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Anticholesterol Bioactive Peptides from Bromelain Hydrolysates of Mangrove Sonneratia alba Protein

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ABSTRACT: Cholesterol with high levels in blood vessels can cause atherosclerosis, stroke, and sudden heart attack. Isolates soy protein have bioactive peptides that have the potential as anticholesterol. This research aims to determine the optimum conditions of hydrolysis and characterize bioactive peptides from soy protein isolate hydrolysates by the bromelain enzyme. Hydrolysis optimization conducted using the enzyme bromelain with levels of 0,2%; and 0,5% (w/v) and variation in incubation time 0, 1, 2, 3, 4, 5 to 6 hours at 45, 50 and 55°C. Protein hydrolysates analyzed the degree of hydrolysis (% DH) and tested anticholesterol activity test through HMG-CoA reductase inhibition test with pravastatin as a positive control. The results showed the optimum conditions hydrolysis of isolate soy protein were obtained at 2 hours, temperature 45 °C with enzyme concentration 0,5% that is by DH value of 40,22%. The highest anticholesterol activity was obtained from the hydrolysate with percent inhibition value of 82,80% (7.371 ppm). SDS-PAGE analysis results show the appearance of bands under 10 KDa and the results of fractionation of bioactive peptide fragments has a molecular weight of 2779 and 2609 Da.

KEYWORDS: Anticholesterol, bromelain, bioactive peptides, isolate soy protein

1. INTRODUCTION

Cholesterol resembles a complex fat compound that has a function to make adrenal cortex hormones, sex hormones, vitamin D, and bile salts that help absorb fat in the intestines. If the total cholesterol level in the blood exceeds normal limits, or high blood cholesterol levels, it will cause various causes such as heart disease, stroke, diabetes mellitus and atherosclerosis. Normal cholesterol levels range from 160-200 mg/dL.

Drugs that are often used to lower cholesterol levels are synthetic drugs, one of which is atorvastatin. Atorvastatin is a statin drug with a long half-life that can induce bile acids, the synthetic enzyme cholesterol-7 alpha-hydroxylase . (CYP7A1) by suppressing farnesoid X receptor (FXR) signaling in the liver and intestines[1]. Continually taking statin drugs can result in several side effects such as moderate/severe liverdysfunction, cataracts, moderate/severe myopathy, and kidney failure [2]. In a recent study, consumption of statins under certain conditions and in excessive doses will lead to an increased risk of developing type 1 diabetes [3].

The existence of side effects of using synthetic drugs, it is necessary to conduct research through the search for food plants or herbal plants that have properties that can lower cholesterol levels and have no side effects. One of the food crops which has the potential to be developed as a source of anticholesterol is soybeans. Soybeans are seeds that can be used for health because they contain high levels of dietary fiber, vitamins, minerals, and protein, and are known as a source of protein. The protein content of soybeans is very high when compared to other nuts, soybean protein reaches 35% based on dry weight, even soybeans with superior varieties have protein levels of 40-43%.

Several studies have shown soy protein has been shown to reduce the risk of heart disease by lowering blood cholesterol (LDL) levels [4]. Pak. et al. [5] , reported that the hydrolysis of pepsin enzymes in soybeans obtained the Ile-Ala-Val-Pro-Gli-Glu-Val-Ala peptide which can inhibit the HMGCoA reductase enzyme with an inhibition value of 45%. The results of Lammi et al[6] showed that the peptides Tyr-Val-Val-Asn-Pro-Asp-Asn-Asp-Glu-Asn and Tyr Val-Val-Asn-Pro-Asp-Asn-Asn-Glu-Asn derived from the protein - Conglycinin can act as a competitive inhibitor of the HMG-CoA reductase enzyme with IC50 of 150 and 200 µM.

Lin et al[7], reported several peptides that have potential as hypocholesterolemic agents having peptide molecular weights of 1384, 1583 and 1586 Dalton, respectively. According to Meinlschmidt et al[8] hydrolysis of soybean protein using papain enzyme at a temperature of 80°C, pH 7, enzyme:substrate concentration of 0.2%, and time variations of 0, 10, 30, 60, and

120 minutes obtained the results of degrees The highest hydrolysis time was 4.9±0.0% at 10 minutes and the soy protein profile decreased from 75 kDa to 25 kDa to < 10 kDa. In his research Zeng et al[9], has carried out hydrolysis of soybean protein using papain enzyme (EC 3.4.22.2, Sigma 80 units/mg) with concentrations of 0.5, 1 and 2% at 37°C and time variations of 5, 15, and 30 minutes obtained the results degrees of hydrolysis was at a concentration of 2% for 30 minutes, which was 4.29%. According to Chang [10] enzymatic hydrolysis of vegetable proteins is safer, and provides a more uniform product through more specific reactions. The papain enzyme can hydrolyze the amide group on the amino acid residues of phenyl alanine, leucine, valine, and tyrosine.

