Preliminary Study of Synthesis and Characterization of Biodegradable Avocado Seed Starch-Chitosan Plastic with Glycerol Plasticizer

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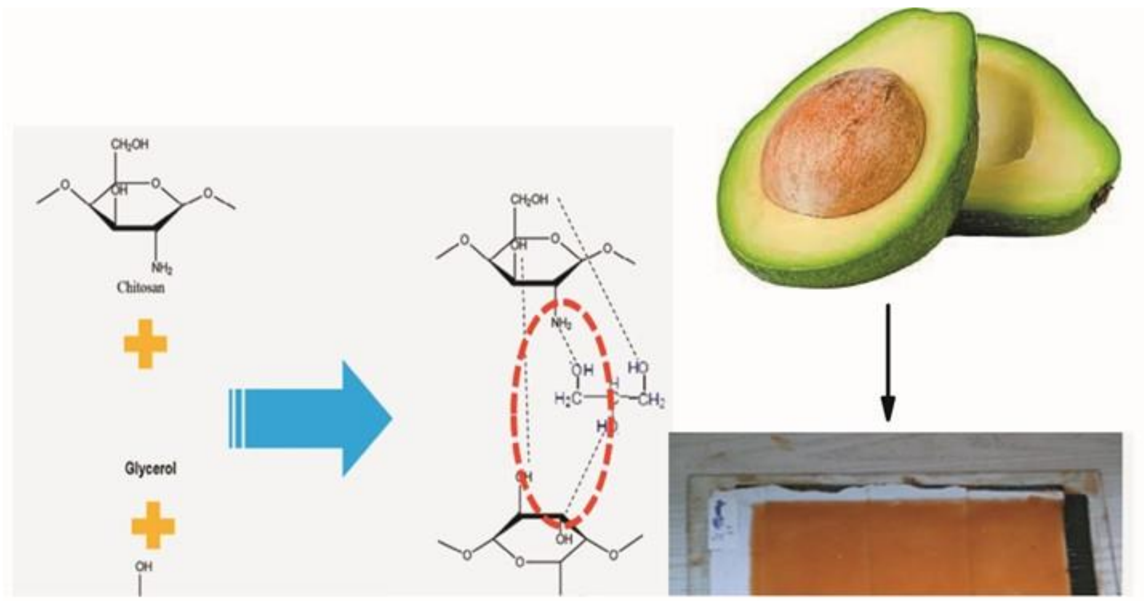
GRAPHICAL ABSTRACT



Preliminary Study of Synthesis and Characterization of Biodegradable Avocado Seed Starch-Chitosan Plastic with Glycerol Plasticizer

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Mechanical characteristics were studied for biodegradable plastics derived from avocado seed starch, emphasizing the influence of chitosan and glycerol. The extraction of starch from avocado seeds began with filtration. Different amounts of glycerol and chitosan were added to the extracted starch of up to 3% by weight. Because this method just uses distilled water instead of chemical chemicals, it was highly environmentally friendly. Tensile testing was done on the generated samples to assess the mechanical properties of this bioplastic; the results indicated that the samples' elongation rates ranged from 21.9% to 134.7%. In spite of this, the bioplastic showed a comparatively low tensile strength, peaking at 3,381 MPa when chitosan-starch ratio was 2:1 and 1.5% glycerol was added.

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Keywords: Mechanical properties; Bioplastic; Avocado seed starch

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INTRODUCTION

Plastic is a material that is widely used in human life today. The use of plastic is very diverse, for example, as food wrappers, various household furniture, and even automotive (Aisyah *et al.* 2021). The widespread choice to employ plastic material is due to the advantages of plastic, which are light, flexible, and water resistant (Syafri *et al.* 2019; Yaradoddi *et al.* 2022; Mashuri *et al.* 2023). Figure 1 shows some groups of sintetic polymers and their application. It seems obvious that polymer applications play a dominant role in packaging, *i.e.*, 39.4% (Coppola *et al.* 2021).

