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# THE HMG-CoA REDUCTASE INHIBITOR ACTIVITIES OF SOY PROTEIN HYDROLYSATES FROM PAPAIN HYDROLYSIS

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## ABSTRACT

The search for an HMG-CoA reductase inhibitor agent as a safe and inexpensive alternative treatment for hypercholesterolemia, without side effects has been carried out using soy protein hydrolysate as an anti-cholesterol bioactive peptides source. This study was performed through an *in vitro* assessment, by testing the inhibition capacity of HMG-CoA reductase enzyme as a key component of cholesterol biosynthesis. Furthermore, sample preparation commenced with soy protein isolation through acid precipitation, and was further separated by centrifugation. The yield was then tested proximately and hydrolyzed with papain enzyme at an enzyme: substrate ratio of 0.2% (w / v), with an incubation time of 0-6 hours and 37, 50, and 55 oC temperature. The protein hydrolysates were subsequently evaluated for hydrolysis degree (% DH), molecular weight profiles with SDS-PAGE (*Sodium Dodecyl Sulphate Polyacrilmaide Gel Electrophoresis*) and anti-cholesterol activity through HMG-CoA reductase inhibition tests. However, the sample with highest inhibition percentage was fractionated using gel filtration *chromatography* (Sephadex G-10). Consequently, the peptide fraction obtained was characterized by LCMS QTOF (*Liquid Chromatography Mass Spectrometry Quadrupole Time-of-Flight*) for molecular weight determination. The results indicated the following optimum hydrolysis conditions of soy protein isolates: 3 hours incubation time, a temperature of 50 °C with a 33.39% DH and a percent inhibition value of 95.65% (protein concentration 1.96 µg / mL). Therefore, SDS-PAGE analysis showed the manifestation of protein hydrolysate bands below 10 kDa. Meanwhile, the fractionated peptide fragments possessed molecular weights 2029 and 1514 Da, and are assumed to have structural similarity with the HMG-CoA (3-hydroxy-3-methyl-glutaryl-coenzyme A) compound. Also, the study showed an intrinsic potential for anti-cholesterol activity by the bioactive peptide.

**Keywords:** Soy protein, papain hydrolysate, anti-cholesterol, HMG-CoA reductase

## 1. Introduction

The presence of high level cholesterol in the blood is a trigger for clogged arteries (atherosclerosis), and further implicated in cardiovascular or coronary heart disease. This condition is a global threat and the number one cause of death worldwide. The report from American Heart Association (AHA) in 2017 show over 17.8 million people die of heart and blood vessel disease, which is 21.1% higher than the data collected ten years ago (Virani et al., 2020). Meanwhile, a reduction in the consumption of fatty foods, performing physical exercises, combined with proper medication helps prevent and treat hypercholesterolemia. Moreover, there is lack of substantive data to prove the direct relationship between high cholesterol levels and death. This disorder often results in atherosclerosis, stroke, and coronary heart disease (Csonka et al., 2016)

According to previous studies, cardiovascular risks, including coronary heart disease and stroke is increased by hypercholesterolemia (Branch, 1991); (Otvos et al., 2006). Furthermore, with regards to age distribution, cardiovascular is mostly diagnosed in people between 65-74 (3.6%) followed by 75 and above (3.2%), 55-64 (2.1%) and lastly 35-44 years old (1.3%), while according to economic status, this disease is common among the lower (2.1%) and middle income class (1.6%) (Trialists, 2005).

Moreover, modern drugs, including lovastatin, pravastatin, gemfibrozil, fenofibrate are used to lower triglyceride and cholesterol levels (Hoie, 2010). However, people prefer to use statins, resulting from the capacity to quickly lower cholesterol levels. According to (Hippisley-Cox & Coupland, 2010) therapeutic applications in certain conditions triggers some side effects especially kidney failure, moderate / severe myopathy, cataracts, and liver dysfunction. The recommended dose in hypercholesterolemic patients is between 10 to 40 mg / day, and long-term usage have been implicated in myopathy, kidney failure, and liver damage (Lyons & Harbinson, 2009), while excess dose and certain conditions increases the risk of developing type 2 diabetes (Huupponen & Viikari, 2013). Previous studies have shown various herbs or food plants capable of reducing cholesterol levels with very minimal side effects.

Currently, there is less exploration of anti-cholesterol sources from natural food plants, where soybeans as one of the few potential resources. The research conducted few decades ago identified beans as a plant-based food product with various antioxidant (Agyei, 2015) antimicrobial (Vasconcellos et al., 2014) and antihypertensive potentials (Shimakage et al., 2012). Meanwhile, several types are also known to possess natural antioxidant-producing agents rich in phenolic compounds (Scalbert et al., 2005).

In addition, supplements manufactured from soy grains help to lower cholesterol levels and soy protein has the capacity to diminish heart disease risks by reducing in blood cholesterol or Low Density Lipoprotein (LDL) levels (Puska et al., 2002). The soybean peptides LPYP, IAVPGEVA and IAVPTGVA have been reported to effectively activate LDLR-SREBP 2 pathway and increase uptake of LDL in blood, therefore inhibiting HMG-CoA reductase activity in HepG2 cells (Lammi et al., 2015). Furthermore, consuming diets low in saturated fat and cholesterol, including 25g of soy protein per day is estimated to reduce the risk of heart disease. The meta-analysis from 38 clinical trials proved the potential to reduce the concentration of total cholesterol, LDL and triglycerides in the blood (Anderson et al., 1995).