Kim [11] stated that enzymatic protein hydrolysis was carried out to obtain bioactive peptides that were safe for food. According to Whitaker [12] bromelain is an enzyme that has a wide cutting specificity for amino acid residues that make up its substrate which includes lysine, arginine, phenylalanine and tyrosine so that it can produce a high degree of hydrolysis. The bromelain enzyme has properties similar to other proteolytic enzymes such as papain, fisin, and rennin enzymes, which have the ability to hydrolyze other proteins [13]. Walker [14] states that the specific activity of the bromelain enzyme is optimum at a temperature of 50°C and at pH 6.5-7 using 1M phosphate buffer pH 6.5, when the temperature is above 50°C and the pH is more than 7, the activity is not within the optimum limit. the bromelain enzyme will decrease. According to Poh and Majid [15], bromelain enzyme has an optimum temperature of 35-45°C. Another study conducted by Indumathy et al [16] regarding the extraction, partial purification and characterization of the bromelain enzyme from pineapple with variations in the buffer solution used, pH and temperature, the optimum conditions for the bromelain enzyme were at 20 °C, pH 4.2 and the buffer used is 0.1M sodium acetate buffer.

In this study, 0.1 M phosphate buffer pH 6.5 and incubation times of 0, 1, 2, 3, 4, 5, and 6 hours at 45°C, 50°C, and 55°C were used. The sample used was smangrove protein isolate that had been dehydrated which was hydrolyzed using the enzyme bromelain with a variation of the enzyme concentration 0.2% and 0.5%. (b/v). The optimum conditions for protein hydrolysis were determined based on the dissolved protein content, the degree of hydrolysis and its anticholesterol activity. Anticholesterol activity testing was carried out using the HMG-CoA reductase enzyme inhibition method [17]. In this study, an anticholesterol bioactive peptide was isolated from yellow soybean protein hydrolyzate by hydrolysis with the bromelain enzyme under different conditions, resulting in a high degree of hydrolysis.

2. METHOD AND EXPERIMENTDENGAN

2.1 Material and Apparatus

Material: S. alba mangrove fruit was obtained from Blitar, East Java, Indonesia. S. alba mangrove fruit has its petals removed, washed under running water, sliced thinly and then dried for 2–3 weeks until it can be broken,were ground and sieved through a 60 mesh sieve

Bromelain enzyme in the form of dry powder extracted from pineapple with specific activity 0.14 Chemicals used with Pro Analysis specifications from Merck (Germany).

The equipment used includes: blender (stainless steel), centrifuge (Yenaco model YC-1180), spectrophotometer (Shimadzu 1600 A), pH meter (Jen Way type 3320, Germany), magnetic stirrer (Stuart Scientific), vortex (Thermolyne type 16700).), refrigerator, water bath (GFL 1083), analytical balance (Ohaus), electric heater (Gerhardt), spatula, vacuum oven, vortex (Maxi Max Type 16700), 80 mesh sieve and glassware.

2.2 Soybean Protein Isolation

Powder S. alba mangrove fruit soaked in technical hexane, at a ratio of 1:5 w/v for 1 hour at room temperature, then centrifuged at 7500 rpm for 15 minutes at 4°C. The supernatant obtained was discarded, the precipitate was re-extracted twice to remove the remaining fat content. The fat-free sample was mixed with distilled water at a ratio of 1:10 w/v, added 2 M NaOH dropwise to pH 8, stirred for 90 minutes at room temperature and centrifuged at 9000 rpm for 30 minutes at 4°C. Take the supernatant and add 2 M HCL drop by drop to pH 4.5 and then centrifuged for 20 minutes. The supernatant obtained was discarded, while the protein precipitate was taken and dried in a freeze dryer. The yield of the protein isolation process from S. alba mangrove fruit was calculated using the equation

$$\text{Rendemen Isolat Protein B (\%)} : \frac{\text{Weight isolate } protein \text{ (g)}}{\text{Weight S. alba mangrove fruit (g)}} \times 100\ \%$$

2.3 Hydrolysis of Protein Isolate

The protein isolate sample was hydrolyzed using the bromelain enzyme at a certain time, bromelain concentration and temperature. Then the solution was heated at 80^0C for 10 minutes with the aim of inactivating the enzyme, then Tris-HCL buffer pH 9. A total of 20 mL was added. The protein content of the isolate was added. The degree of hydrolysis of protein was measured by the Hoyle and Merrit method.

2. 4. Determination of Protein Levels in Protein Isolates

A total of 5 ml of protein isolate was added to 5 ml of Biuret reagent. The mixture was homogenized and incubated for 20 minutes at room temperature. Then measure the absorbance with a spectrophotometer at a wavelength of 540 nm. The concentration of protein in the protein isolate was calculated using a standard curve with the equation y = bx + a.