On the other hand, this massive use of plastic certainly creates problems, especially for the environment. Furthermore, plastic is a material that is difficult to decompose and some even take hundreds of years to decompose in the environment. This of course causes environmental pollution if the material fails to be efficiently recycled or reused. One solution to this problem is to make environmentally-friendly plastics (bioplastics). Bioplastics are plastics in which all or almost all of their components come from renewable raw materials (Asyraf *et al.* 2022; Mohammed *et al.* 2023). Though they are made from plant materials, it is well known that not all bioplastics are biodegradable under typical conditions in soils or waters (Bhatia *et al.* 2021; Yamada *et al.* 2020).

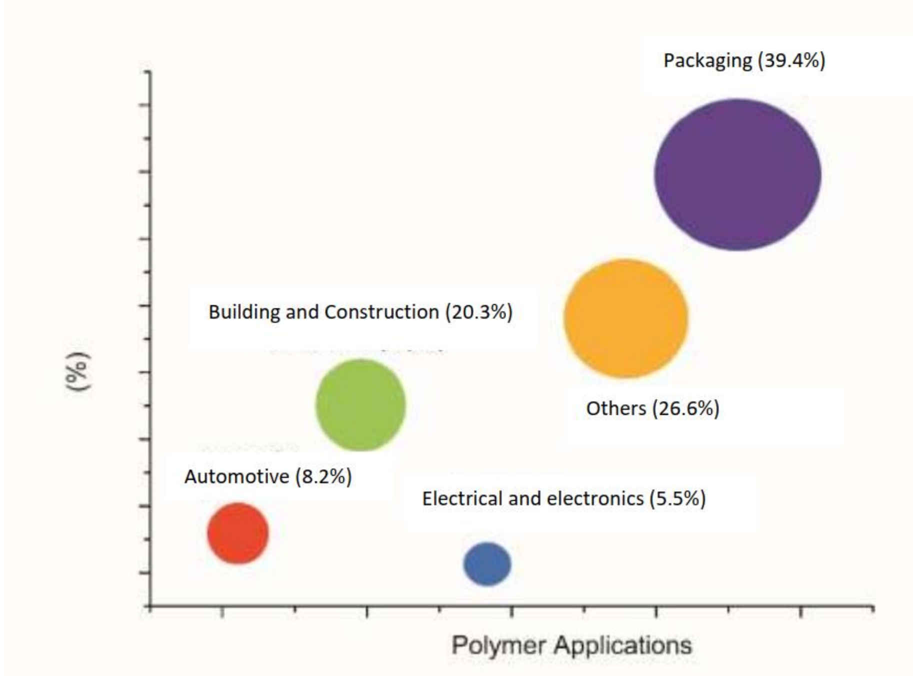


Fig. 1. Typical applications of polymers

One of the materials used in the manufacture of bioplastics is starch. Starch is a polymer of d-glucose and is found as a storage carbohydrate in plants. Starch is in the form of small grains of various sizes and shapes that are typical for each plant species (Maniglia *et al.* 2021). Starch is the most versatile and abundant polysaccharide on earth. Starch is obtained quite easily because it is abundant and the price is cheap. According to Winarti and Purnomo (Winarti and Purnomo 2006), avocado seeds contained 80.1% starch with 43.3% amylose and 37.7% amylopectin. This high starch content has the potential to be used as a raw material for bioplastics. The use of avocado seeds was intended to reduce waste from avocado seeds and maximize the utilization of avocado seed waste to be more efficient.

Another material used in the manufacture of bioplastics is chitosan. Chitosan is a non-toxic biodegradable polymer with a high molecular weight. Chitosan is a promising renewable polymer material for wide application in the pharmaceutical and biomedical industries as an immobilized enzyme (Azmana *et al.* 2021). Chitosan is used in wastewater treatment and the food industry as food formulations such as binders, gelling agents, emulsifiers, and stabilizers (Priyadarshi and Rhim 2020). Because it has a positive ionic charge, chitosan is a fascinating natural biopolymer that sets itself apart. Chitosan's positive charge creates interesting opportunities for interactions with negatively charged polymers. For example, interactions between oxidized starches and chitosan might result in the creation of polyelectrolyte complexes. These complexes are more than simply a simple mixture of components; they provide an intriguing synergy that can greatly improve the final substances' qualities. The interactions between the positive and negative charges can result in improved mechanical strength, solubility, and even bioactivity of the materials. This unique capability of chitosan to form complexes with various polymers expands its potential applications in fields such as biomedical engineering, food packaging, and environmental science. As researchers continue to explore these interactions, the versatility and utility of chitosan in developing innovative solutions become increasingly evident.

