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Film (patch) based on starch compound hydrolise by amylase from saliva and bacteria

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Abstract. Research on the determination of amylase enzyme activity on hydrolysing film (patch) made from sugar palm starch with the addition of STPP and Temu putih (*curcuma zedoaria*) freeze drying powder have been done. The purpose of this study was to determine amylase enzyme activity of saliva and amylase from bacteria in hydrolysis of sugar palm starch and edible film made from palm starch with the addition of STPP and Temu putih freeze drying powder. Amylase enzyme activity was analysed using UV-Vis spectrophotometer and DNS reagent. Determination of amylase enzyme activity in hydrolysing edible film was carried out by reacting enzyme with various edible film at optimum condition. The result of amylase enzyme activity from saliva and bacteria was obtained at temperature 35 °C, pH 7, substrate concentration 2.5%, and incubation time 95 min. The enzyme was reacted in various concentration against edible film at optimum condition. Amylase enzyme activity of saliva gave optimum edible film hydrolysis in the addition of STPP of 10.26 unit/mL, whereas the best enzyme activity of amylase from bacteria achieved with the addition of STPP and 3% freeze drying powder of Temu putih at 23.09 units/mL. Data analysis using one-way analysis of variance obtained F-value less than F-table at significance level 0.05. The result implied there is no significant difference on amylase enzyme activity from saliva and bacteria when it used to hydrolyse sugar palm starch and edible film from sugar palm starch with the addition of STPP and Temu putih freeze drying powder.

1. Introduction

Starch basically can be hydrolyzed by amylase enzyme produced by human saliva or other living creatures isolate. Starch industry use amylase enzyme to hydrolyze polysaccharide into simple sugars. Amylase specifically is catalyzing hydrolysis reaction of α 1,4-glicosidic polysaccharide to produce oligosaccharide, maltose and D-glucose. Jayanti [1] reported α-amylase enzyme activity isolated from Bacillus licheniformis able to hydrolyze bran porridge and show high enzyme activity at 40-90 °C and then decrease at temperature above 100 °C. Another result Dida G et al regarding amylase enzyme activity isolated from soil bacteria achieve high activity 0.94 unit/mL at pH 7 and 35-40 °C. High α-amylase enzyme activity at 40 °C, pH 7.5 at 15.76 and 31.11 Unit/mL while at substrate concentration 2%, the enzyme showed activity 8.23 Unit/mL. Enzyme has optimum temperature and pH to obtain optimum result. The deviation from optimum condition would cause reduce in enzyme activity [17].

Sugar palm starch can be used as the basic ingredients for preparing edible film. During the preparation process, granules of starch break down hence the physical and chemical property of palm starch is different. To enhance the physical characteristic as well as functional of the starch film, biopolymer or other similar material must be added into the raw material. Modified starch is a starch resulted from chemical, physical and enzymatical modification and used as food additive as well as non-food application. Here, we reported the addition of sodium tripolyphosphate (STTP) to improve starch physical property. Edible film can be made active anti-bacterial with the addition of antibacterial substance such as curcuma zedoaria. The difference between amylase enzyme from saliva and from bacteria was evaluated through hydrolysis of sugar palm starch and edible film from sugar palm starch with the addition of STTP and curcuma zedoaria freeze drying powder.

2. Materials and Methods

Sugar palm starch and curcuma zedoaria powder were obtained from local resource whereas STTP, sodium sulphate, sodium hydroxide, CMC, glycerol, glucose, buffer solution, liquozyme supra amylase enzyme and DNS reagent were purchased in analytical grade. Amylase enzyme from saliva was obtained from respondence.

2.1. Edible film preparation from sugar palm starch

Sugar palm starch (8 g) was dissolved in 160 mL distilled water and then stirred to obtain starch solution which than added with glycerol 5 mL and CMC powder 3 g. Starch was heated at 70 °C on a hotplate for 30 minutes. The suspension formed was placed on a glass plate and then heated in an oven at 50 °C for 24 hours. Edible film result was collected and stored in desiccator.

2.2. Starch preparation with the addition of STTP

Sugar palm powder as much as 7.5 g were dissolved in 50 mL distilled water and added with STTP 1%. The mixture was stirred, filtered using vacuum filtration and the solid residue was collected. The solid was washed 2 times in 100 mL distilled water and then dried in an oven at 50-60 °C for 12 hours. The result was grinded and reduced its size into a fine powder.

2.3. Edible film preparation from starch with STTP addition

Sugar palm starch (4 g) added with STTP were dissolved in 50 mL distilled water, stirred and after forms a solution was added with 2 mL glycerol and 2 g CMC. The suspension was heated using hotplate at 70 °C for 30 minutes. The suspension was poured on a glass plate and dried in an oven at 50 °C for 24 hours. The product was cooled in room temperature and stored in desiccator.