The consumption of soy-based products has significantly reduced blood lipid content based on the composition. These include the protein isolates rich in isoflavones, soy fiber, cotyledons and phospholipids (Anderson & Hoie, 2005). However, the reduction mechanism resulting from the presence of soy protein remains unclear, hence the need for further investigation. The use of supplements manufactured from soy has produced substantial efficacy and tolerability. In addition, many of these products currently in the market are claimed to lower cholesterol levels, although there is less valid scientific data to support this claim. This study, therefore, explores the potential protein hydrolysate product of soy beans as an anti-cholesterol, and was tested by hydrolyzing papain. Furthermore, hydrolysis was performed using an enzyme: substrate ratio of 0.2% (w / v) with variations in incubation times of 0, 1, 2, 3, 4, 5 and 6 hours at 37, 50 and 55°C (Mutamimah et al., 2018a). The optimum treatment condition was determined based on dissolved protein content, degree of hydrolysis value and anti-cholesterol activity. These were evaluated through an in vitro approach, using the enzyme HMG-CoA (3-Hidroxy-3-Methylglutaryl-Coenzyme A) reductase. This is an important factor in determining cholesterol biosynthesis, especially for the formation of mevalonic acid from hydroxy Methylglutaryl-Coenzyme A (HMG-CoA), and also reduce blood cholesterol levels (Lyons & Harbinson, 2009); (Rinto et al., 2019). Furthermore, pravastatin was used as a positive control for HMG-CoA reductase inhibitors, which played a key role in suppressing cholesterol synthesis (Gotto, 2003). Consequently, the products' potentials with bioactive peptides from

enzymatic hydrolysis are determined and expected to be applied as an alternative, cheaper and safer anti-cholesterol agent.

## **2. RESEARCH METHODS**

### **2.1 Sample preparation**

The soybeans used in the study were obtained from supermarkets in the South Jakarta area. These legumes were cleaned and blended in cold water, then precipitated with 1 M hydrochloric acid to obtain the hydroxylates. The mixture was further centrifuged at a speed of 12000 rpm for 15 minutes to produce protein precipitates. Therefore, dialysis was performed for 24 hours, before freeze-drying, and storage at 4 °C.

### **2.2 Proximate Test**

The proximate test was performed on soy protein isolates after precipitation. This involved the assessment for crude protein, crude fat, moisture, and ash contents (AOAC International, 2016).

### **2.3 Measurement of protein content**

The measurement of protein precipitate levels was conducted with the Bradford method using BSA solution as a standard (He et al., 2015). This sample solution vortexed as the Bradford reagent was added and incubated at room temperature for 10 minutes. Furthermore, blue color with a 595 nm wavelength was produced, and the protein content was consequently extrapolated from the standard curve using the formula:  $y = ax + b$  where:

y = absorbance, x = concentration.

### **2.4 Hydrolysis of Soy Protein Isolates**

The papain enzyme was utilized in the enzymatic hydrolysis of soy protein isolates. This was performed using a 7.5 pH phosphate buffer at 0.1 M concentration, as well as temperatures of 37, 45, and 50 °C. The ratio of substrate used was 0.2% (w / v) with an incubation time of 0-6 hours (Mutamimah et al., 2018b). Furthermore, 5 mL of the mixture was obtained at intervals of 0, 2, 4, 8, and 12 hours during this process, then the degree of hydrolysis was measured. Subsequently, each hydrolysate mixture and the enzyme were inactivated by adding 0.5 M Tris-HCl with pH 8 and heated at 80 °C for 5 minutes.

### **2.5 Determination of Hydrolysis Degree**

The degree of hydrolysis was calculated using the SN-TCA method (Hoyle & Merritt, 1994). Approximately 5 mL of protein hydrolysate was added with 20mL TCA 10% (w / v). This mixture was then allowed to settle for 30 minutes and centrifuged at a speed of 12000 rpm for 15 minutes. The supernatant protein content was determined with the Bradford method, and consequently calculated with the following formula:

$$N\ HCl = \frac{\text{TCA dissolved protein 10\%}}{\text{the total protein sample}} \times 100\%$$

### **2.6 Anticholesterol Activity Test (Liang et al., 2015)**

#### **Reagent preparation**

The reagent used in this test contained an enzyme solution of HMG-CoA reductase dissolved in 550 µl buffer. This substrate along with NADPH was dissolved in 1.3 ml and 440 µl dH<sub>2</sub>O, respectively. Subsequently, all diluted reagents and a 10 mM pravastatin inhibitor were stored at -20 °C and room temperature respectively, before use.

### Sample preparation and measurement of inhibition %

The 5 mL protein hydrolysate sample was prepared and filtered using a 0.45 µm membrane. Therefore, the filtrate was used as an inhibitor in the HMG-CoA reductase inhibition test with the help of an assay kit, where pravastatin was utilized as a positive control. The % measurement was performed at an elisa reader wavelength of 340 nm. Subsequently, reagents were added according to the details in Table 1.

