laboratory UIN SGD Bandung. The following is the percentage of water absorption obtained in this study can be seen in **Table 2**.

Table 1. Water Absorption Edible Film Resistance Test Results

Sample	Water Absorption (%)
Ca-alginate non WP	112,08 %
Ca-alginate + WP 5 %	89,65 %
Ca-alginate + WP 7 %	78,35 %
Ca-alginate + WP 9 %	74,71 %
Ca-alginate + WP 11 %	63,62 %

Based on the data obtained the value of the level of absorption of water on edible film with the addition of whey protein and without whey protein has a very significant difference decreasing. Where, the higher the whey composition is added, the lower the absorption value of the water will be. Edible films without whey have a large absorption compared to the addition of whey. This is because edible film has hydrophilic properties which cause it to be less effective as a barrier to moisture. While the addition of whey protein tends to increase the resistance of edible film water, this is because the addition of whey protein by combining lipids in the emulsion can cause edible film to be effective as a barrier to moisture because whey and lipid proteins are hydrophobic substances that can improve the hydrophobicity of edible films. Comparison of the value of water absorption of each composition can be seen clearly in **Figure 4**

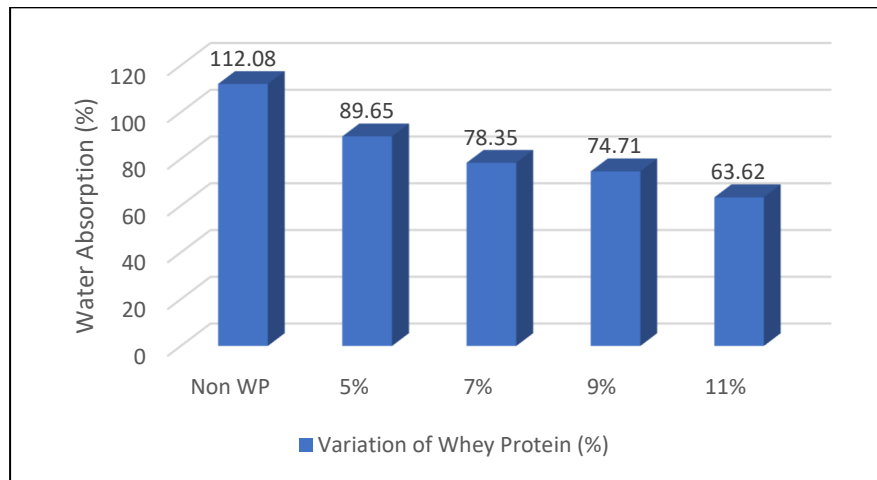


Figure 4. The relationship of Whey Protein Variations to the Water Absorption of Edible Film

In the process of the formation of Ca-alginate there is a possibility that Na-alginate which binds to Ca^{2+} ions has not formed a perfect complex which causes a lot of empty space so that the mass density between chains in Ca-alginate is not too large and still carry the hydrophilic nature of Na-alginate which causes water absorption is quite large. The addition of whey protein can increase the mass density of edible films and cause the amount of water absorbed to be smaller. The empty space will be filled by whey protein which has hydrophobic properties so that the inter-chain bonds in the edible film are more tightly tight and make water resistance higher.

4.2.3 Mechanical Properties

The mechanical characteristics of edible films can be studied based on three parameters, among others; tensile strength, elongation, and elasticity. These parameters can explain how the mechanical characteristics of edible film are related to their chemical structure. Mechanical characteristics show an indication of the integration of edible film under stress conditions that occur during the formation process mechanical properties are influenced by the formulation of edible film constituents namely, Na-alginate, CaCl_2 , glycerol and whey protein. The mechanical test results of Edible film can be seen in **Table 3.** below:

