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Research Article

Anticancer Activities of Bromelain Hydrolysate of Soy Protein Against Breast Cancer Cells MCF-7

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Abstract

Soybeans contain proteins that have the potential to produce anticancer bioactive peptides. This study aims to determine the anticancer activity of soy protein hydrolysate against MCF-7 breast cancer cells. Soybean protein hydrolyzed by bromelain enzyme 0.5% (w/v) at the optimum temperature and pH for protein hydrolysis using the Bergmeyer and Grassl method. The degree of hydrolysis value of protein hydrolysate was determined by the Alder-Niesen method and the protein profile was analyzed by SDS-PAGE. The hydrolysate with the best degree of hydrolysis value was analyzed for anticancer activity against MCF-7 breast cancer cells by the Presto Blue assay method, and fractionation of protein hydrolysates by gel filtration chromatography (Sephadex G-15). The molecular weight of the peptide was characterized by LCMS/MS. Soy protein hydrolysis using 0.5% (w/v) bromelain enzyme was optimum at 65 ºC and pH 7.0 for 4 hours, with a hydrolysis degree value of 20.57%. The SDS-PAGE analysis showed that the protein hydrolysates had quite thick protein bands in the range of <35 kDa with an IC_{50} value of 70.37 mg/mL. Based on the LCMS/MS results, the peptide from fractionation has a molecular weight of 5.133 kDa.

Keywords: Anticancer; bioactive peptide; bromelain; MCF-7; soybean

1. INTRODUCTION

Cancer is a disease that arises due to uncontrolled cell growth resulting in changes from body tissue cells to cancer cells[1]. Based on the GLOBOCAN (Global Burden of Cancer) data on cancer incidence and deaths in 2020 obtained from the International Agency for Research on Cancer (IARC), it is estimated that there were 19.3 million new cancer cases and nearly 10 million cancer deaths occurring worldwide in 2020[2]. The most frequently diagnosed cancer is breast cancer in women. Many methods for treating cancer have been found, including surgery, radiotherapy, chemotherapy, immunotherapy, and gene therapy[3], but these treatments are considered ineffective because they cause many side effects due to the low selectivity of the drug used, where the drug can be toxic to other normal cells that are not cancer cells[4]. Therefore, it is necessary to explore alternative treatments that are more natural and effective both in terms of safety and efficacy.

Soybean is a grain that contains high protein (40%) and is a potential source of bioactive peptides. The protein content in soybeans can be increased by modifying the protein into peptide compounds. Peptides isolated from soybeans have been shown to have benefits for the body's biological functions, including antioxidants, inhibitors of ACE activity, anti-obesity, and anti-cancer[5]. One example of a potential anticancer bioactive peptide in soybean is lunasin which was isolated by Odani et al (1987) and reported to contain 43 amino acids[6,7,8]. Based on further research by Galvez & De Lumen it is known that lunasin has an apoptotic effect, namely programmed cell death, on MCF-7 breast cancer cells[9].

Bioactive peptides can be isolated through enzymatic hydrolysis with protease enzymes such as bromelain, papain, trypsin, pepsin, and alcalase[10]. Enzymatic hydrolysis was chosen because it can reduce the molecular weight of proteins and has advantages in

process stability, high catalytic efficiency, and can reduce amino acid damage[10]. Rayaprolu et al (2017) conducted research on the anticancer activity of hydrolyzed soybean bioactive peptides using alcalase enzymes against MCF-7 breast cancer cells and obtained IC_{50} values of 654 μg/mL (peptide fraction 5-10 kDa)[11]. Chen et al (2018) conducted a study on hydrolyzed black soybean protein using an alcalase enzyme and obtained the Leu/Ile-Val-Pro-Lys peptide with MCF-7 anticancer activity with an IC_{50} value of 276 μg/mL (peptide fraction <4 kDa)[12].

In this study, we have tried to explore more deeply the enzymatic hydrolyzation of soy protein by using bromelain as the hydrolyzing enzyme. The bromelain enzyme was chosen because the specificity of bromelain enzyme cutting is quite broad, which can cut peptide bonds from carbonyl groups such as lysine, arginine, phenylalanine, and tyrosine[13]. The main focus of this study was to determine the optimum conditions for hydrolysis of soy protein by bromelain enzymes and their anticancer activity against MCF-7 breast cancer cells. The bioactive peptides from the hydrolysate will be characterized based on their molecular weight using LCMS/MS.

2. RESEARCH METHODS

Material

Soybeans obtained from a local market in the South Tangerang area, bromelain enzyme CAS No. 9001-00-7 EINECS No. 253-387-5 Active Unit: 50,000U/G-200,000U/G were produced from Wuhan Recedar Biotechnology Co., Ltd. China, distilled water, sodium hydroxide, Bradford solution, BSA (Bovine Serum Albumin) (Sigma-Aldrich), tyrosine (Merck), 0.1 M phosphate buffer pH 7.5, Tris-HCl buffer pH 8 0.5 M, 10% (w/v) TCA, microtube, tube, T-flask, 96 well plate, cisplatin, ceftriaxone, dimethyl sulfoxide (DMSO), phosphate buffered saline (PBS), PrestoBlue cell viability reagent, Roswell Park Memorial institute Medium (RPMI), fetal bovine serum (FBS), trypsin-EDTA, trypan blue, MCF-7 cancer cell line (ATCC HTB-22), acrylamide solution gel (bio-rad), bis-acrylamide, stacking gel, resolving gel, sodium dodecyl sulfate (SDS) 10% (w/v) and 20% (w/v), ammonium peroxide disulfate (APS) 10% (w/v), N, N, N'N'-Tetramethylethylenediamine (TEMED) (Sigma-Aldrich), methanol, acetic acid, staining solution coomasie blue (bio-rad), protein marker (thermofisher #26643), and Sephadex G-15 (Sigma-Aldrich).