2.4. Preparation of edible film incorporated with freeze drying powder of Temu Putih (curcuma zedoaria)

Sugar palm starch (4 g) made with the addition of STTP was dissolved in 50 mL distilled water, stirred at 70 °C to obtain starch solution. CMC (2 g) and 2 mL glycerol were added into solution. The gelatinized product was added with freeze drying powder of Temu putih (*curcuma zedoaria*) in various amount 0.5, 1, 2 and 3% repectively. The suspension was poured on a glass plate and the dried in an oven at 50 °C for 24 hours and then cooled at room temperature and finally stored in a desiccator.

2.5. Physical and chemical characterization of edible film made from sugar palm starch: tensile strength and elongation percentage

The tensile strength and elongation percentage was measured by using universal testing machine Zwick/z 0.5 using procedure by ASTM D882-02 (ASTM, 2001).

2.6. Edible film thickness test

Thickness test was conducted by using micrometer manual (Mitutoyo, Japan) 0.001 mm precision. The thickness value obtained was average of 5 times measurement on random position point.

2.7. Water content determination (SNI 01-3182-1992)
Edible film was weighted 3 g placed in crucible porcelain and then heated in an electric oven with temperature control at 105 °C for 5 hours. The sample was cooled in a desiccator to achieve room temperature and then weighted. Sample heating was repeated (3-4 times) until its weight is constant or reduce only by 0.001 g. Calculation of water content was based on following equation:

$$\text{Water content} = \frac{m_0 - m_1}{m_1} \times 100\%$$

where m_0 is initial weight of edible film (g), m_1 is final weight of edible film (g).

2.8. Collecting amylase enzyme extract from saliva
The amylase enzyme was taken directly from a respondent at the same time every day and immediately used for the determination of amylase enzyme activity.

2.9. Enzyme activity determination: glucose standard curve
1% glucose stock solution was made by dissolving 0.5 g of glucose in 50 mL of distilled water. The glucose solution was then diluted to obtain concentrations of 0.01; 0.015; 0.02; 0.025; 0.03 and 0.04 respectively by taking aliquot as much as 100 µ, 150 µ, 200 µ, 250 µ, 300 µ, and 400 µ placed in 10-flask flask mL. Each solution of various concentrations was reacted with 1 mL of DNS reagent, heated at 100 °C for 10 minutes and then cooled in cold water for 10 minutes. The absorbance of the sample was measured at λ 540 nm. distilled water was used as blank. The glucose concentration was determined based on the sample absorbance and converted to concentration using standard solution curve.

2.10. Determination of protein using Biuret method
A total of 4 mL of amylase enzyme taken from bacteria and saliva was added with 5 mL biuret reagent and 1 mL of distilled water. The mixture was then stirred and incubated at 37ºC for 30 minutes. The absorbance measurements were carried out using a UV-Vis spectrophotometer at pre-determined λ_{max} . The distilled water mixed with biuret reagent was used as blank.

2.11. Determination of optimum condition for amylase enzyme activity to break down starch: Optimum temperature
A total of 0.5 mL starch (2% dissolved starch in 0.05 M glycine-NaOH buffer pH 7) was added to the amylase enzyme from saliva and amylase enzyme from bacteria each of 1 mL and 0.5 mL. The starch and enzyme solutions were incubated in an incubator at a temperature varied from 20, 25, 30, 35, 40 and 45 °C for 10 minutes. The reaction was stopped after 10 minutes by adding to each test tube 1 ml of DNS reagent and then diluted with distilled water in a 10 mL measuring flask. The tube is heated at 100o C to boil for 10 minutes, after which the mixture is cooled for 10 minutes. Each solution in the test tube was measured its absorbance using a UV-Visible spectrophotometer at 540 nm wavelength.

2.12. Optimum pH determination
Amylase enzymes from bacteria as much as 0.5 mL and amylase enzyme from saliva as much as 1 mL were put into the test tube. Starch substrate 0.5 mL (2% dissolved starch in 0.05 M buffer glycine-HCl pH 3, 4, 5, 6) was added with 0.05 M glycine-NaOH buffer with variations in pH 7, 8, 9 and 10 as much as 0.5 mL. Each sample was incubated in an incubator using the optimum temperature from the previous results for 10 minutes. 1 mL of DNS reagent was added to each tube. The tube is then heated at 100 °C for 10 minutes, after which it is cooled for 10 minutes. Each solution in the test tube was measured its absorbance at 540 nm using UV-Vis spectrophotometer.

2.13. Determination of optimum substrate concentration
The substrate was prepared at starch concentrations of 0.5; 1; 1.5; 2; 2.5; 3 and 4% dissolved in 0.05 M of optimum pH glycine buffer and then vortexed to homogenize. Amylase enzyme from saliva and bacterial amylase enzyme was added 1 mL and 0.5 mL respectively and then vortexed to homogenize.