Table 2. Edible film mechanical test results

Sample	Tensile Strength (MPa)	Elongation (%)	Elasticity (MPa)
Ca-alginate + non WP	8,93	27,97	31,93
Ca-alginate + WP 5 %	10,74	16,08	66,79
Ca-alginate + WP 7 %	14,11	25,66	54,99
Ca-alginate + WP 9 %	15,63	28,98	53,93
Ca-alginate + WP 11 %	17,59	35,84	49,08

a. Tensile Strength

Tensile strength indicates the maximum value of the force produced when pulling until the edible film persists before breaking or tearing. The higher the force produced, the greater the tensile strength. Edible films with high tensile strength will be able to protect the products they pack from mechanical interference well. Based on **Table 3.**, the tensile test value of edible film was varied, whey protein composition varied with increasing concentration of whey protein added. This can be seen in the graph of the relationship between the variation of whey protein to the tensile strength of the edible film shown in **Figure 5** below:

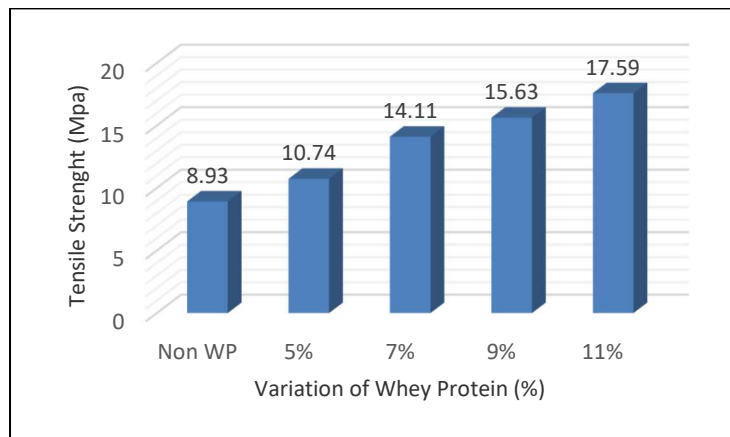


Figure 5. The Relationship of Whey Protein Variations to the Tensile Strength of Edible Films

From **Figure 5.** it can be seen that the higher composition of whey protein will cause the gel to be more stable so that the value of tensile strength obtained will be even greater because the edible film produced will be more rigid. The higher the whey composition is given, the higher the tensile strength, because the number of particles in the edible film is getting denser so the space between the molecules is getting smaller which causes higher tensile strength. Edible films with high tensile strength will be better at protecting products that are packaged from mechanical disturbances (withstand mechanical damage). The resulting tensile strength increases with the addition of whey protein, this can be caused by tightly bonding between chains so that it is harder to break, the use of concentrations of whey protein used will also affect the tensile strength of the edible film produced. Based on **Table 4** the tensile strength produced

in this study is 8.93-17.59 MPa. While the value of commercial tensile strength of edible film is 15.53 MPa. In the Japanese Industrial Standard, it is stated that the range of tensile strength values that can be applied to standard edible films is between a maximum of 40 MPa. Thus, the value of the tensile strength of edible films produced in this study falls into a predetermined standard value.

b. Elongation

Percent extension or elongation shows the maximum stretch of edible film ability. From the results of the study it was found that the value of elongation of edible film with variations in the concentration of whey protein had increased, this was due to the increase in the concentration of whey protein increasing the value of elongation, making the film matrix flexible. This can be seen in the graph of the relationship between the variation of whey protein to the elongation of edible film shown in **Figure 6**

Based on **Table 4** the strength of elongation produced in this study is 27.97–35.84. Kroctha and Johnston (1997) state that percent elongation or percent extension for standard edible films ranges from 10–50%. Thus the value of the edible film extension produced in this study has reached the standard edible film value.