**Table 1.** Addition of anti-cholesterol activity test reagents

Mixture	buffer (µL)	pravastatin (µL)	NADPH (µL)	HMG- CoA (µL)	HMGR (µL)	Hydrolysate (µL)
Blank	184	-	4	12	-	-
Negative control	182	-	4	12	2	-
Positive control	181	1	4	12	2	-
Sample	172	-	4	12	2	10

Approximately 200 µL of each mixture was read at a wavelength (340 nm) every minute for 10 minutes. The enzyme activity was then calculated using the equation:

$$\text{Unit/mgP} = \frac{(\Delta A_{340}/\text{minsample} - \Delta A_{340}/\text{minblank}) \times TV}{12,44 \times V \times 0,6 \times LP}$$

Description:

12,44 = it needs 2 NADPH during the reaction. (the coefficient for NADPH at 340 nm is 6.22 / mM.cm)

TV = Total reaction volume (1 mL)

V = Volume of enzymes used

0,6 = Enzyme concentration in mg-protein (mgP)/mL

LP = *Ligh path* (1 cm for *cuvettes* and 0,55 cm for *plate*)

Furthermore, inhibition % was calculated using the equation:

$$\text{Inhibition \%} = \frac{\text{Enzyme activity (non inhibitor)} - \text{Enzyme activity (pravastatin/sample)}}{\text{Enzyme activity (non inhibitor)}} \times 100\%$$

### 2.7 Analysis of SDS-PAGE protein hydrolysate (Laemmli, 1970)

The protein profile for soybean hydrolysates was determined using the SDS-PAGE method with a 7.5-17.5% resolving and 4% stacking gel solution in a buffer of 1.5 M Tris HCl at pH 8.45. These samples were denatured with a buffer, including 1% Coomassie brilliant blue, 25% glycerol, 6.8 pH Tris-HCl 1M, 20% SDS, and boiled at 90 °C for 2 minutes where the ratio with protein was 1:1. The electrophoresis device was prepared using a resolving and stacking solution with a concentration of 1.5 M and 0.5 M at pH 8.8 and 6.8, respectively. The resulting sample was termed Tris-HCl. Subsequently, 1.5 % bisacrylamide and 48% acrylamide were added, and the electrophoresis process commenced for 55 minutes at a voltage of 150 volts with a Biorad protein marker, where the range of 7.7-204.0 kDa was compared. Also, 0.1% (w / v) dye solvent was used for protein staining and the process yield were washed using a 7.5% acetic acid and 40% methanol solvent.

## **2.8 Separation and purification of anti-cholesterol peptides from hydrolysates (Li et al., 2008)**

The soybean protein hydrolysate with the highest anti-cholesterol activity value of 15 ml was concentrated by freeze drying. This was consequently fractionated using a G-10 sephadex column to separate the peptide portion below 3 kDa. The molecular weight was then analyzed using an LCMS / MS QTOF Mass Analyzer.

## **2.9 Peptide identification using LCMS QTOF Mass Analyzer (Zhou et al., 2016)**

The total of 5 µL purified peptides were obtained and filtered using a 0.2 µm syringe filter then injected into the Analyzer. Furthermore, ionization was performed using ESI electrospray ionization mode. The ESI parameters used include Column C-18 with a 1.8 µm x 2.1 x 100 mm size, 0.2 mL / min flow rate, and the acetonitrile mobile phase. The process also utilized a 1: 1 v / v water, source voltage range of 4 - 50 volts and a capillary temperature of 50 ° C. The molecular weights of peptides were analyzed by mass spectroscopy, while the deconvolution calculations were conducted using ESIPROT online ([www.bioprocess.org/esiprot/](http://www.bioprocess.org/esiprot/)).

# **3 RESULTS AND DISCUSSION**

## **3.1 Proximate Value of Soy Protein Isolates**

Table 2 shows the proximate analysis results of soy protein isolates, indicating the presence of crude protein, crude fat, moisture and ash contents.

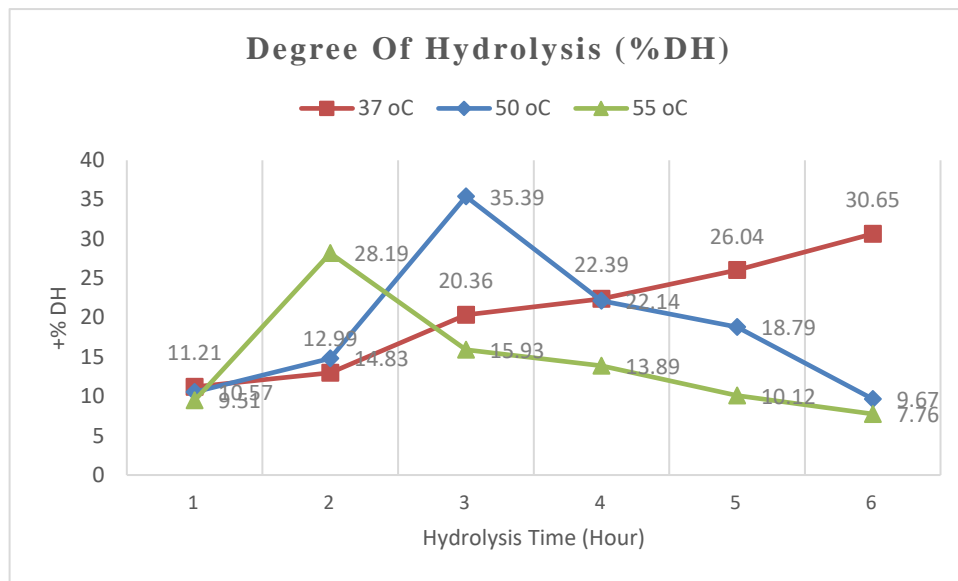
**Table 2.** Proximate composition of soybean protein isolates