All substrates were incubated at optimum temperature and pH for 10 minutes. The reducing sugar products formed were measured by the DNS method. Incubation results were added with 1 mL of DNS solution then heated at 100 °C for 10 minutes and followed by cooling for 10 minutes. 20 mL of distilled water is added to the mixture. The absorbance of the solution was measured at 540 nm using a UV-Vis spectrophotometer.

2.14. Incubation time determination

The bacterial amylase enzyme of 0.5 mL and 1 mL of amylase enzyme from saliva were added into 0.5 mL starch substrate (2% dissolved starch in 0.05 M optimum pH glycine buffer). The mixture was incubated at the temperature according to the optimum condition obtained for various duration i.e. 10, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 95 minutes respectively. Reducing sugar (glucose) products were determined using 3.5 dinitro salicylic acid (DNS). 1 mL of the product was diluted by distilled water in 10 mL measuring flask, then heated at 100 °C for 10 minutes and cooled for another 10 minutes. The absorbance solution was measured at wavelength of 540 nm using UV-Vis spectrophotometer.

2.15. Amylase enzyme activity determination on edible fil hydrolysis

Edible film is crushed, smoothed and dissolved in hot water to be used as substrate. 0.5 mL substrate from optimum condition (2% dissolved starch in 0.05 M optimum pH glycine buffer solution) was added with amylase enzyme from bacteria 0.5 mL and 1 mL amylase enzyme from saliva then incubated at optimum conditions. The reducing sugar product was determined by adding 1 mL dinitro salicylic acid (DNS) and then diluted in a 10 mL measuring flask. The mixture was heated at 100 °C for 10 minutes then cooled to room temperature for 10 minutes. The sample absorbance was measured using a UV-Visible spectrophotometer at a wavelength of 540 nm.

3. Results and Discussion

Edible film containing palm starch is formed as a thin sheet of clear white, hard textured and not too flexible. In terms of appearance, the results obtained with the addition of STPP and the addition of freeze-drying powder of curcuma zedoaria shows no significant difference with edible film from sugar palm starch (no addition of STTP). The results of edible film made from sugar palm is shown on Figure 1.

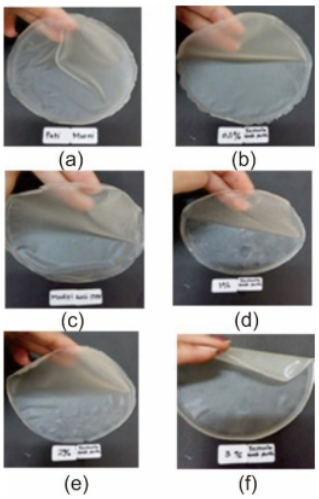

Figure 1. Film (Patc) from sugar palm starch (a) sugar palm starch only (control), (b) with STPP addition, (c) 0.5% (Temu Putih/*curcuma zedoaria*) addition, (d) 1% Temu Putih, (e) 2% Temu Putih, (f) 3% Temu Putih

3.1. Test Results for Physical and Chemical Properties of Edible Film: Tensile Strength Test

Tensile strength testing is done to determine the magnitude of the force achieved in the maximum pull in each unit of edible film area to stretch or elongate. The results of the analysis of tensile strength testing of edible film are display in Figure 2.

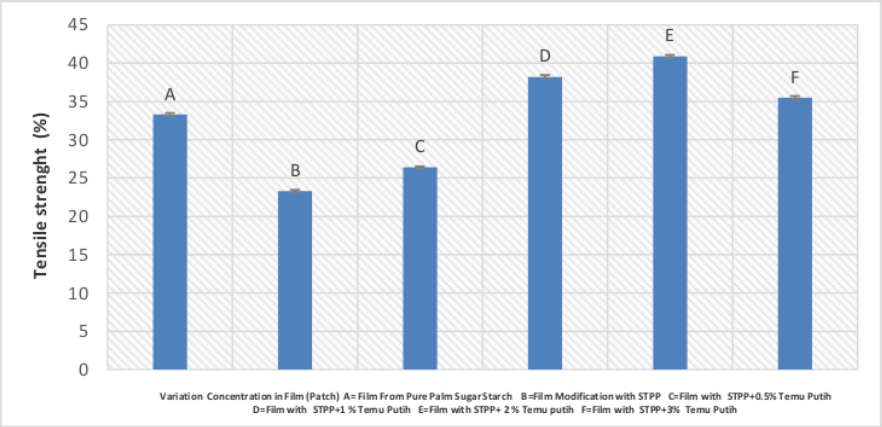

Figure 2. The Effect of Composition in Edible Films on the Tensile Strength of Edible Film