2.5 Determination of the Degree of Hydrolysis of Isolate Protein

Soy protein isolate hydrolyzate was taken as much as 10 mL and added with 10 mL of 10% (w/v) TCA solution. Next, the mixture was allowed to stand for 30 minutes, then centrifuged for 15 minutes at a speed of 12000 rpm. The supernatant was tested for protein content based on the method of Bradford (1976). The degree of hydrolysis is calculated using the equation:

$$\text{Degree of hydrolysis} = \frac{\text{Protein on the hydrolysis of protein isolate}}{\text{Protein on protein isolate}} \times 100\%$$

2.6 Antichosterol Hydrolyzate Isolate Protein Test

Testing of anticholesterol activity was carried out according to the work steps made by Sigma-aldrich. The reagents used were buffer, pravastatin, NADPH, HMG-CoA, HMG-CoA reductase (HMGR) enzyme, and hydrolysate. Before starting the test, the Elisa reader was set the wavelength to 340 nm. The reagents were added according to the procedure listed in Table 1.

Table 1. Sample formulation for HMGR . activity inhibition test

Sampel	Buffer (µL)	Pravastin (μL)	NaDPH	HMG-CoA	HMGR	Hidrolisat
Blank	184	-	4	12	-	-
Control negative	182	-	4	12	2	-
Control positive	181	1	4	12	2	-
Isolat 45	172	-	4	12	2	10
Isolat 50	172	-	4	12	2	10
Isolat 55	172	-	4	12	2	10

Every minute for 10 minutes, 200 µL of sample was measured for absorbance with an elisa reader (340 nm). The amount of enzyme activity is determined by the equation:

$$\text{Enzyme activity Unit/mgP} = \frac{(\Delta A\text{sample}) - (A\text{blank}) \times TV}{12.44 \times V \times 0.6 \times LP}$$

Information:

12.44 = 2 NADPH requirements during the reaction. (the coefficient for NADPH at 340 nm is 6.22/mM.cm)

TV = Total volume (mL)

V = Volume of enzyme used

0.6 = Enzyme concentration in mg-protein (mgP)/mL

LP = Light path (cuvet)

Asample = Absorbance sample at time tn - Absorbance sample at time tn+1

Asample = Absorbance blank at time tn - Absorbance blank at time tn+1

The percentage of inhibition is calculated by the equation:

$$\% \text{ inhibition} = \frac{(\text{Negative control enzyme activity}) - (\text{sample enzyme activity})}{(\text{Negative control enzyme activity})} \times 100\%$$

3. RESULT AND DISCUSSION

3.1 S. Alba Mangrove Fruit Protein Isolate

S. alba mangrove fruit protein was isolated by precipitation method using NaOH and HCl solution. The test results showed that the protein content in S. alba mangrove fruit was quite high, namely 47.28%. The total protein content of S. alba mangrove fruit isolate used in this study did not meet Codex Standard 175-1989 which stated that the minimum protein content of soy protein isolate was 90%≤. The low protein isolate content was possible because during the isolation process a lot of protein was wasted or dissolved in water during the washing and purification process of the protein.

3.2 Hidrolisis Isolate Protein

3.2.1 Effect of Time on Degree of Hydrolysis

The hydrolysis of S. alba mangrove fruit protein isolate was carried out with various variations of temperature, time and bromelain enzyme concentration. The time variations used in this hydrolysis step were 1, 2, 3, 4, 5, and 6 hours, and the concentrations of the bromelain enzyme used were 0.2% and 0.5%. with temperatures of 45^{0}C, 50^{0}C and 55^{0}C. The results of measuring the degree of hydrolysis of the hydrolyzate of S. alba mangrove fruit protein isolate at a temperature of 450C with different hydrolysis times and enzyme concentrations can be seen in Figure 1