<b>Proximate composition</b>	<b>Soy protein isolate (%)</b>
<b>Crude protein content</b>	36,59±0,01
<b>Crude fat content</b>	10,24±0,26
<b>Moisture content</b>	8,22±0,06
<b>Ash content</b>	4,26±0,08

The proximate analysis results show a crude protein content of <50%. This low level was probably due to the lack of an optimal isolation process for soy protein, and most were assumed to be soluble and not precipitated. This study was performed using an imported variant with a possible difference in chemical composition from the local form. Furthermore, results from the moisture analysis and ash contents indicated a relatively low moisture range for the isolates. The highest value tends to make the microbes grow easier and trigger legume damage with mold. However, total ash content determination was conducted to establish the quality of soybeans processing. This parameter represents the presence of important minerals, including organic and inorganic salts where a high amount consequently influenced the enzyme activity in food production.

## **3.2 Hydrolysis of Soy Protein Isolates**

The hydrolysis of soy protein isolates was performed using the 0.2% (w / v) papain enzyme with incubation temperatures of 37, 50, and 55 ° C (Mutamimah et al., 2018a). Figure 1 shows the calculation for percentage of protein hydrolyzed based on the degree of hydrolysis (% DH).



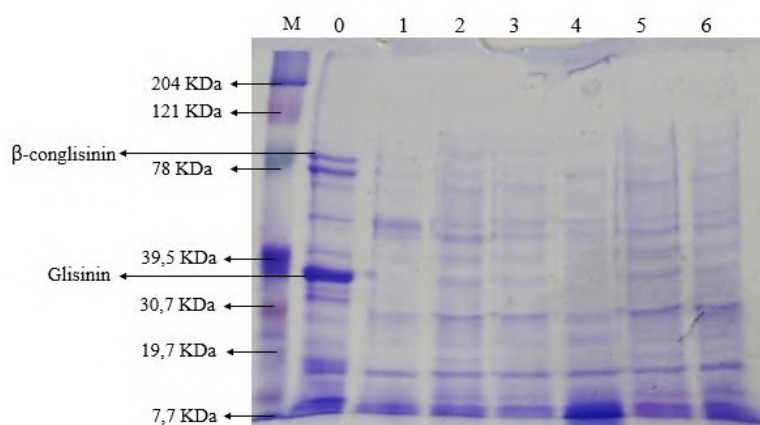
**Figure 1.** Soy protein isolate hydrolysis degree value at 37, 50, and 55 °C hydrolysis temperatures

The hydrolysis degree test results showed the highest % DH values at 50 °C, with an incubation time of 3 hours. However, the most significant value obtained at 37 °C was observed at 6 hours, while 2 hours was recorded for treatments with 55 °C. The differences in % DH value at different temperature conditions were attributed to the increased heat estimated to affect enzyme activity. However, optimum enzyme activity was achieved at 50 °C. In addition, the hydrolysis degrees value reported at 37 °C increased linearly over 1-6 hours, while a steady decline was observed with exposure to 50 and 55 °C, which was prominent after 3 and 2 hours. This phenomenon occurs due to the tendency for higher hydrolysis temperature to increase the kinetic energy of enzymes and substrate. Therefore, the molecular collisions tends to become faster and substrate enzyme complex formed produces an ideal activation energy for the inception of a reaction. This condition leads to better effectiveness, and consequently higher product yield at the optimum temperature (Schowen, 2003)

In contrast with a study by Anggraini & Yuniarta, (2015) the highest hydrolysis degree value of 35.39% was produced using the papain enzyme at 50 °C. This was stipulated as the temperature for optimal activity (Ashie et al, 2002). However, the highly significant hydrolysis degree was associated with the amount of product, and was estimated to have a directly proportional relationship with the amount of dissolved protein or free amino groups (Sun, 2011). Marcela, (2017) ascribed the elevated values to an increase in peptides and amino acids dissolved in TCA, following the incidence of peptide bonds breakage during protein hydrolysis. The extent of degradation recorded in this study is indicated by the effect of enzyme concentration and the time required. Therefore, the most efficient papain intensity involved in the hydrolysis of soy milk protein was 0.2%, over a 3 hours process duration.

### 3.3 Soy Protein Hydrolysate Profile Results of SDS-PAGE analysis

The SDS-PAGE analysis of soy protein hydrolysate was conducted to assess the protein profile before and after hydrolysis. Figure 2 shows the analysis results obtained at optimum temperature (50 °C).