Various studies have been carried out to develop bioplastics as a solution to environmental problems. Darni (2011) studied sorghum-based bioplastic synthesis and showed the best formulation at a stirring speed of 375 rpm. This resulted in a percent elongation of 19.3% and a tensile strength of 143 MPa. In other work, Ren *et al.* (2017) demonstrated the effect of the addition of chitosan on the elongation of corn starch bioplastics. The elongation at break of the corn starch/chitosan films increased with more chitosan. It reached an optimum at 41 wt%, then dropped at higher chitosan addition. In the earlier work of Sinaga *et al.* (2014), the role of glycerol on tensile strength and elongation of putua bioplastic from taro bulb starch exhibited the best mechanical properties at 1% glycerol, *i.e*., tensile strength 18.5 MPa and elongation at break 2.1%. In addition to the mechanical properties that must be considered in the preparation of bioplastics, the ability to be degraded is also very important to explore. Thus, in 2021, the authors analyzed the effect of adding glycerol and chitosan to bioplastics from avocado seeds on the biodegradable (Devi *et al.* 2021). The mechanical strength obtained was able to increase by 10% from pure chitosan, namely 1.38±0.05 MPa with a composition of 3:1 for chitosan:avocado starch. The prepared bioplastics were degraded for 60 days. However, further testing related to its physical properties has not been carried out. Whereas the addition of chitosan and glycerol greatly affects the mechanical properties of bioplastics, the mechanical properties of plastic from avocado seed starch will be investigated further.

So that a thorough study of bioplastics from avocado seed starch will be obtained as alternative biodegradable packaging, and as a follow-up study from previous research (Devi *et al.* 2021), this study sought to understand mechanical attributes of avocado seed starch-based bioplastics, considering the effects of chitosan and glycerol additives.

EXPERIMENTAL

Materials

The purpose of this research was to produce alternative biodegradable packaging using avocado seeds. In this study, avocado starch was extracted from mashed avocado seeds. The type of avocado seeds used was Wina avocado, a type of butter avocado with shiny and thick green skin. At its core is a buttery type of Avocado pit with thick, glossy green skin. Chitosan was purchased from HiMedia Laboratories Pvt.Ltd ($(C_6H_{11}NO_4)_n$ Molecular Weight: 3800 to 20000 Daltons; degree of deacetylation ≥75%). Glycerol 85% was produced by Merck (Glycerol CAS 56-81-5 anhydrous (vegetable) EMPROVE® EXPERT Ph Eur, BP, JP, USP, ACS).

First, avocado seeds were blended using distilled water as a medium. The ratio of distilled water and avocado seeds was 2 liters and 1 kg, respectively. Starch filtrate was obtained by separating starch precipitates and suspends. In this study, the starch suspension that passed the filter was deposited for 1 h to obtain a starch filtrate. Then, the starch filtrate was dried in an oven at 100 °C for 24 h. From previous studies (Devi *et al.* 2021), the starch content of synthesized avocado seeds was 76.9%.

Furthermore, the avocado starch obtained was processed into bioplastics. This process is similar to what has been done in previous studies (Devi *et al.* 2021). Avocado starch solution (mixed with distilled water) was mixed with chitosan solution (chitosan dissolved in 1% acetic acid) with the ratio of chitosan to starch composition 3:1; 2:1; 1:1;

1:2; 1:3. Then, glycerol (1; 1.5; 2; 2.5; 3 %) was added in a variation of the mixture and stirred at 65 °C stirring for 70 min. The plastic film mixture was then molded and cooled at room temperature for 24 h. Figure 2 shows the representative of molded bioplastic. To understand the morphology and distribution of bioplastics, images are taken using an optical camera. Optical images were obtained by a microscope-mounted digital camera (Digitus-DA-70351) with 10 times magnification.