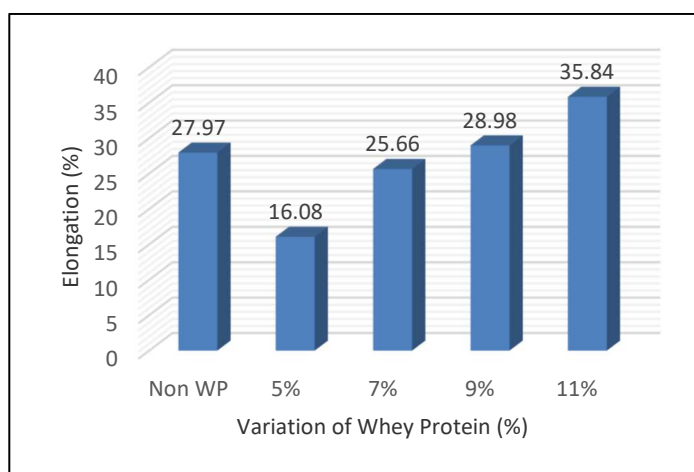


Figure 6. The Relationship of Whey Protein Variations to the Elongation of Edible Film

In this study the value of the extension of each edible film all entered into the standard value. The higher the whey composition is given, the greater the elongation, because the number of particles contained in the edible film is getting looser, the greater the space between the molecules that causes greater elongation [8].

But with the increasing percentage of elongation the weaker edible film is formed because it is easy for edible films to stretch and extend, this can cause the surface of the film to be thinner so that the edible film produced is more easily torn or damaged. Therefore, this can be corrected by adding glycerol. With the addition of glycerol, it can increase the elongation value so that the fragility of edible film decreases and its permeability increases [9]. The greater the addition of the plasticizer, the greater the percentage of elongation [7].

c. Elasticity

In testing the elasticity it will be known is the stiffness level of an edible film. Stiffness level can be known by measuring the elasticity of the edible film. From the calculation results, it can be seen that the small modulus value indicates that the edible film is elastic. This can be seen in the graph of the relationship between the variation of whey protein to the elasticity of edible film shown in **Figure 7** below

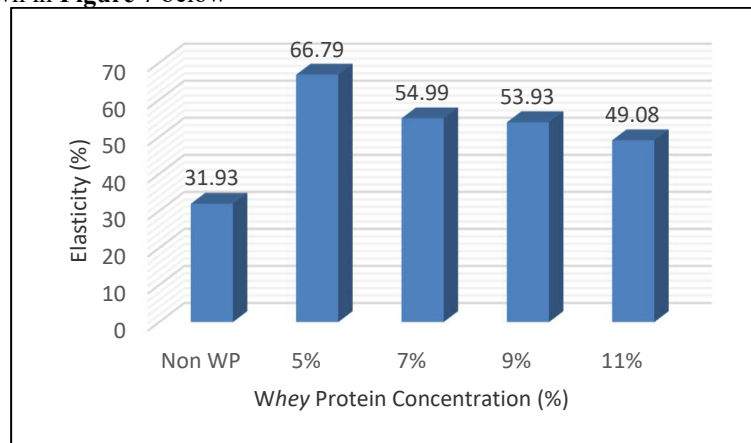


Figure 5 The relationship of Whey Protein Variations to the Elasticity of Edible Film

The elasticity is the opposite of the elongation because it will decrease with increasing amounts of whey protein. Elasticity which decreases means edible flexibility will increase. So that the elasticity value of Ca-alginate + WP 5% has high stiffness so that its nature is not too flexible [10].

4 Conclusion

The addition of whey protein causes a decrease in the value of water absorption from a concentration of 5%; 7%; 9% and 11% respectively 89.65%; 78.35%; 74.71% and 63.62%. Furthermore, the tensile strength values increased from a concentration of 5%; 7%; 9% and 11% respectively of 10.74 MPa; 14.11MPa; 15.63 MPa and 17.59 MPa. As well as the elongation value decreased at a concentration of 5% and 7% by 16.08% and 25.66%, then an increase in the concentration of 9% and 11%, namely 28.98% and 35.84%. While the elasticity has increased from a concentration of 5%; 7%; 9% and 11% respectively of 66.79 MPa; 54.99 MPa; 53.93 MPa and 49.08 MPa.

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