**Figure 2.** Soy protein hydrolysate electrophoregram (M = Marker, 0 = before hydrolysis, 1-6 = hydrolysis time 1-6 hours)

Figure 2 demonstrates the different sizes / molecular weights of protein bands in the soy protein profile before and after hydrolysis. In addition, several major bands appeared below 39.5 KDa at 0 hours, and were assumed to be the glycinine type (Wang et al., 2014). Moreover, other protein varieties were observed above 78 kDa, therefore indicating the presence of  $\beta$ -conglycinin forms (Barać et al., 2011). These bands detected tend to decrease in intensity and fades away after hydrolysis at 1-4 hours, followed by an increase in thickness to below 10 KDa over time. Furthermore, this outcome indicates the complete breakdown of glycinine and  $\beta$ -conglycinin present in soy protein isolates. However, the bands subsequently reappeared with lower intensity at 5-6 hours. This finding is in line with the results obtained during hydrolysis degree evaluation at 50 Oc, where another decline in DH value was recorded 5-6 hours after the optimum condition was reached at 3 hours. Based on the soy protein isolate profile, an extended hydrolysis time using papain enzyme leads to more significant conversions of longer protein structures into shorter peptides.

The soybean protein is known to have a  $\beta$ -conglycinin fraction, consisting of  $\alpha$   $\beta$ -conglycinin,  $\beta$   $\beta$ -conglycinin and  $\gamma$   $\beta$ -conglycinin. These components possess molecular weights of 80.22 KDa, 48.42 KDa and 46.24 KDa, respectively. Particularly, the single protein fraction termed glycinine is characterized by A-glycinin and B-glycinin, with molecular weights of 34-35 KDa and 18-22 KDa, respectively (Barać et al., 2011). This glycinine molecule model is a hexamer comprising of five sub-units, including Gly I (A1A B1B), Gly II (A2 B1B), Gly III (A1B B2), Gly IV (A4 B5 B3) and Gly V (A3 B4), where each has two polypeptide components, encompassing acid and base. Meanwhile,  $\beta$ -conglycinin is a glycoprotein present in soybeans with a trimeric molecular model, and is Known to possess 3 subunit types, including the  $\alpha$ ,  $\alpha$  and  $\beta$  (Wang et al., 2014).

### 3.4 HMG-CoA Reductase Soy Protein Hydrolysate Inhibitory Activity

Figure 3 shows the results of HMG-CoA reductase inhibitory activity test for soybean hydrolysate and pravastatin samples, which was used as a positive control. The analysis showed highest inhibition activity of 95.65% at 50 °C, with a concentration of 1.96 ppm. This was followed by treatments at 37 °C (78.29%) and 55 °C (52.37%). Figure 3 showed a lower value with the standard pravastatin at 43.57%.



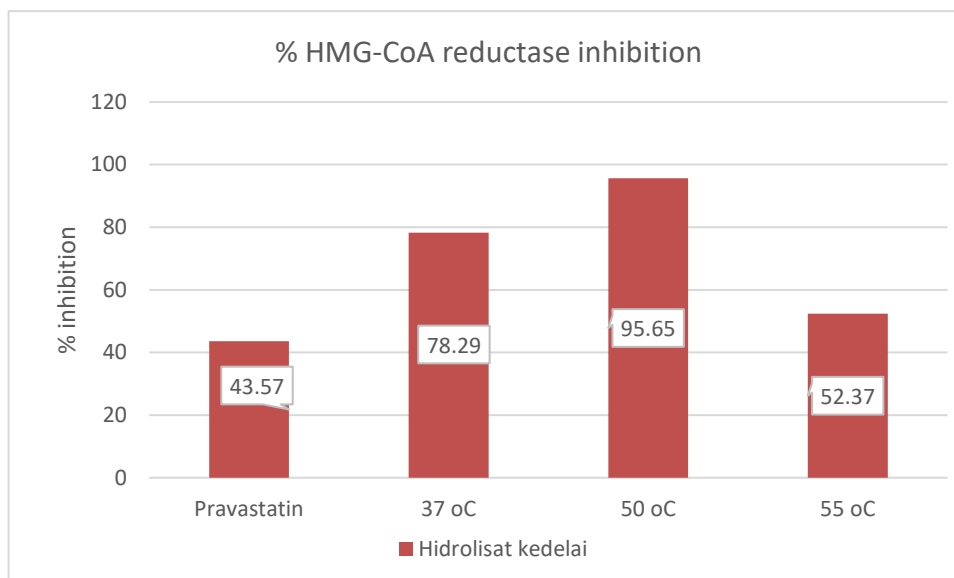


Figure 3. Percent inhibition of HMG-CoA reductase from soy hydrolysate

The low percentage value of pravastatin inhibition was attributed to the decreased concentration used, compared to the hydrolysate samples at 37, 50 and 55°C. The high hydrolysis degree at 50°C was probably due to the relatively different peptide fragments produced in each hydrolysate, which varied between sample. According to Pak et al., (2005) soybeans hydrolysis using the enzyme pepsin produces anti-cholesterol activity, with a 45% inhibition value (protein concentration of 1 ppm).

Moreover, HMG-CoA Reductase (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase) is a controlling enzyme in cholesterol biosynthesis (EC 1.1.1.88; NADPH dependent, EC 1.1.1.34) produced from the mevalonate pathway. The introduction of catalysts of this reaction facilitates the conversion of HMG-CoA to mevalonic acid, and is considered the first step in cholesterol biosynthesis (Figure 4).