The results obtained from testing the tensile strength of edible films showed that at 1% addition of STPP there was an increase of 44% compared to palm starch. Chemical crosslinking substances can increase tensile strength due to the formation of new bonds between starch molecules [8]. However, the addition of freeze-drying powder of curcuma zedoaria at 0.5% to 3% causes a decrease in tensile strength level of 10-20%. This is probably due to the fact that Temu putih (*curcuma zedoaria*) added in powder creates inhomogeneity within film matrix. This inhomogeneity causes the film cannot hold the pressure exerted on the film [18]. The tensile strength test results on edible film from sugar palm starch with the addition of STPP is 0.001412 MPa and edible film with the addition of STPP and 3% freeze-drying powder of curcuma zedoaria is 0.000874 MPa. This result is not sufficient for the application as edible film. The range of tensile strength values that can be applied to standard edible films is between 10 – 100 MPa [6].

3.2. Elongation percentage test

Percent of elongation of edible film represents a condition when the film breaks after experiencing a change from actual length due to being stretched. This parameter shows the ability of edible film to withstand against loads given before edible film breaks [5]. Elongation percentage of edible film is measured by cutting the edible film to a size of 8 cm x 2 cm and the film was pulled using a load. The results of the analysis of percent length of edible film can be seen in the following figure.

The elongation percentage of edible film at the addition of 0.5% curcuma zedoaria decreased by 50%. The decrease in elongation is due to the increased tensile strength which indicates its strength, but its flexibility decreases. The addition of curcuma zedoaria with a higher concentration causes the percent of elongation to increase by 65% because the tensile strength of edible film decreases. The greater the elongation percentage, the better the edible film produced because it represents its elasticity and does not torn easily. The elongation percentage above 50% is considered to be good and if the value is less than 10% it is considered as poor [6]. Edible film made from sugar palm starch with the addition of STPP and curcuma zedoaria has a good percent of elongation.

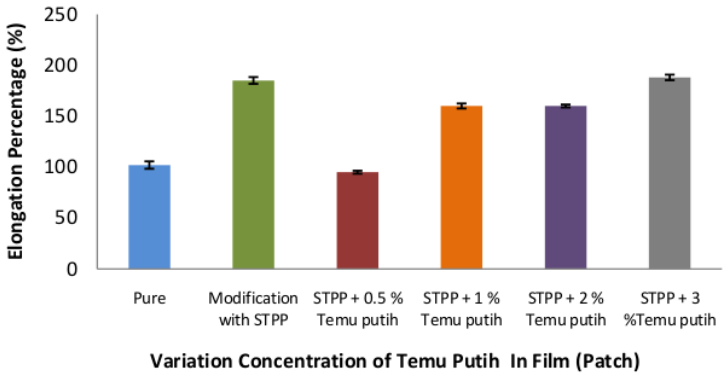

Figure 3. The effect of edible film composition on the elongation percentage

3.3. Average thickness test

Thickness of edible film was measured using a manual micrometer with accuracy of 0.001 mm. Thickness is an important parameter that influences the formation of edible film. Thickness affects the water and gas vapor transmission rate. The thickness value obtained is the average of measurements at 5 measurements. The following are the analysis results of the edible film average thickness.

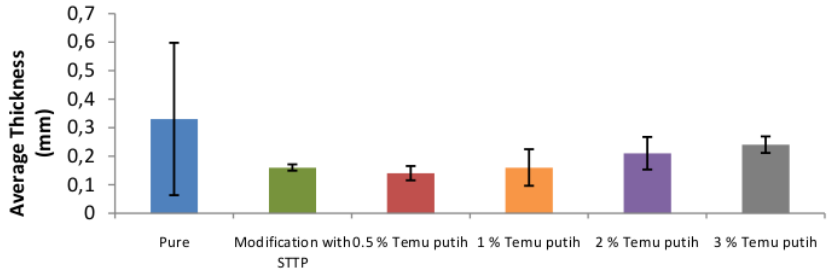

Figure 4. The effect of edible film composition on average thickness

The average value of edible film thickness is directly proportional to the concentration added. In edible films with the addition of 1% STPP and edible film with the addition of 1% powder freeze drying the white meeting showed the same average thickness i.e. 0.16 mm. The increase in thickness is due to differences in the concentration of edible film raw materials. The higher the concentration is added, the higher the total amount of solids in the solution, resulting in a thicker film. The total solids in the film increase after drying and the polymers that make up the film matrix are also increase. The standard thickness of edible film is 0.25 mm. The thickness value of edible film added with STPP 1% and the addition of curcuma zedoaria powder 0.5% - 3% of the weight of sugar palm starch approached the required standard value, furthermore the edible film of sugar palm starch showed a value that exceeded the standard.

3.4. The effect of water content

The result of water content determination is displayed on figure 5.

