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Potential of Vanname Shrimp Skin Astanxanthin Hydrolyzate as Alpha Glucosidase Enzyme Inhibitor

Potensi Hidrolisat Astanxanthin Kulit Udang Vanname sebagai Penghambat Enzim Alfa Glukosidase

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Abstract

The purpose of the study was to analyze hydrolysate astaxanthin as an inhibitor of the enzyme alf glucosidase. The method used is an experimental method by applying a Complete Random Design (RAL) The treatment used is a concentration of different papain enzymes, consisting of 3 treatment levels namely t1 (3%), t2 (6%), and t3 (9%). Observations made are 1) proximal analysis; 2) analysis of hydrolysate astaxanthin and its antioxidant activity; 3) analysis of the inhibition test of the enzyme alpha glucosidase and its inhibitory mechanism. The results showed that the proxmat of vanname shrimp skin flour is; water content (9.20% bb), ash (31.46% bk), fat (1.64% bk), protein (37.60% bk), and carbohydrates (29.29% bk) (by different) with a yield of 36.22%. The highest astaxanthin content at the 3% papain enzyme concentration of 3.57 µg/g with the best antioxidant activity has the smallest IC50 value of 88,793 ppm and astaxanthin shows inhibition activity of alpha glucoside enzyme by 49.38% - 58.87% and astaxanthin has a competitive enzyme inhibition mechanism resulting in a decrease in postrandial blood glucose levels.

Keywords: Astaxanthin, Antioxidants, Alpha Glucosidase

Abstrak

Penelitian ini bertujuan menganalisis hidrolisat astaxanthin sebagai penghambat enzim alf glukosidase. Metode yang digunakan adalah metode eksperimen dengan menerapkan Rancangan Acak Lengkap (RAL) Perlakuan yang digunakan adalah konsentrasi enzim papain berbeda, terdiri dari 3 taraf perlakuan yaitu t1 (3%), t2 (6%), dan t3 (9%). Pengamatan yang dilakukan yaitu 1) analisis proksimat; 2) analisis hidrolisat astaxanthin dan aktivitas antioksidannya; 3) analisis uji penghambatan enzim alfa glucosidase dan mekanisme penghambatannya.Hasil penelitian menunjukkan bahwa proksimat tepung kulit udang vanname yaitu; kadar air (9,20% bb), abu (31,46% bk), lemak (1,64% bk), protein (37,60% bk), dan karbohidrat (29,29% bk) (*by different*) dengan rendemen sebesar 36,22%. Kandungan astaxanthin tertinggi pada konsentrasi enzim papain 3% sebesar 3.57 µg/g dengan aktivitas antioksidan terbaik memiliki nilai IC50 terkecil yaitu 88.793 ppm serta astaxanthin menunjukkan aktivitas penghambatan enzim alfa glukosida sebesar 49.38% - 58.87% dan astaxanthin memiliki mekanisme penghambatan enzim kompetitif sehingga terjadi penurunan peningkatan kadar glukosa darah postrandial.

Kata kunci: Astaxanthin, Antioksidan, Alfa Glukosidase

1. Introduction

Diabetes Mellitus (DM) is a chronic hyperglycemic condition accompanied by various metabolic disorders due to hormonal disorders that cause chronic complications of the eyes, kidneys, nerves and blood vessels (Mansjoer, 2000). Ewadh (2014) states that DM is a metabolic disorder characterized by high glucose concentrations in the blood (hyperglycemia). One source of antioxidants that can be utilized is astaxanthin using the DPPH method, which is thought to be able to help regenerate pancreatic tissue damaged by free radicals and can also have the potential to act as an inhibitor of the alpha-glucosidase enzyme, preventing the formation of glucose in the human body's metabolism so that it can lower blood glucose levels and is very useful for DM sufferers (Karnila, 2011). By inhibiting the action of the alpha-glucosidase enzyme, blood glucose levels can be returned to normal limits (Bosenberg, 2008).

Astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione) is a carotenoid pigment such as zeaxanthin, lutein, and β-carotene but specifically as a red-orange pigment. It belongs to the xanthophyll group with hydroxyl and carbonyl functionalities, making it an excellent source of antioxidants (Davinelli et al., 2018). This compound can be found in marine biotas such as salmon, shrimp, crayfish and the microalgae *Haematococcus pluvialis*. Shrimp shells have potential as antioxidants (Pratiwi, 2018) because they can donate one or more electrons to free radicals so that these free radicals can be suppressed. Antioxidants can provide electrons to bind and end free radical chain reactions (Sadeli, 2016). Shrimp shells can produce astaxanthin, which has widespread use in various fields and has much better added value.