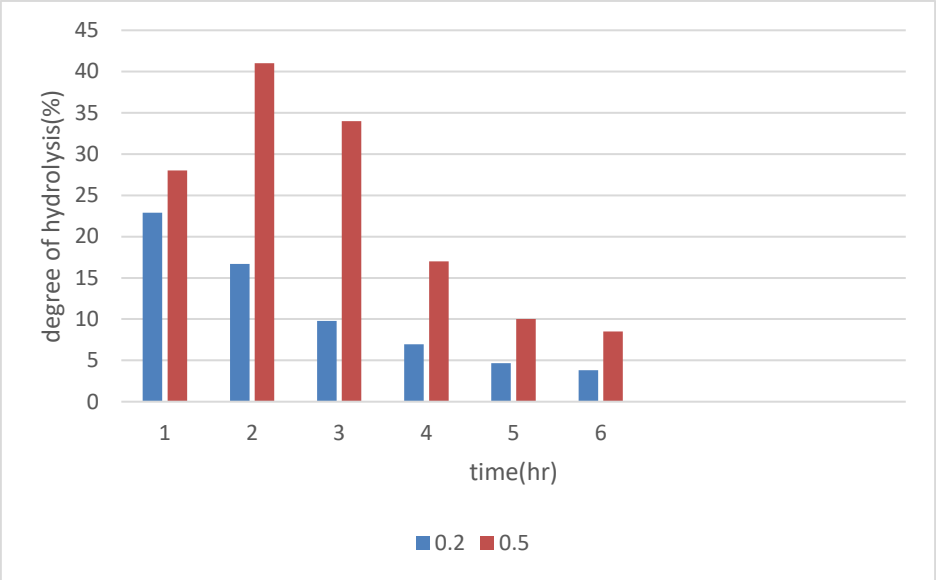

Fig 1. Effect time hydrolysis on degree of hydrolysis at temperature 45^{0}C

The value of the degree of hydrolysis at 45°C and the enzyme concentration of 0.5% in Figure 1 shows an increase in the value of the degree of hydrolysis at 1 to 2 hours and decreases at 3 to 6 hours. At 0.2% enzyme concentration showed a decrease in the value of the degree of hydrolysis with increasing hydrolysis time. The value of the most optimum degree of hydrolysis at 45^{o}C was obtained in 2 hours with an enzyme concentration of 0.5% where the value of % degree of hydrolysis obtained was 40.22%. According to Murray et al [18] enzyme concentration affects the rate of reaction between the substrate and the enzyme so that it will affect the resulting product. However, if the concentration of the enzyme used is too high or excessive, it can also cause the enzyme not to work optimally because of the presence of free enzymes that cannot bind to the substrate, so that the hydrolysis process becomes inefficient[19].If the temperature is increased to 50^{0}C, the value of the degree of hydrolysis at a temperature of 50 °C and the enzyme concentration of 0.2% in Figure 2 shows an increase in the value of the degree of hydrolysis of the enzyme 0.2% in Figure 2 shows an increase in the value of the degree of hydrolysis at time 1 to 6 hours, while the 0.5% enzyme concentration showed an increase in the time interval between 1 to 5 hours and decreased at 6 hours. The value of the largest degree of hydrolysis was obtained at the time of hydrolysis of 5 hours and the enzyme concentration was 0.5% with a value of % degree of hydrolysis of 25.09%. 2.1% as shown in Figure 2

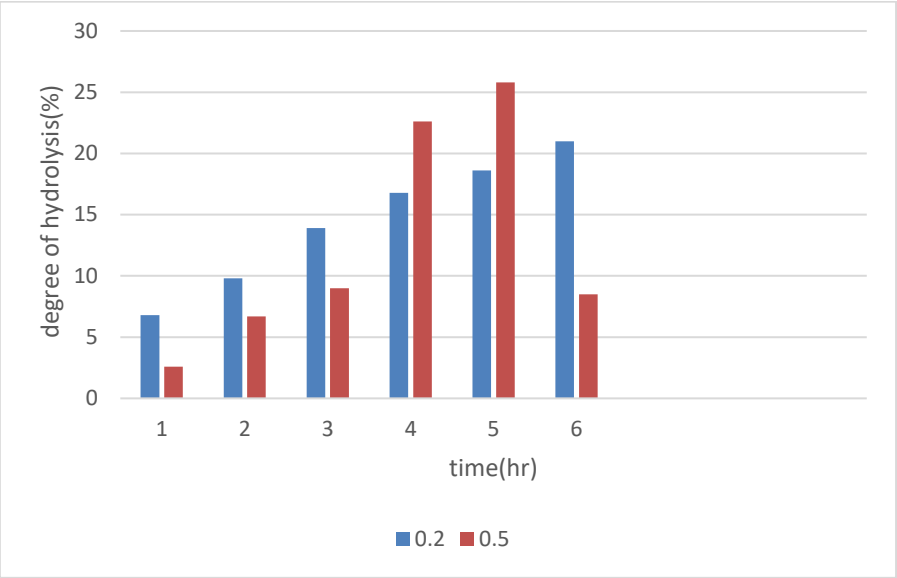

Fig 2. Effect time hydrolysis on degree of hydrolysis at temperature 50^{0}C

By increasing the hydrolysis temperature to 55^{0}C, with an enzyme concentration of 0.2%, it showed an increase in the value of the degree of hydrolysis at 1 to 2 hours and decreased again at 3 to 6 hours. On the other hand, the 0.5% enzyme concentration showed a decrease in the value of the degree of hydrolysis at a time interval of 1 to 6 hours. The value of the largest degree of hydrolysis was obtained at the time of hydrolysis of 2 hours and the enzyme concentration was 0.2% with a value of % degree of hydrolysis of 29.63%, while the smallest value of the degree of hydrolysis was found at the time of hydrolysis for 6 hours and the enzyme concentration was 0.5% with the value of % degree of hydrolysis. by 0.78% as shown in Figure 3.

In addition to the enzyme concentration, the optimum temperature of the enzyme and the time of hydrolysis are also influential factors in the hydrolysis process [20]. According to Poh and Majid[21] at a temperature of 35-45°C, the bromelain enzyme will work fully by releasing maximum kinetic energy which causes the collision between enzyme molecules and the substrate to accelerate, resulting in a sufficiently large amount of dissolved protein and is characterized by the percentage degree of hydrolysis is quite large as well. According to Zarei et al[22] when hydrolysis reaches optimum conditions, there will be a decrease in dissolved protein levels, because enzymes have a certain time or optimum time to work to hydrolyze substrates so that there can be an imbalance between enzymes and substrates and causes the hydrolysis process to fail. optimum again.