Mechanical Test

Mechanical tests were carried out to determine tensile strengh and elongation using autograph (Shimadzu Autograph). This method is based on JIS-L1015 and R-7601. Here, the bioplastic sample was prepared with the dimensions 6 cm × 1 cm. Clamps were used to secure the sample at both ends. From the tensile test, information about the tensile strength and elongation of the bioplastic will be obtained. The calculation (Eq. 1) was as follows:

Tensile strength (MPa)

$$\sigma = \frac{force\ (F)}{Area\ (A)\ x\ 100} \tag{1}$$

In Eq. 1, σ is the tensile strength (MPa), F is the force (N), and A is the area (m^2).

Elongation (%)

The elongation measurements were carried out in the same way as the tensile strength test. Elongation is expressed as a percentage (Eq. 2),

$$\varepsilon\ (\%) = \frac{l - lo}{lo} x\ 100\% \tag{2}$$

where ε is the elongation (%), l is the length after break up (m), and l_o is the initial length (m).

Fig. 2. Representative example of a molded bioplastic

RESULTS AND DISCUSSION

As a sequel of the previous results (Devi *et al.* 2021), it is known that the higher the content of chitosan, the slower the degradation process, while the higher the composition of starch and the percentage of glycerol, the faster the biodegradation process. Thus, bioplastics in chitosan composition variations 1:3 starch can be completely decomposed for 60 days in the EM4 degrading medium. Furthermore, the mechanical characteristics (tensile strength and elongation) will be detailed as follows.

Tensile Strength

Figure 3 shows the effect of the chitosan composition to starch with the percentage of glycerol on the tensile strength of bioplastics. The more composition of chitosan, the greater was the value of the tensile strength.

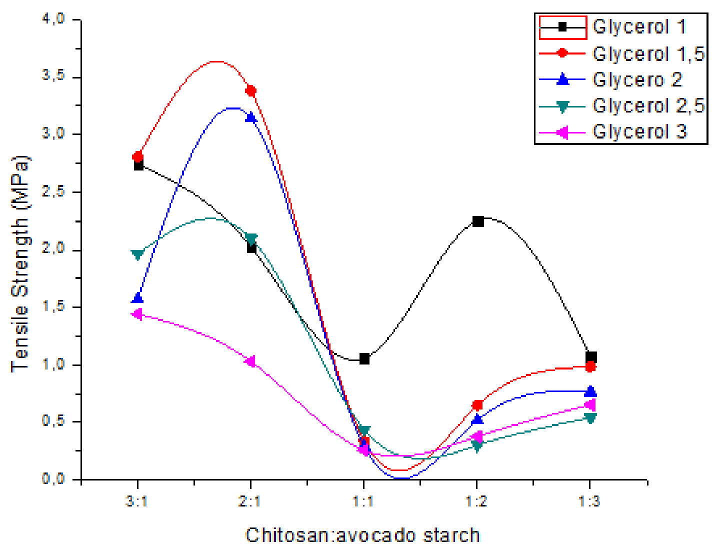


Fig. 3. Tensile strength of bioplastics to chitosan composition and percent of glycerol