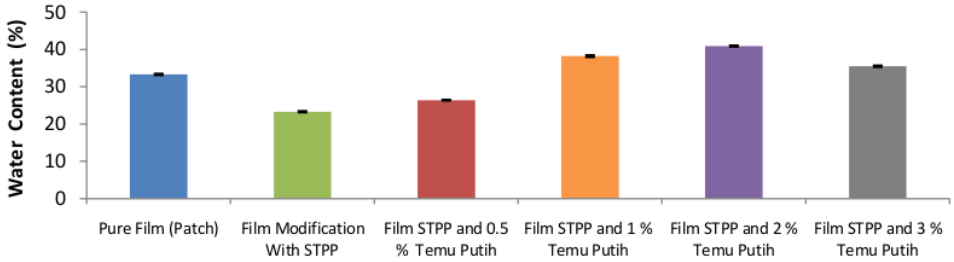

Figure 5. The effect of edible film composition on water content

The calculation results show that the lowest water content (23.3%) obtained when edible film is added with 1% STPP, while the highest water content is obtained on edible film with the addition of 2% freeze drying powder of curcuma zedoaria i.e. 40.9%. The increase in water content probably occur due to the nature of the molecular freeze-drying powder of curcuma zedoaria which is hydrophilic, the higher the concentration of powder is added the higher the water content of the edible film. The high content of water in the edible film decrease the quality of edible film, because water is causing moisture formation in food hence shortens the food storage durability.

3.5. Protein determination of amylase extracted from saliva and bacteria by using Biuret method

Protein content in the amylase enzyme from bacteria is 0.0134 g/mL while amylase enzyme from saliva is 0.0025 g / mL. Environmental conditions with high protein levels trigger high enzyme activity on contrary, conditions of low protein levels make enzyme activity also low[10, 11].

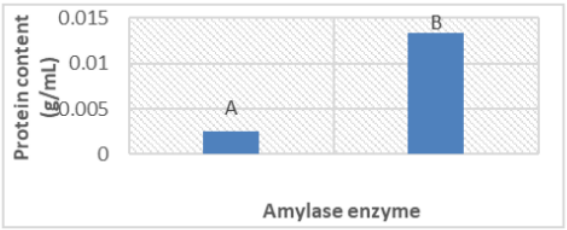

Figure 6. The protein content of amylase enzyme from saliva (A) and bacteria (B)

3.6. The optimum temperature of amylase enzyme activity against sugar palm starch

Color changes due to the breakdown of the polysaccharides and produce monosaccharides using the DNS reagents are obtained in the following image.

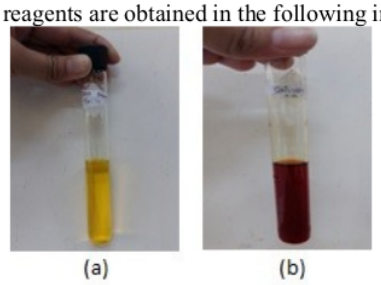

Figure 7. The color of starch, enzyme and DNS mixture (a) before and (b) after heat treatment

Prior hydrolysis, the color of the solution is yellow. This color indicates polysaccharide retain itself hence the color shown is the color of the DNS reagent. In a solution where hydrolysis occurred, the color turns brick red. This shows the breakdown of polysaccharides into monosaccharides of the type of reducing sugars that react with DNS reagents to form 3 amino-5-nitrosalicylic. The presence of

reducing sugars in the sample is responsible in producing a red brick color. The polysaccharide hydrolysis reaction to disaccharide can be seen in the following figure.

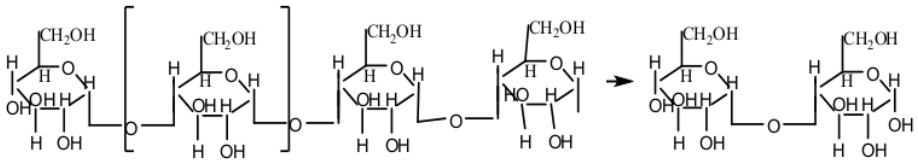

Figure 8. Hydrolysis of polysaccharide into disaccharide

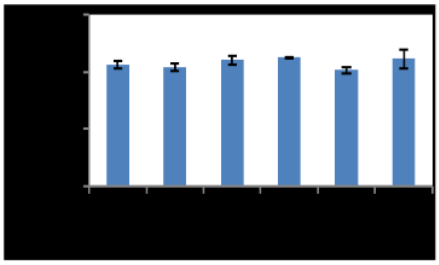

Figure 9. The effect of incubation temperature on enzyme activity extracted from bacteria

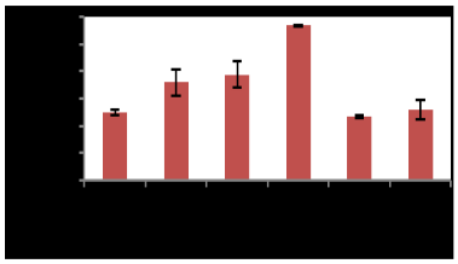

Figure 10. The effect of incubation temperature on enzyme activity isolated from saliva