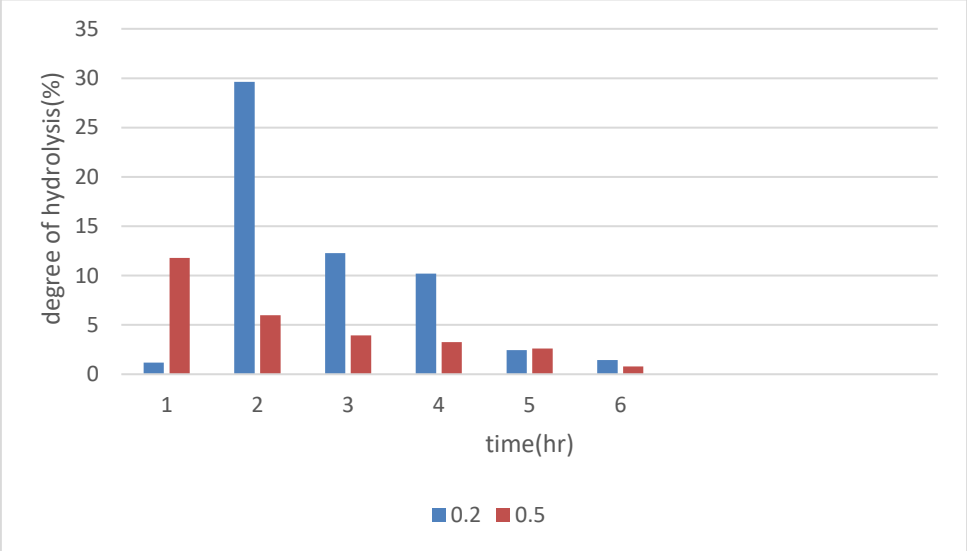

Fig 3. Effect time hydrolysis on degree of hydrolysis at temperature 55^{0}C

Whitaker [23] , stated that a high degree of hydrolysis can be produced because the bromelain enzyme has a wide cutting specificity for amino acid residues that make up its substrate which includes lysine, arginine, phenylalanine and tyrosine. Meanwhile, a lower degree of hydrolysis resulted in a hydrolyzate with a higher molecular weight fraction, which showed better emulsification and aeration properties but showed greater hydrophobicity [24].

According to Kamau and Lu[25], if the amount of enzyme concentration used to hydrolyze protein is increased, the value of % degree of hydrolysis will also increase. This happens because with the increasing amount of enzyme concentration, the speed of hydrolysis also increases. The greater the concentration of the enzyme used, the more peptide bonds that can be hydrolyzed quickly resulting in a high degree of hydrolysis, but on the other hand, if the concentration of the enzyme used is low, the value of the degree of hydrolysis produced also tends to be low because the amount of substrate availability is greater than the amount of enzyme. used, causing the peptide bond cutting speed to be slower .According to Himonides et al.[24] the degree of hydrolysis is defined as the percentage of the number of broken peptide bonds in the protein hydrolyzate and can determine the nature of the protein hydrolyzate. Factors that affect the degree of protein hydrolysis are enzyme concentration, type of enzyme used, temperature, pH and hydrolysis time [20].The enzymatic hydrolysis method was chosen because it can produce peptide products with specific amino acid composition and sequence according to the type of protease used. Enzymatic hydrolysis also works at neutral pH so it does not damage the amino acids produced . Enzymatic hydrolysis is important to pay attention to the physico-chemical conditions of the substrate before hydrolysis is carried out, where the temperature and pH of the solution must be in accordance with optimal conditions for enzyme work [26].

Based on the results of the three hydrolysis degree measurements above, the highest % hydrolysis degree value was taken, namely at a temperature of 45 °C with a hydrolysis time of 2 hours and an enzyme concentration of 0.5%, which was 40.22%. The results of the hydrolysis at the optimum conditions were then tested for anticholesterol properties. The samples used were hydrolyzate at 45 °C with an enzyme concentration of 0.5%; the hydrolyzate temperature was 50 °C, the enzyme concentration was 0.5% and the hydrolyzate temperature was 55°C the enzyme concentration was 0.2%.