According to Desiana (2000), proteolytic enzymes can hydrolyze astaxanthin from shrimp shell waste. Protease enzymes help break protein bonds with astaxanthin to obtain free astaxanthin (Chakrabarti, 2002). Therefore, this research was conducted to prove vannamei shrimp shell astaxanthin hydrolyzate as an inhibitor of α-glucosidase enzyme activity in vitro to be used as an anti-hyperglycemic agent.

2. Material and Method

2.1. Time and Place

This research was conducted at the Fishery Products Chemistry Laboratory, Faculty of Fisheries and Marine, Universitas Riau, Pekanbaru.

2.2. Methods

The method used is a method experiment with Completely Randomized Design (CRD). The treatments used were different concentrations of the papain enzyme, consisting of 3 levels, namely T1 (3%), T2 (6%), and T3 (9%). The treatment used three repetitions, so the number of experimental units was 9.

2.3. Procedure

2.3.1. Identification of Astaxanthin using the UV-Vis Spectophotometric Method

Determination of carotenoid levels using the PORIM (1995) method. Hydrolysis was weighed as much as 0.1 g and put into a 25 ml measuring flask. The sample was dissolved with n-hexane until the tera mark. Then, stir until homogeneous. The sample's absorbance was then measured at a wavelength of 450 nm using a spectrophotometry tool, and then the total calculation was carried out using the following equation.

$$T = \frac{\text{Concentration x sample vol x Fp}}{\text{sampel Weight}}$$

Information:

T = Total carotenoids; Concentration = mg/mL; Fp = Dilution factor

2.3.2. Antioxidant Test

The antioxidant activity of the resulting hydrolyzate was analyzed using the DPPH (1,1-Diphenyl-2-picryl Hydrazil) method using a two-fold dilution microplate reader with a wavelength of 515 nm. This test consists of several stages, namely, the resulting hydrolyzate is weighed at 30 mg and dissolved in 30 ml methanol so that a concentration of 1 mg/ml is obtained. Adding m ethanol was carried out to dilute samples with concentrations (1000, 500, 250, 125, 62.5 ppm). Antioxidant activity at a predetermined concentration was pipetted as much as 4.5 ml using a micropipette and then homogenized. The mixture was left for 30 minutes in a dark place. The absorbance was measured using a UV-Vis spectrophotometer with a wavelength of 515 nm.

2.3.3. Preparation of Acarbose Standard Solution Curve

5 mg arcabose was dissolved in 50 ml n-hexane with a 100 mg/l concentration. Then, make standard series solutions with various concentrations, namely 0.28; 0.65, 1.09; 1.52; 1.96 ppm. The absorption of each solution was measured using UV-Vis spectrophotometry at a wavelength of 410 nm. The absorbance value of each standard solution was made into a calibration curve.

2.3.4. Glucosidase Enzyme Inhibitory Activity Test

Data was analysed by calculating the percentage of α-glucosidase inhibition obtained from absorbance measurements. IC50 calculations use linear regression equations, sample concentration as the x-axis and % inhibition as the y-axis. The IC50 value can be calculated from the equation y = bx + a using the formula: IC50 = 50-2/b (Sugiwati et al., 2009).

2.3.5. Kinetic Test of Alpha Glucosidase Enzyme Inhibition

Data analysis was carried out by calculating p-NP, then the p-NP results were used to calculate enzyme activity.

2.4. Data Analysis

The data obtained was tabulated in tables and graphs and processed statistically using analysis of variance (ANOVA). From the calculations, an F-count will be obtained, determining whether the proposed hypothesis is accepted or rejected. If the F-count is smaller than the F-table at the 99% confidence level, then H0 is accepted. If the F-count is greater than the F-table at the 99% confidence level, then H0 is rejected, and a further BNJ test is carried out.

3. Result and Discussion

3.1. Vanname Shrimps Shell Flour Proximate

Nutritional content analysis (proximate) was carried out to determine the fat, protein, ash content and water content contained in shrimp shell flour using fresh shrimp shell as raw material. The results of the nutritional content analysis (proximate) can be seen in Table 1.