The activity of amylase enzyme extracted from saliva and bacteria after incubated for 10 minutes at various temperatures resulted in the highest activity at 35 °C i.e. 0.56 units/mL and 4.53 units/mL respectively. Amylase enzyme from the roots of *alang-alang* rhizome (*Imperata cylindrica*) which showed highest activity 0,94 unit/mL enzyme at 35 °C. Low temperatures is not an optimum condition for amylase enzyme activity because the energy absorbed by the enzyme does not suffice to hydrolyze the substrate hence enzyme activity is low. At high temperature, the enzyme experience denaturation i.e. disruption of the active part of the enzyme which cause decrease in reaction speed [2,9,10].

3.7. Optimum pH of amylase enzyme activity against sugar palm starch

The analysis results showed that the highest amylase activity extracted from saliva and bacteria is at pH 7 i.e. 3.80 units/mL and 0.37 units/mL respectively. The results of amylase enzyme activity against pH can be seen at Figure 11 and Figure 12.

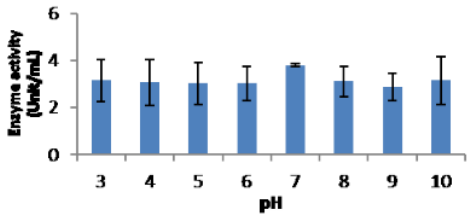

Figure 11. The effect of pH on the amylase enzyme activity extracted from bacteria

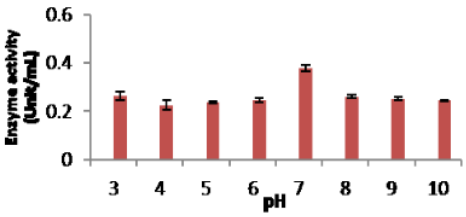

Figure 12. The effect of pH on the amylase enzyme activity isolated from saliva

Enzymes show maximum catalytic activity in a certain pH range called the optimum pH. Amylase enzymes from saliva and bacteria shows highest activity at pH 7. This result is similar to research in case crude amylase activity of *Bacillus subtilis* from crater of Darajat mountain isolate shows optimum result at pH 7 i.e. 57.346 units/mL [3]. Optimum pH of amylase varies from 2 – 10.5 and particularly active well at pH 5 – 8 [13, 17].

3.8. The effect of optimum substrate concentration on sugar palm starch

The analysis results obtained from the testing of amylase enzyme activity extracted from saliva and bacteria is stable at substrate concentration 2.5%.

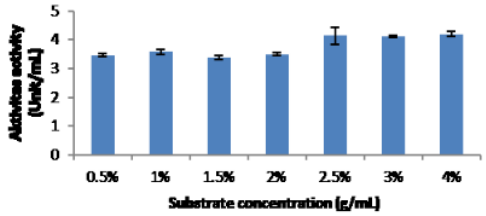

Figure 13. The effect of substrate concentration on amylase enzyme activity extracted from bacteria

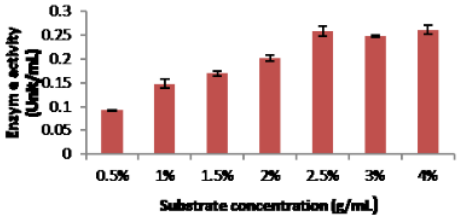

Figure 14. The effect of substrate concentration on amylase enzyme activity isolated from saliva

The picture shows at a fixed concentration, the addition of substrate concentration increases enzyme activity to achieve maximum. In these conditions all enzymes are saturated with the substrate, so the addition of the substrate does not increase the activity. At 2.5% substrate concentration, the amylase enzyme from saliva has an activity of 0.25 units/mL while in the amylase enzyme extracted from bacteria 4.14 units/mL. The increase in activity occurs at concentrations of 0.5-2.5% and reaches constant value at 3-4% substrate concentration. At this point, the active side of the enzyme is equal to the amount of substrate. α-amylase from actinomycetes isolates from East Kalimantan gave the highest activity at 2% substrate concentration 8.23 unit/mL and decreased at a concentration of 2.5% [16].

A low substrate concentration causes low rate of enzyme work. Conversely, if high substrate concentrations available, enzyme work in higher rate. In excess substrate conditions, the enzyme's work is constant [17]. To determine the relationship between substrate concentration and amylase enzyme activity or reaction speed, Lineweaver-Burk method was used, Vmax and Km values can be calculated. Vmax is the reaction speed when the enzyme is saturated with the substrate, Km shows the substrate concentration which causes the enzymatic reaction to reach half the maximum. Substrate concentration activity can be seen in the figure 15.