3.3 Anticholesterol Activity of S. alba Mangrove Fruit Hydrolyzate

Anticholesterol activity was tested on three hydrolysates which had the highest degree of hydrolysis through an in vitro test using the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA reductase) enzyme inhibition method. The basic principle of this anticholesterol activity test is the ability to inhibit the HMG-CoA reductase enzyme by peptide substrates and compared to the positive control (pravastatin), the higher the inhibitory ability of the enzyme, the greater the anticholesterol activity. The mechanism of pravastatin works as an anticholesterol by competitively inhibiting the HMG-CoA reductase enzyme, because it has a structure similar to HMG-CoA. The HMG-CoA reductase enzyme works as a catalyst for HMGCoA compounds into mevalonic acid with the help of NADPH. If there is an inhibitor, the amount of remaining NADPH will be more than NADP+. The control has more NADP+ than NADPH. The results of the percentage inhibition (anti-cholosterol) test are shown in Table 2, Table 3 and Figure 4

Table 2. Data for enzyme activity

No	sample	$\Delta Asample$ (n=10)	$\Delta Ablanko$	TV	V	LP	Enzyme activity unit/mgP
1	Control negative	0.02925	0.00465	0.2	0.002	0.55	0.599
2	Pravastin	0.01855	0.00465	0.2	0.002	0.55	0.338
3	Isolat 45	0.0089	0.00465	0.2	0.002	0.55	0.103
4	Isolat 50	0.0125	0.00465	0.2	0.002	0.55	0.191
5	Isolat 55	0.0191	0.00465	0.2	0.002	0.55	0.364

Table 3. Data for % inhibition

No	sample	Enzyme activity unit/mgP)	% inhibiton
1	Control negative	0.599	-
2	Pravastin	0.338	43.57
3	Isolat 45	0.103	82.80
4	Isolat 50	0.191	68.11
5	Isolat 55	0.364	39.23

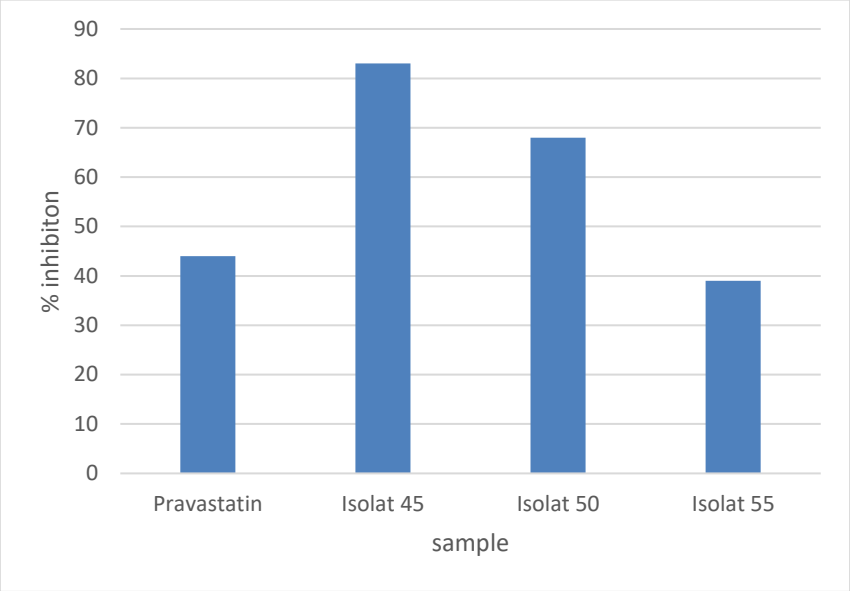

Fig 4. % inhibiton from hidrosilat isolate protein soy bean

The results of the anticholesterol activity test based on Figure 4 show that for the standard pravastatin with a concentration of 0.02125 g/mL, the percentage inhibition of HMG-CoA was 43.57% while the percentage inhibition of 82.8% was obtained from the hydrolyzate at 45°C, the concentration 0.5% enzyme, and the concentration of hydrolyzate was 7.371 g/mL. While the hydrolyzate sample with a temperature of 50 °C, 0.5% enzyme concentration and 4.598 g/mL hydrolyzate concentration obtained a percentage of inhibition of 68.11%, and the hydrolyzate with a temperature of 55 °C, 0.2% enzyme concentration and hydrolyzate concentration 5.4315 g/mL, the percentage inhibition was 39.23%. However, the percentage of inhibition of the samples obtained in this study is still relatively better when compared to the research of Pak et al.,[5] where the anticholesterol activity with % inhibition values of 31, 43, 45 and 50% was obtained from soybean hydrolyzate with a concentration of 1 g/mL which was hydrolyzed using pepsin enzyme at a concentration of 0.4%. The enzyme activity in the control was greater than the enzyme activity in the sample, the lower the enzyme activity value, the higher the inhibitory power[27].

Research conducted by Lin et al[7] showed that in silico peptide synthesis has anticholesterol activity by inhibiting the HMGCoA reductase enzyme. The peptide has the sequences Thr-Pro-Met-Ala-Ser-Asp (Hexapeptide), His-Phe-Lys-Trp (Tetrapeptide) and Pro-Met-Ala-Ser (Tetrapeptide). Each of the three peptides had IC50 values of 80 M, 80 M and 68 M with a peptide concentration of 100 M. Another study conducted by Wenny et al.[27] peptides derived from cowpea and modified with the Gln-Asp-Phe sequence (tripeptide) have anticholesterol activity. The peptide had an IC50 value of 12.8 M and a %inhibition value of 85.8% with a peptide concentration of 250 M. If seen from previous studies that peptides with amino acid residues 3 to 6 have anticholesterol activity. Peptide has a function as a bioactive component as an anticholesterol by inhibiting the HMG-CoA reductase enzyme which has a structure similar to the structure of HMG-CoA (3-Hydroxy-3-methyl-glutaryl-coenzyme A) or pravastatin. The characteristics of a peptide that is anticholesterol when viewed from its reactive group has a hydroxyl and carboxyl/carbonyl group as well as the groups in pravastatin and HMG-CoA [28].