Table 1. Chemical composition of vannamei shrimp shell flour

Content	Percentage
Water (%)	9.20
Ash (%)	28.57
Protein (%)	34.14
Fat (%)	1.49
Carbohydrates (by different) (%)	35.80

Based on Table 1, it can be seen that from 3 measurements, the fat content of vannamei shrimp shell flour is an average of 1.49%. Shrimp heads have a fat content of around 1%. The demineralization process in HCl cannot reduce the fat content of shrimp shells. Differences in fat content are influenced by the type of shrimp and the life stage of the shrimp at harvest. Shrimp in the molting phase contain higher fat levels. Protein content 34.14% showed that the shell protein content of *Litopenaeus vannamei* shrimp was 40.35% (bb). Differences in shrimp protein levels are influenced by the level of food eaten by shrimp in the waters and protein levels are also influenced by calculating the wet weight and dry weight of a material. The water content of vannamei shrimp shell flour is 9.20%. The water content of *Penaeus notables* shrimp shells, based on research by Emmanuel et al. (2008) is 13.3%. The difference in water content was influenced by the different types of shrimp and the level of dryness of the samples used in the study, the ash content of vanname shrimp shell flour averaged 28.57%. The difference in ash content values is thought to be caused by differences in habitat and living environment, the carbohydrate content of vannamei shrimp shell flour is an average of 35.80%.

3.2. Astaxanthin Hydrolyzate

Vanname shrimp shell flour hydrolyzate uses different concentrations of the papain enzyme to produce different amounts of hydrolyzate. Data regarding hydrolysates produced from the hydrolysis process with different enzyme concentrations can be seen in Table 2.

Table 2. Vanname shrimp shell astaxanthin hydrolyzate

Test	Treatment		
	T_1	T_2	T_3
1	3.50	2.70	1.60
2	3.50	2.80	1.70
3	3.70	2.60	1.70
Average (μg/g)	3.57 ^{c}	2.70 ^{b}	1.67 ^{a}

Based on Table 2, the hydrolyzate value in treatment T1 produces more hydrolyzate than treatments T2 and T3. According to Desiana (2000), the optimum concentration of the papain enzyme for shrimp shell hydrolyzate is 2-3%; this concentration increases enzyme activity and more peptide bonds are broken down. Concentrations more significant than that decrease enzyme activity, resulting in less than optimal breakdown of peptide bonds.

Based on analysis of variance, it shows that the vanname shrimp shell astaxanthin hydrolyzate produced based on the concentration of the papain enzyme has a very significant effect on the amount of hydrolyzate assembled, where calculated F (305.375) > F table (10.92) at the 99% confidence level, then H0 is rejected and carried out further test (BNJ).

Astaxanthin hydrolyzation obtains astaxanthin from materials containing astaxanthin pigment, such as shrimp shells. Astaxanthin in shrimp shells is a complex compound that binds non-covalently to protein (Gimeno et al., 2007 in Karnila and Heriansyah, 2020). T1 treatment with a papain enzyme concentration of 3% had an effect on astaxanthin hydrolyzate from vannamei shrimp shells of 3.57 µg/g, so it can be seen that this concentration increases enzyme activity and breaks down more peptide bonds.

3.3. Antioxidant Activity of Vannamei Shrimps Shell Astaxanthin

Antioxidant activity was tested on vanname shrimp shell astaxanthin hydrolyzate using the DPPH (2,2-diphenyl-1- picrylhydrazyl) method using concentrations of 1000, 500, 250, 125, and 62.5 ppm. The results obtained from testing antioxidant activity using the DPPH method will reveal the relationship between the concentration of astaxanthin hydrolyzate and the per cent inhibition. The data obtained can be seen in Table 3.