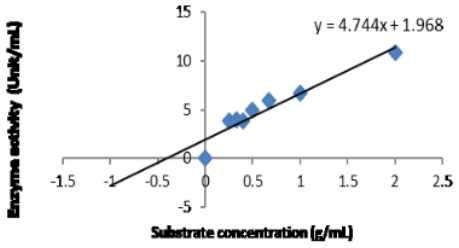

Figure 15. Relationship curve between substrate concentration (sugar palm starch) with amylase enzyme reaction rate isolated from saliva

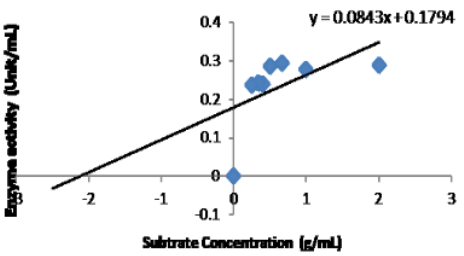

Figure 16. Relationship curve between substrate concentration (sugar palm starch) with amylase enzyme reaction rate extracted from bacteria

Based on the curve, the linear equation is obtained Y = 4.744x + 1.968, Vmax value is 0.508 unit/mL and Km is 2.409 g/mL. The Vmax value 0.508 units/mL shows the maximum speed of the amylase enzyme when it was saturated with the amylose substrate, while the constant Michalis-Menten when the amylase enzyme reaches half of its maximum velocity is 2.409 mg/mL.

The plot of substrate concentration vs. enzyme activity produced a linear equation Y = 0.084x + 0.179. According to the equation, the Vmax value is 5.586 units/mL and Km is 0.469 g/mL. The Vmax value of 5.586 unit/mL shows the maximum speed of the amylase enzyme when being saturated with the amylose substrate, while the Michalis-Menten constant when the enzyme amylase activity

half of its maximum speed is 0.469. The difference in Vmax and Km values in the amylase enzyme from saliva and bacteria is caused by differences in the purity of the enzyme and the experimental conditions.

Thermostable α-amylase from Bacillus stearothermophilus TII-12 gave Vmax and Km values of 1.21 units/mL and 1.06%, respectively [7]. Activity of amylase enzyme from Bacillus Subtilis KCX 006 attained Vmax value of 23.69 unit/mL and Km value of 0.291% [1].

3.9. The effect optimum incubation time on amylase enzyme activity from sugar palm starch

Amylase enzyme activity obtained from both saliva and bacteria increased at 40 minutes incubation time and decreased at 60 minutes incubation. At the incubation time of 70 minutes to 95 minutes an increase in activity were detected. The results of the analysis of amylase enzyme activity on incubation time can be seen in the figure 17.

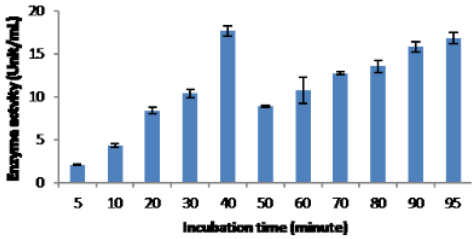

Figure 17. The effect of incubation time on enzyme activity extracted from bacteria

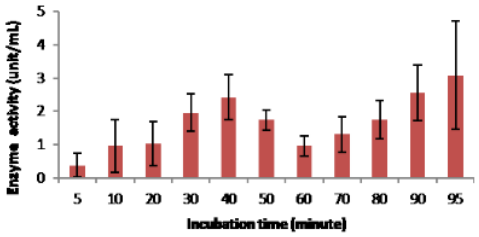

Figure 18. The effect of incubation time on enzyme activity extracted from saliva

The picture shows the highest amylase enzyme activity from saliva obtained 2.42 units/mL at 40 minutes incubation time and 3.08 units/mL at 95 minutes incubation time. While the highest amylase enzyme activity from bacteria was 17.71 units/mL at 40 minutes incubation time and 16.87 units/mL at 95 minutes incubation time. The activity of amylase enzyme extracted with ethanol gave the highest enzyme activity of 39.26 units/mL after 120 minutes incubation time [4]. The enzyme requires enough incubation time to reach its maximum activity because the substrate must enter the active center of enzyme to form product. Therefore, this study used 95 minutes as the optimum incubation time.

3.10. Amylase enzyme activity on sugar palm starch hydrolysis at optimum condition

Data on the results of amylase enzyme activity in hydrolysing sugar palm starch can be seen in figure 19.