Research by Lammi et al.[6] reported that 2 peptides from soybean -conglycinin namely YVVNPDNDEN and YVVNPDNNEN can induce hypocholesterolemia in HepG2 cells. The two peptides have molecular weights of 1178,158 Daltons and 1177.174 Daltons. Other research conducted by Lin et al [7], reported several peptides that have potential as hypocholesterolemic agents have amino acid sequences DHIHWITPSHPG, DHYSYTWFSWPT and QLEWSYWPQLSR with peptide molecular weights of 1384, 1583 and 1586 Dalton, respectively.

Soybean protein hydrolyzed using protease enzymes from Bacillus amyloliquefaciens bacteria can induce hypocholesterolemia using cultured cells in vitro [29]. The results of the analysis showed that hydrolysis of yellow soybeans using papain enzymes at 37 and 50°C had anticholesterol activity with inhibition of 78.29 and 95.65%, respectively. The inhibition value can be classified as very strong and the peptide content contained in the hydrolyzate can inhibit the enzymeHMG-CoA reductase, because according to Rinto et al., [29] the inhibition value can be classified as strong if it has a value greater than 50%, while the inhibition value is medium if it has a value greater than 25%. In this study, pravastatin was used as the standard, because pravastatin has anticholesterol activity and has a molecular structure similar to the HMG-CoA molecule (substrate).

4. CONCLUSION

Based on the results of the research conducted, the optimum conditions for hydrolysis of soybean protein isolate with bromelain enzyme which produced the best anticholesterol activity were obtained at 2 hours and a temperature of 45°C at a sample concentration of 7.371 ppm with a percent inhibition value of 82.80%. while the standard pravastatin has a lower percentage of inhibition (43.57%) at a concentration of 0.02125 ppm.In the study, soy protein isolate hydrolyzed using bromelain enzyme had good anticholesterol activity compared to that which was not hydrolyzed. Soy protein isolate which is hydrolyzed will produce specific peptides such as peptides that have a cyclic structure. Peptides that have a cyclic structure will be resistant to proteolytic enzymes in the body and also have a faster absorption orally.

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REFERENCES

1) Fu Zidong Donna , Julia Yue Cui , Curtis D Klaassen .2014. Atorvastatin induces bile acid-synthetic enzyme Cyp7a1 by suppressing FXR signaling in both liver and intestine in mice. *J Lipid Res*. **55**:2576-86.
2) Hippisley-Cox & Coupland, 2010. Unintended effects of statins in men and women in England and Wales: population based cohort study using the *QResearch database. BMJ*. **20;**340: c2197
3) Huupponen R and Viikari J. 2013. Statins and the risk of developing diabetes. *BMJ* **23**; 346:f3156
4) Høie LH, Morgenstern ECA, Gruenwald J, Graubaum HJ, Busch R, Lueder W, & Zunft HJF. 2005. A double blind-blind placebo-controlled clinical trial compares the cholesterol lowering effects of two different soy protein preparations in hypercholesterolemic subjects. *European Journal of Nutrition* **44**: 65-71.
5) Pak, V.V., Koo, M.S., Kasymova, T.D. & Kwon, D.Y. 2005. Isolation and 63 Identification of Peptides from Soy 11S-Globulin with Hypocholesterolemic Activity. *Chemistry of Natural Compounds***,41**: 710-714
6) Lammi, C., Zanoni, C., & Arnoldi, A. 2015. IAVPGEVA, IAVPTGVA, and LPYP, three peptides from soy glycinin, modulate cholesterol metabolism in HepG2 cells through the activation of the LDLR-SREBP2 pathway. *J. Funct. Foods* **14** :469– 478.
7) Lin, SH., Chang, DK., Chou, MJ., Huang, KJ., & Shiuan, D. 2014. Peptide inhibitors of human HMG-CoA reductase as potential hypocholesterolemia agents. *J. Biochemical and Biophysical Research Communications.***456** :104-109
8) Meinlschmidt, P., Sussmann, D., Schweiggert-weisz, U., & Eisner, P. 2015. Enzymatic treatment of soy protein isolates: effects on the potential allergenicity , technofunctionality , and sensory properties. *Food Sci Nutr* **4**:11-23.
9) Zeng Maomao, Benu Adhikari, Zhiyong He, Fang Qin, Xiaolin Huang & Jie Chen 2013 Improving the Foaming Properties of Soy Protein Isolate Through Partial Enzymatic HydrolysisDrying Technology *An International Journal* 31 : 1545-1552
10) Chang Liu**,** Mamata Bhattarai, Kirsi S. Mikkonen, and Marina Heinonen.2019 Effects of Enzymatic Hydrolysis of Fava Bean Protein Isolate by Alcalase on the Physical and Oxidative Stability of Oil-in-Water Emulsions. *J. Agric. Food Chem.* **67**: 6625–6632
11) Kim, SK. 2013. Marine Proteins and Peptides, Biological Activities and Applications. John Wiley and Sons, Ltd. Oxford (UK).
12) Whitaker, J.R. 2018. Principles of Enzymology for the Food Sciences Second Edition. New York : Routledge
13) Steven J.Taussig,, Stanley Batkin.1988. Bromelain, the enzyme complex of pineapple (*Ananas comosus*) and its clinical application. An update. *Journal of Ethnopharmacology*,**22** : 191-203
14) Walker A.F.R.Bundy,S.M.Hicks,R.W.Middleton.2002.Bromelain reduces mild acute knee pain and improves well-being in a dose-dependent fashion in an open study of otherwise healthy adults. *Phytomedicine* **9** : 681-686
15) Poh, S.S., and Majid A. 2011. Thermal stability of free bromelain and bromelain polyphenol complex in pineapple juice. *International Food Research Journal* **18**:1051-1060.
16) Indumathy, S., K. Kiruthiga, K. Saraswathi, and P. Arumugam. 2017. "*Indo American Journal of Pharmaceutical Research*, 2231- 6876.
17) Ana A. Arantes, Pedro L. Faléa, Larissa C.B. Costa, Rita Pacheco, Lia Ascensãoe, Maria Luísa Serralheiro.2016 Inhibition of HMG-CoA reductase activity and cholesterol permeation through Caco-2 cells by caffeoylquinic acids from Vernonia condensata leaves .*Brazilian Journal of pharmacology***26** : 738-743
18) Murray, R. K., Granner, D. K., & Rodwell, V. W. 2009. Biochemistry harper. Book of medicine EGC. Jakarta
19) Pelczar, M.J., & Chan E.C.S. 2005. Basic of Microbiology. Jakarta : UI Press.