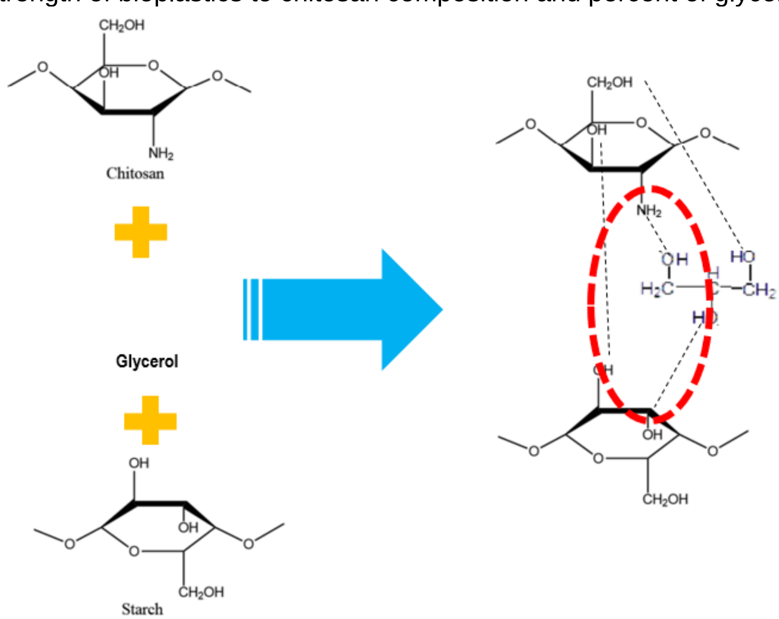


Fig. 4. Formation of Hydrogen bonds between chitosan and avocado starch

Based on Fig. 3, the significant increase of tensile strength was shown by the 1:2 chitosan:starch composition with the addition of 1% glycerol. Additionally, the coherent improvement pattern in tensile strength was also exhibited by the composition of chitosan:starch 1:2 and 1:3 with the addition of glycerol 1.5% to 3%. This phenomenon is attributed to the higher addition of chitosan, which is expected to yield harder bioplastics. However, different results were seen in bioplastics with 2:1 composition of chitosan:starch with the addition of 1.5 and 2% glycerol. The composite with 1:1 composition had the lowest tensile modulus value. This corresponds to the tensile strengths of each composite material, *i.e.*, the tensile strength of avocado starch has been reported as 19.37±5.31 MPa (Jiménez *et al.* 2021), while the tensile strength of chitosan is 1.08±0.44 MPa (Wang *et al.* 2005). Avocado starch provides extra strengthening, which results in a rise in the composite's tensile modulus in compositions above 1:1. The results shown in Fig 3 reveal an obvious anomaly. This anomaly may be due to inhomogeneous mixing. More specifically, as explained by Chen *et al.* (2019), the increase tensile strength can be attributed to hydrogen bonds in the dominating chitosan:starch composition. The $-NH_2$ groups on the chitosan would tend to form weaker hydrogen bonds (Navarro *et al.* 2019).

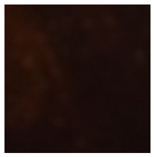
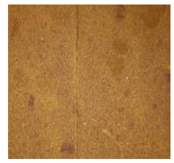
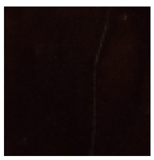
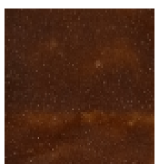
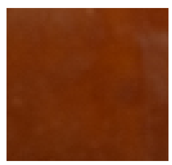
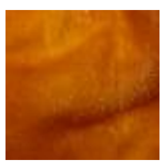
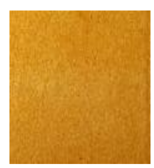
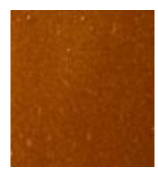
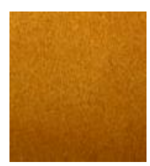
Initially, chitosan will form hydrogen bonds with avocado seed starch. This formation of hydrogen bonds is in line with previous research (Susilowati and Lestari 2019; Cruz-Balaz *et al.* 2023). Hydrogen bonds are formed when the amino group from chitosan and the hydroxyl group from starch interact (Fig. 4). However, this hydrogen bond will make the bioplastic more brittle. Therefore, to improve the performance of bioplastics, glycerol is added to the bioplastic as a plasticizer.

In the current study, recognizing the effect of chitosan and glycerol can be seen from Fig. 4. In the natural, starches are fragile and difficult to be processed. Thus, there is a need for additives to make it easier to process bioplastics from starch. Futhermore, the role of glycerol as plasticizer is to improve the flexibility, processing, and workability of polymers. From the literature (Navarro *et al.* 2019), glycerol will intersperse between polymer chains, break hydrogen bonding and distribution the mobility of the polymer chains apart, which enhance flexibility. This causes the plastic film to be tougher and more difficult to break (Bajer *et al.* 2020).