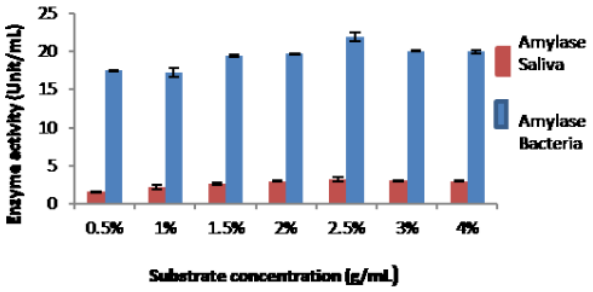

Figure 19. Amylase enzyme activity from saliva and from bacteria used on sugar palm starch hydrolysis in optimum conditions

The highest activity of amylase enzyme from saliva and bacteria was obtained at 2.5% substrate concentration. At this concentration the number of active sides of the enzyme is equal to the amount of binding on the substrate catalyzed hence the addition of more substrate won't be hydrolyzed by the enzyme because no more active side available within the enzyme.

3.11. The amylase enzyme activity on edible film hydrolysis

The results of the analysis of amylase enzyme activity in the hydrolysis of edible film can be seen in the Figure 20.

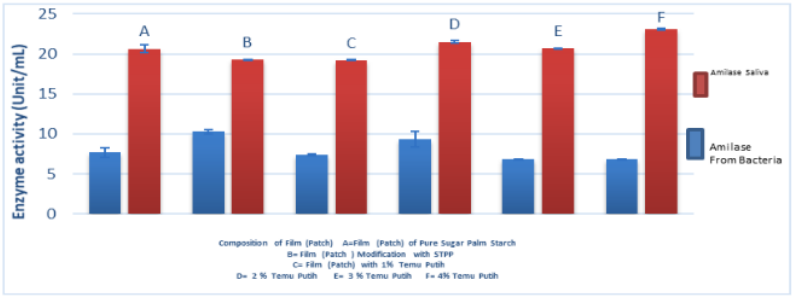

Figure 20. Effect of edible film composition on enzyme activity from saliva and bacteria in hydrolysis of film (patch)

Figure 20 informs the amylase enzyme from saliva used in hydrolysis of edible film made from sugar palm powder with the addition of STPP has the highest activity 9.37 units/mL. Films made from sugar palm powder with the addition of STPP possess phosphate groups which is hydrophilic and able to interact with other molecules such as CMC and glycerol. This group causes more water molecules to be released in edible film and help the work of the amylase enzyme from saliva to break down the starch substrate. The highest enzyme activity of the amylase from bacteria is 23.09 Unit / mL. The relationship between amylase enzyme activity and the addition of antibacterial active substances has never been studied before. The addition of antibacterial active substances will disturb enzyme activity [12,14].

In this study, the addition of small amount of antibacterial active substances i.e. curcuma zedoaria does not affect the activity of the enzyme amylase in hydrolyzing the substrate. The molecules from the curcuma zedoaria is hydrophilic hence it can interact with other molecules such as CMC and glycerol molecules. The molecules therefore not easily bind to water molecules. The non-bound water molecules assist the activity of the amylase enzyme in hydrolyzing the substrate and result in high activity.

Data analysis of amylase enzyme from saliva applied on hydrolysis of sugar palm starch, hydrolysis of edible film made with addition of STPP, hydrolysis of edible film with addition of STPP and freeze-drying powder of curcuma zedoaria in various concentration were conducted using one-way ANOVA. F-count = 0.7613 which is smaller than F-table at significance level of 0.05. Similar analysis for amylase enzyme activity from bacteria obtained F-count of 0.2807 which is smaller than F-table at significance level of 0.05. This concludes that there is no significant difference in the activity of amylase enzymes from saliva or bacteria in hydrolysis of sugar palm starch, edible film with the addition of STPP and edible films with the addition of STPP and freeze-drying powder of curcuma zedoaria.

4. Conclusion

The results of characterization of physical and chemical properties: tensile strength test gave the best results on edible film with the addition of STPP, elongation percentage gave the best results on the addition of STPP edible film, the average thickness test obtained best result on edible film added with STPP and edible film added with freeze-drying powder of curcuma zedoaria in various concentration, the best water content test obtained on edible film with the addition of STPP.

Determination of the optimum conditions for the activity of amylase enzymes from saliva and bacteria against sugar palm starch was obtained at 35 °C, pH 7, substrate concentration 2.5% and incubation time of 95 minutes. Vmax and Km values of sugar palm starch obtained in the amylase enzyme activity from saliva were 1.015 units/mL and 1.039 g/mL respectively, whereas in amylase from bacteria the values of Vmax and Km were 12.658 units/mL and 0.430 g/mL.

The best activity of amylase enzyme from saliva was found out on hydrolysis of edible film with the addition of STPP, while the amylase enzyme from bacteria was found out on hydrolysis of edible film by addition of STPP and freeze-drying powder of Temu putih (*curcuma zedoaria*) 3%. One-way ANOVA analysis concludes, the F-count is smaller than F-table which confirms that there is no significant difference in the activity of amylase enzymes from saliva or bacteria on hydrolysis of sugar palm starch and edible film made with various composition.

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