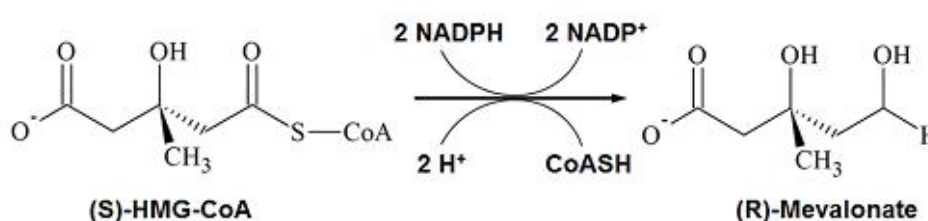


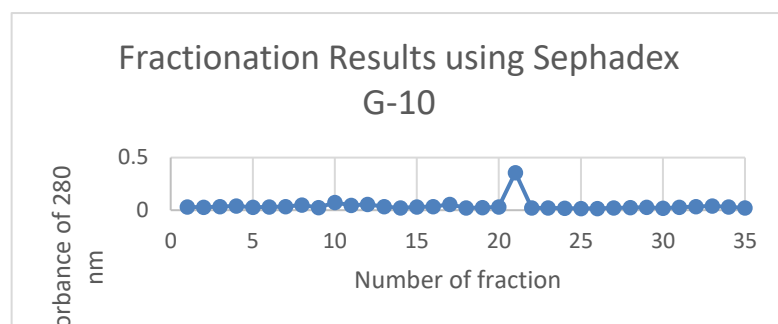
Figure 4. The formation reaction of mevalonic acid from HMGCoA using HMGCoA reductase enzyme and 2 molecules of NADPH through the transfer of 2 hydride ions. (Son et al., 2013)

The HMG-CoA reductase enzyme functions by catalyzing the HMG-CoA compounds into mevalonic acid, with the help of NADPH, estimated to serve as a precursor in cholesterol formation. Meanwhile, the inhibitors of this enzyme tend to effectively reduce cholesterol biosynthesis, and are considered valuable hypolipidemic agents, therefore the drug of choice for atherosclerosis. In addition, pravastatin is a polyketide bioactive component synthesized by the microorganism *Bacillus megaterium*, and is commercially used as one of the drugs to reduce cholesterol biosynthesis. The medication activity is executed by inhibiting the HMG-CoA

reductase enzyme, due to the structural characteristics with close resemblance to HMG-CoA. However, the enzyme inhibition in the liver instigates an increase in hepatic LDL receptor expression, which reduces plasma LDL cholesterol levels. Therefore, a large number of HMG-CoA reductase inhibitor studies use pravastatin as a positive control while treating hypercholesterolemic disorders. (McFarland *et al.*, 2014).

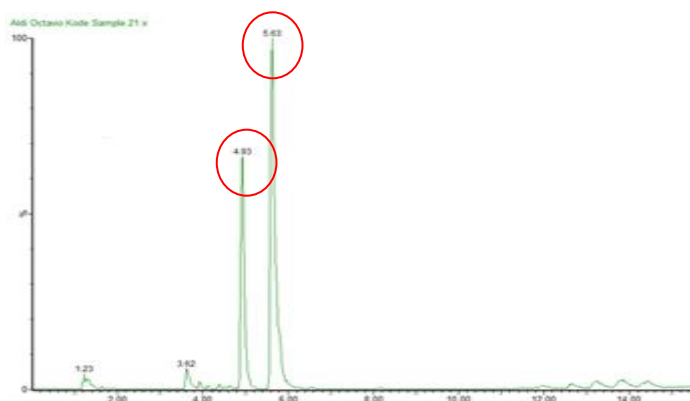
### 3.5 Results of Soy Protein Hydrolysate Separation and Molecular Weight Analysis

The soy protein hydrolysate with the highest HMG-CoA reductase inhibitory capacity was purified and separated by gel filtration chromatography (Sephadex G-10). Figure 5 shows the fractionation output, and the 21st fraction reportedly produced the most significant absorption value, with an absorbance of 0.36. This yield is assumed to contain protein/peptide with smaller molecular weight compared to others. In addition, further analysis was performed for molecular weight using the LCMS QTOF Mass Analyzer, and the results are shown in Figure 6.



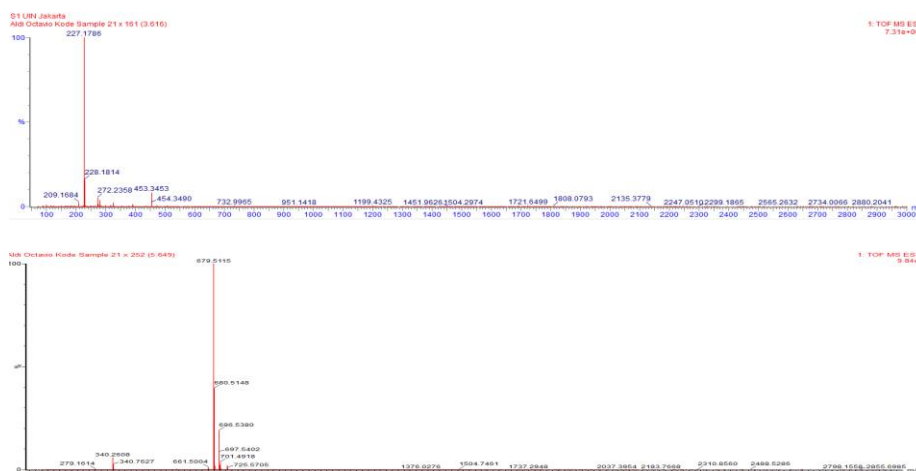
**Figure 5.** Fractionation results of soy protein hydrolysate (temperature of 50°C)

Figure 6 showed the LCMS analysis results of the 21st fraction, and two dominant peaks with retention times of 4.82 and 5.53 minutes were obtained.