In more detail, if one looks at Fig. 3, there were contrary results from previous results. The tensile strengh decreased in the composition of chitosan:starch 3:1 with the addition of glycerol 1.5% to 2.5%. This leads to the imperfect mixing process of chitosan. The less homogeneous mixing process resulted in an uneven distribution of molecules making up the bioplastics.

The bonds formed between starch and chitosan, glycerol and starch, or even chitosan and chitosan, play a crucial role in enhancing the properties of biopolymer composites. When chitosan, with its unique positive ionic charge, interacts with starch, which typically carries a negative charge, they can create polyelectrolyte complexes. This interaction is not merely physical; it often involves the formation of hydrogen bonds between the hydroxyl (-OH) groups present in both starch and chitosan. These bonds contribute to the overall stability and functionality of the resulting material. Similarly, when glycerol is introduced into the mix, it acts as a plasticizer, facilitating the formation of additional bonds with starch. This interaction can improve the flexibility and processability of the starch-based materials. The presence of glycerol enhances the intermolecular interactions, allowing for a more cohesive structure that can withstand various environmental conditions. Thus, the resulting material decreases in tensile strength and is also seen in its rough surface texture which indicates that it is less homogeneous (Table 1).

Table 1. Morphology of Bioplastics as Shown by Optical Imaging

Glycerol (%)	Chitosan : Starch				
	3:1	2:1	1:1	1:2	1:3
1					
1.5					
2					
2.5					
3					

The addition of glycerol also affected the tensile strength of bioplastics (the stress-strain values shows in Table 2, in detail). The addition of glycerol caused the value of the tensile strength to decrease because high amounts of plasticizer cause the material to become elastic with longer strain, thereby reducing the tensile strength of the material (Cazón *et al.* 2018). According to previous research from Wahyudi *et al.* (Wahyudi et al. 2020), the addition of glycerol as a plasticizer, the plasticizer molecules in the solution are located between the biopolymer bond chains and can interact by forming hydrogen bonds in the bond chains between polymers, causing interactions between biopolymer molecules to decrease. This causes a decrease in the tensile strength of bioplastics with the addition of glycerol plasticizer.

The highest tensile strength results in this study were found when the ratio of chitosan : avocado starch was 2:1 with the addition of 1.5% glycerol, with a tensile strength value of 3.381 MPa. The lowest tensile strength results in this study were found in the ratio of chitosan : avocado starch 1:1 with the addition of 3% glycerol with a tensile strength value of 0.258 MPa. The tensile strength value according to the Indonesian National Standard (SNI) is 24.7 to 100 MPa for bioplastic (Gabriel *et al.* 2021); thus, the tensile strength value of the bioplastic in this study was not able to meet the Indonesian National Standard. In this research, it does not meet national standards, so reinforcement and agglomeration control are still needed so that the distribution of reinforcement will be more homogeneous and it is hoped that the performance of bioplastics will meet Indonesian national standards.

Table 2. Stress-strain Values from Bioplastics

Glycerol Composition	Ratio of chitosan: Avocado starch	Elongation	Tensile Strength (MPa)
1%	3:1	5.35	2.744
	2:1	19.7	2.024
	1:1	134.7	1.055
	1:2	42.7	2.251
	1:3	12.2	1.074
1.5%	3:1	14.6	2.805
	2:1	15.9	3.381
	1:1	62.4	0.336
	1:2	21.9	0.648
	1:3	16.5	0.982
2%	3:1	12.7	1.568
	2:1	40.3	3.14
	1:1	62	0.29
	1:2	43.1	0.517
	1:3	31	0.765
2.5%	3:1	10.5	1.964
	2:1	20.8	2.098
	1:1	98.2	0.438
	1:2	24.4	0.301
	1:3	23.8	0.54
3%	3:1	17.7	1.441
	2:1	16	1.029
	1:1	79.1	0.258
	1:2	61.1	0.376
	1:3	42.5	0.652

Elongation

Figure 5 describes the effect of the comparison of the composition of chitosan: starch with the percentage of glycerol on the elongation value of bioplastics. If the weight of chitosan is increased or excessive, it will cause a decrease in the value of plastic elongation. This is because the addition of a small amount of chitosan can improve its tensile strength and elongation properties.