Table 3. IC50 value of antioxidant activity of vannamei shrimp shell astaxanthin

Test	Treatment		
	T_1	T_2	T_3
1	87.08	124.82	672.68
2	91.84	128.82	672.62
3	87.46	124.82	435.06
Average (ppm)	88.79[c]	126.155[b]	593.42[a]

Table 3 shows that the highest IC50 value was produced by the T3 treatment, which was 593.42 ppm and the lowest by the T1 treatment, which was 88.79 ppm. Therefore, the lower concentration of the papain enzyme will affect the antioxidant activity of the vannamei shrimp shell astaxanthin hydrolyzate. The lower the concentration of the papain enzyme, the lower the IC50 value, which indicates the better the antioxidant activity. The IC50 value for each treatment was subjected to analysis of variance. Based on the analysis of variance, it shows that different concentrations of the papain enzyme have a very significant effect on antioxidant activity where calculated F (1047.98) > F table (10.92), so H0 is rejected at the 99% confidence level. Knowing which treatment is different, then proceed with the BNJ further test.

According to Molyneux (2004), a compound is said to be a powerful antioxidant if the IC50 value is <50 ppm, it is said to be vital if the IC50 value is between 50-100 ppm, a medium antioxidant if the IC50 is between 100-150 ppm, and a weak antioxidant if the IC50 value is between 150-200 ppm. Treatment T3 has an IC50 value of 593.42 ppm, which is classified as weak; treatment T2 has an IC50 value of 126.15 ppm, which is classified as moderate; and treatment T1 has an IC50 value of 88.79 ppm, which is classified as strong.

3.4. Alpha Glucosidae Enzyme Inhibition Activity Test

Testing of the inhibitory activity of astaxanthin hydrolyzate against the alpha-glucosidase enzyme was carried out using varying concentrations. They were testing at this concentration to see how increasing the hydrolyzate concentration improves the inhibitory power. In this test, acarbose was used as a standard. Testing of astaxanthin hydrolyzate samples was divided into five concentrations, namely 0.28, 0.65, 1.09, 1.52, and 1.96 mg/ml. Tests were carried out at several different concentrations to see the effect of increasing concentrations on the increase in inhibiting the alpha glucosidase enzyme. The percentage of alpha glucosidase inhibition by astaxanthin hydrolyzate is shown in Figure 1; the hydrolyzate shows inhibition of the alpha glucosidase enzyme.

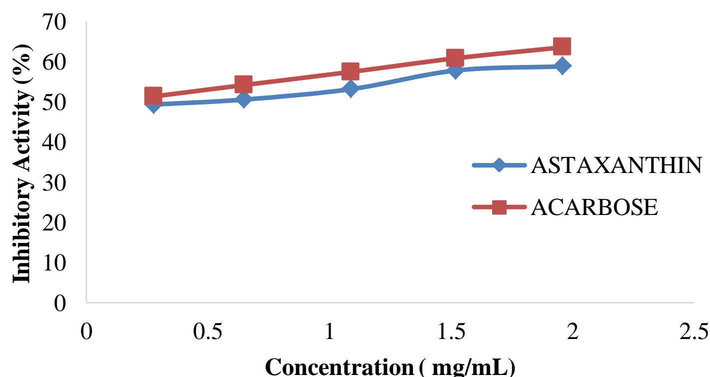

Figure 1. Percentage of alpha glucosidase enzyme inhibitory activity

The percentage value of astaxanthin hydrolyzate inhibition varied, namely 49.38% - 58.87% for the lowest and highest concentrations. Acarbose is used as a standard drug for alpha-glucosidase inhibition. Acarbose was used as a comparison because it has been clinically proven to inhibit the alpha-glucosidase enzyme. Acarbose showed inhibitory activity of 51.41% - 63.62%. Compared to the activity of acarbose, the activity of astaxanthin hydrolyzate can inhibit the alpha-glucosidase enzyme but is not as good as acarbose.

Research (Bharathkumar, 2014) shows inhibitory activity that is 67% higher than this study. The differences obtained are due to the ingredients and concentration factors used. In this study, the acarbose used was pure acarbose originating from Glucobay Bayer AG (Germany), and the color reagent used was glucose oxidase. Meanwhile, this research used 50 mg acarbose tablets and 3,5-dinitro salicylic acid color reagent.

4. Conclusions

Based on the results of research on the effect of astaxanthin hydrolyzate using different enzyme concentrations, information was obtained that the highest astaxanthin content was at a 3% papain enzyme concentration, namely 3.57 µg/g with the best antioxidant activity with the smallest IC50 value, namely 88,793 ppm, which was classified as vital. Also, testing of astaxanthin showed that the inhibitory activity of the alpha glucoside enzyme was 49.38% - 58.87%, resulting in a decrease in the increase in postprandial blood glucose levels.

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