**Figure 6.** Chromatogram for the 21st fraction of LCMS QTOF-MS analysis results.

Figure 7 demonstrated mass spectra with  $m/z$  data, while the molecular weight was analyzed using ESIprot online ([www.bioprocess.org/esiprot/](http://www.bioprocess.org/esiprot/)).



**Figure 7.** Mass spectrum of peptide compound fraction 21 with (a) tR 4.82 minutes (b) tR 5.53 minutes.

Based on the deconvolution results using ESIprot online ([www.bioprocess.org/esiprot/](http://www.bioprocess.org/esiprot/)), the molecular weights of the first and second peptide compounds were (<3 kDa, at 1514 Da and 2029 Da, respectively. According to Lammi et al., (2015) a research by soybean hydrolysis using pepsin and trypsin enzymes produces peptides weighing 1178 Da and 1177 Da, correspondingly. Therefore, both yields were considered to be of similar mass. The results prompt the speculation of possible similarity between the two peptides isolated and pravastatin. Hence, there is a tendency for identical inhibitory potentials against HMG-CoA reductase enzyme activity. However, further studies to determine the peptide structure is required to ascertain this assertion.

## 1. CONCLUSION

The inhibitory activity of HMG-CoA reductase by soy protein hydrolysate from hydrolysis using papain enzyme has enormous potential as an anti-cholesterol agent. This product anticipated to serve as a replacement for statin drugs. Therefore, further in vivo testing and studies on peptide interactions with HMG-CoA enzyme is expected to influence future drug development.

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**Table 1.** Addition of anti-cholesterol activity test reagents

Mixture	buffer ( $\mu\text{L}$ )	pravastatin ( $\mu\text{L}$ )	NADPH ( $\mu\text{L}$ )	HMG- KoA ( $\mu\text{L}$ )	HMGR ( $\mu\text{L}$ )	Hidrolisat ( $\mu\text{L}$ )
Blank	184	-	4	12	-	-
Negative control	182	-	4	12	2	-
Positive control	181	1	4	12	2	-
Sample	172	-	4	12	2	10

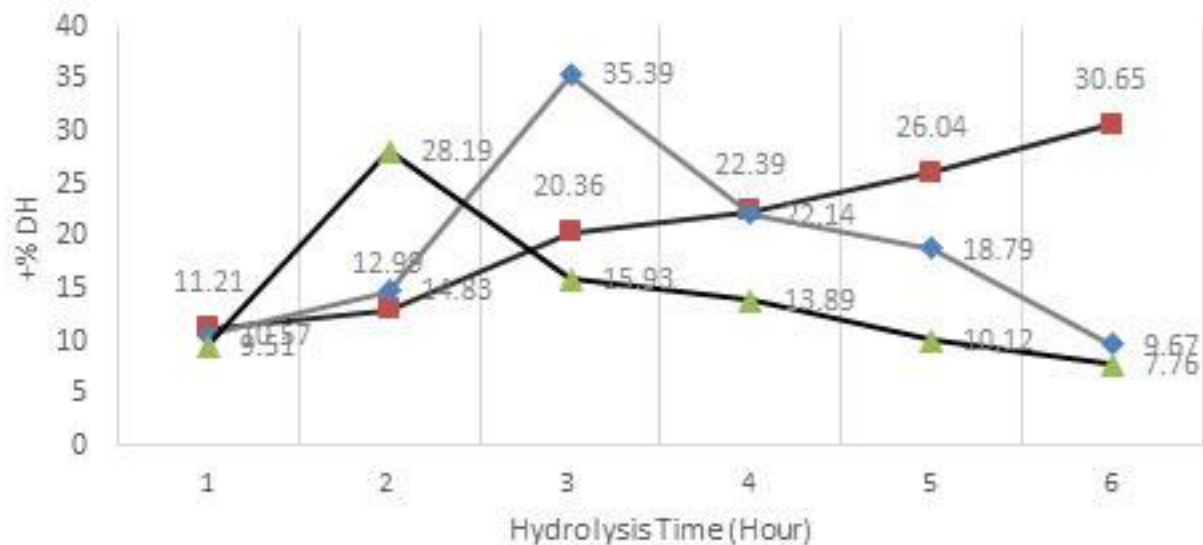
**Table 2.** Proximate composition of soybean protein isolates