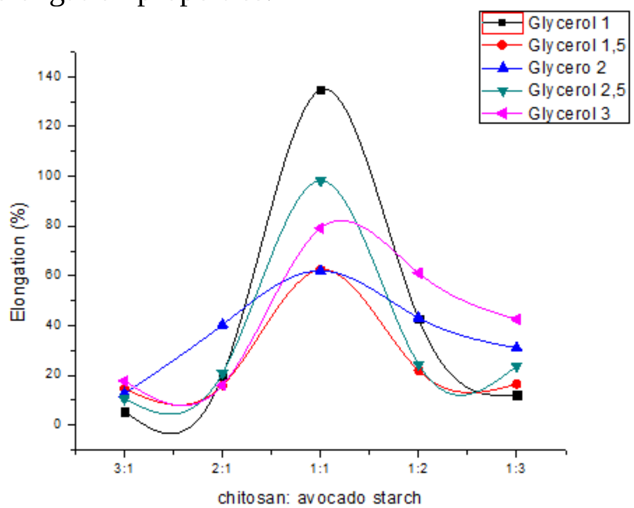


Fig. 5. Elongation (%) of bioplastics to chitosan composition and percent of glycerol

In the percent elongation test, the results of the ratio of chitosan: starch 3:1 increased and decreased at a ratio of 1:1. The elongation test value that goes up and down is caused by several factors, such as less homogeneous mixing so that the insertion of the plasticizer into the composite plastic matrix has not been perfect and the resulting elongation at break is not optimal (El-Hadi 2017). Nofianto (2019) reported that the variation of chitosan added to bioplastics had an effect on decreasing the elongation value (Nofianto 2019). The concentration of dissolved chitosan affects the number of hydrogen interactions in bioplastics.

In addition, chitosan has a linear polymer chain structure, where the linear chain structure tends to form a crystalline phase because it is able to arrange regular polymer molecules (Fig. 4). The crystalline phase can provide strength, stiffness, and hardness. However, it also causes the bioplastic film to become more brittle so that it is easy to break. Therefore, with the addition of more chitosan, the elongation value will decrease. The addition of glycerol can make the bioplastic film more elastic so that the elongation value can increase. Glycerol, which functions as a plasticizer, the two polymers have less interaction directly with each other, which makes the mixture less stiff. This reduces the hydrogen bonds between chitosan-starch and is replaced by hydrogen interactions between chitosan-glycerol and glycerol-starch according to the illustration above. Thus, the bioplastic will be more elastic so that the elongation value tends to increase.

The results of the highest percent elongation can be seen for the composition of chitosan: starch by 1:1 with 3% glycerol, which was 135%. Meanwhile, the lowest percentage of elongation can be seen for the composition of chitosan: starch by 3:1 with 1% glycerol, which is 5.5%.

The uneven surface of the bioplastic resulted in increased elongation when the force was not too large. In addition, the drying process also has an effect, if the bioplastic is too dry, the increase in length of the bioplastic when given a force will be very small in value. During the drying process, the bioplastic is not evenly distributed (Yamada *et al.* 2020).

CONCLUSIONS

Avocado starch-chitosan bioplastics added with different plasticizer composition (glycerol) were studied in this paper. The higher the chitosan composition, the greater the tensile strength value, while the higher the starch and glycerol composition, the lower the tensile strength value. The tensile strength values were inversely proportional to the elongation values. Among the prepared bioplastics, the ratio of chitosan:avocado with the addition of 1.5% glycerol, exhibited the highest tensile strength, *i.e.*, 3.38 MPa. Meanwhile, the elongation value of all sample variations exhibited a value range of 21.9% to 135%. All these results confirmed that produced bioplastics had met the SNI standard. The bioplastics produced from this research produce bioplastics that can be degraded.

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