20) Haslaniza, H., Maskat, M.Y., Wan Aida, W.M. dan Mamout, S. 2010. The effects of enzyme concentration, temperature and incubation time on nitrogen content and degree of hydrolysis of protein precipitate from cockle (Anadara granosa) meat wash water. *International Food Research Journal*, **17:** 147- 152.
21) Poh, S.S., and Majid A. 2011. Thermal stability of free bromelain and bromelain polyphenol complex in pineapple juice. International Food Research Journal 18(3):1051-1060. [22].Zarei, M., Ebrahimpour, Abdul Hamid, A., Anwar, F., Abu, F., Philip, R., & Saari, N. 2014. Identification & Characterization of Papain-Generated Antioxidant Peptidas from Palm Kernel Cake Proteins. *Journal Food International*, **62**: 726-734.
22) Whitaker, J.R. 2018. Principles of Enzymology for the Food Sciences Second Edition. New York : Routledge
23) Himonides, A.T., A.K.D. Taylor, & A.J. Morris. 2011. A Study of the Enzymatic Hydrolysis of Fish Frames Using Model Systems. *Food and Nutrition Sciences.* **22:** 575-585.
24) Kamau, S.M. dan Lu, R. 2011. The Effect of Enzymes and Hydrolysis Conditions on Degree of Hydrolysis and DPPH Radical Scavenging Activity of Whey Protein Hydrolysates. *Current Research in Dairy Sciences*, **3**: 25-35.
25) Bhat, Z. F., Kumar, S., & Bhat, H. F. 2015. Bioactive Peptides of Animal Origin : a Review. *Journal Food Science Technology,* **52** :5377-5392
26) Wenny Silvia L. Br. Sinaga, Wangsa T. Ismaya, Debbie S. Retroningrum , Raymond R. Tjandrawinata , Maggy T. Suhartono.2020.Peptides Hydrolysate Derived from Collagen of Snakehead Murrel (Channa striata) Skin Demonstrate Anti-cholesterol and Antioxidant activities.*Hayati* **27** : 136-141
27) Cho Seong-Jun , Sung-Hoon Oh, R. David Pridmore,Marcel A. Juillerat, and Cherl-Ho Lee.2003.Purification and Characterization of Proteases from *Bacillus amyloliquefaciens* Isolated from Traditional Soybean Fermentation Starter . *Agric. Food Chem.* **5**1: 7664–7670
28) Rinto, Ratih Dewanti, Sedarnawati Yasni, Maggy Thenawidjaja Suhartono. 2017.Novel HMG-CoA Reductase Inhibitor Peptide from Lactobacillus acidophilus Isolated from Indonesian fermented food Bekasam . *Journal of Pharmaceutical, Chemical and Biological Sciences* **5**:195-204