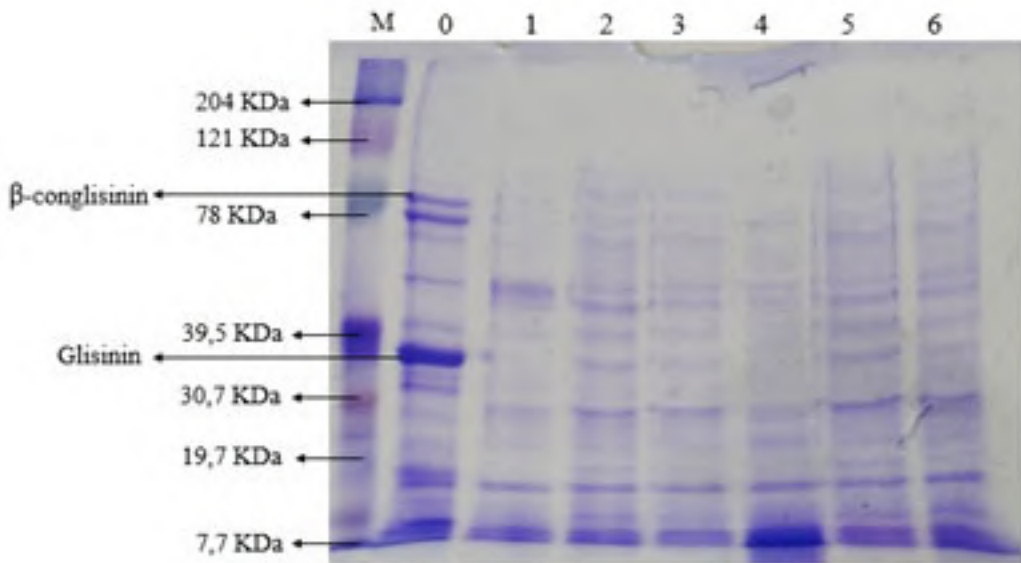
<b>Proximate composition</b>	<b>Soy protein isolate (%)</b>
<b>Crude protein content</b>	36,59±0,01
<b>Crude fat content</b>	10,24±0,26
<b>Moisture content</b>	8,22±0,06
<b>Ash content</b>	4,26±0,08



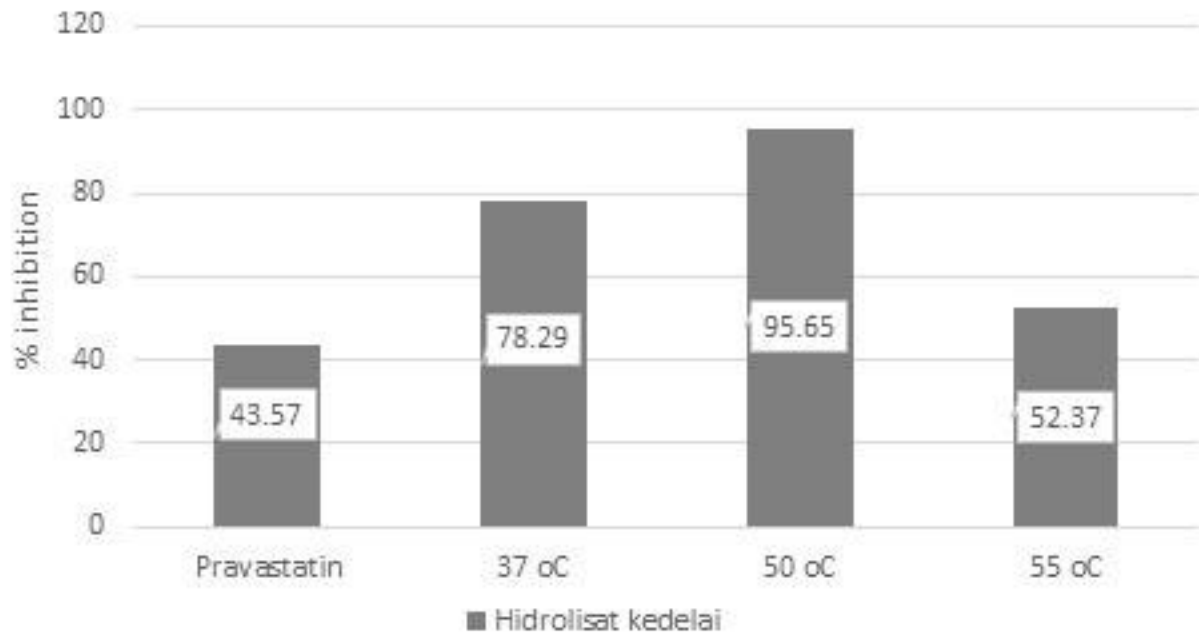
## Degree Of Hydrolysis (%DH)

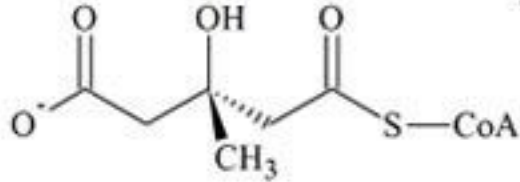
—■— 37 oC —◆— 50 oC —▲— 55 oC



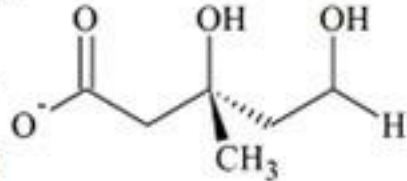
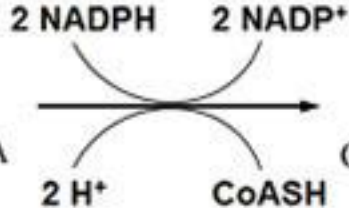


# % HMG-CoA reductase inhibition





**(S)-HMG-CoA**



**(R)-Mevalonate**

# Fractionation Results using Sephadex G-10