Soybean Protein Extraction and Purification

A total of 50 g of dried soybeans was ground using a grinder mill to produce soybean flour. The fat from soybean flour is removed by Soxhlet extraction using *n*-hexane (technically) (ratio 1:5 w/v) for 5 hours, then the solvent is evaporated by decantation and the flour is air-dried until all solvents disappear. Fat-free soybean flour was added with 0.05 M PBS (phosphate buffered saline) buffer pH 7.4 and then homogenized using a homogenizer at 4 °C for ± 5 minutes. The homogenate was centrifuged at 4,000 rpm at 4 °C for 30 minutes. The supernatant obtained was freeze-dried.

Bromelain Enzyme Activity Test

Determination of optimum temperature

A total of 1 mL of 5% (w/v) BSA was added to 1 mL of 0.1 M phosphate buffer with 0.2 mL of bromelain enzyme extract pH 7.0, then incubated for 10 minutes at 45 ºC, 50 ºC, 65 ºC, and 70 ºC to determine the optimum temperature. The solution was added 2 mL of 10% (w/v) TCA then incubated for 10 minutes at room temperature and centrifuged at 6,000 rpm for 10 minutes. The resulting supernatant was added with 5 mL of Na_2CO_3 and 1 mL of Folin's reagent and allowed to stand for 5 minutes, then the absorbance was measured at a wavelength of 578 nm.

Determination of Optimum pH

A total of 1 mL of 5% (w/v) BSA was added to 1 mL of 0.1 M phosphate buffer and 0.2 mL of bromelain enzyme extract, then incubated for 10 minutes at the optimum temperature obtained with a pH of 5.5, 6.0, 6.5, 7.0 and 7.5. The solution was added 2 mL of 10% (w/v) TCA then incubated for 10 minutes at room temperature and centrifuged at 6,000 rpm for 10 minutes. The resulting supernatant was added with 5 mL of Na_2CO_3 and 1 mL of Folin's reagent and allowed to stand for 5 minutes, then the absorbance was measured at a wavelength of 578 nm.

One unit of enzyme activity is defined as the amount of enzyme required to hydrolyze a substrate per unit of time under experimental conditions. Enzyme activity calculation follows equation 1.

Protein Hydrolysis

Samples of soy protein isolate were hydrolyzed using a proteolytic enzyme, bromelain. The hydrolysis process was carried out using 0.5 g of protein isolate and dissolved in phosphate buffer solution 0.1 M, under optimum conditions of pH and temperature of enzyme. Next, bromelain 0.5% (w/v) was added and incubated for 0, 1, 2, 3, 4 and 5 hours. During the incubation process, shaking or agitation was carried out at 200 rpm. Each hydrolysate mixture was heated at 80 °C for 10 minutes and 0.5 M Tris-HCl buffer was added with pH 8.0 to inactivate the enzyme. Each hydrolysate obtained was then measured in the degree of hydrolysis and the hydrolysate with the highest degree of hydrolysis was freeze-dried using the freeze-dry method.

$$\text{Enzyme activity} = \frac{(Absorbance\ sample - Absorbance\ blank)}{(Absorbance\ control - Absorbance\ blank)} \text{ x } \frac{1}{(Incubation\ time)} \text{.....(1)}$$

Calculation of Degrees of Hydrolysis

The degree of hydrolysis was calculated based on the calculation of dissolved protein content[14]. Soy protein isolate hydrolysate was taken as much as 5 mL and added 5 mL of 10% (w/v) TCA solution, then allowed to stand for 30 minutes until precipitation occurred. Then, it was centrifuged for 20 minutes at 4,000 rpm. The resulting supernatant was tested for protein content using the Bradford method (1976)[15]. The degree of hydrolysis of each hydrolysate can be calculated by the following formula:

$$\%DH = \frac{(10\%\ TCA\ soluble\ protein)}{(Total\ sample\ protein)} \text{x } 100\% \text{ ……(2)}$$

Protein Profile Analysis with SDS-PAGE

The protein profile for soybean hydrolysates was determined using the SDS-PAGE method with a 7.5-14.5% resolving gel and 4% stacking gel solution in a buffer of 1.5 M Tris HCl at pH 8.45[16]. 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 ºC 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. Subsequently, 1.5 % bis-acrylamide and 48% acrylamide were added, and the electrophoresis process commenced for 55 minutes at a voltage of 150 volts with a Biorad protein marker, in the range of 7.7-204.0 kDa. After the electrophoresis was complete, 0.1% (w / v) dye solvent was used for protein staining and the process yield was washed using 7.5% acetic acid and 40% methanol solvent.

Anticancer Activity Test

The prepared cells were then cultured into 96 well plates with Roswell Park Memorial Institute (RPMI) liquid culture medium containing 10% (v/v) FBS (fetal bovine serum) and 50 L of antibiotics. The positive control used in this method is cisplatin. Samples and controls were added with PrestoBlue reagent which then produced a red fluorescent color that could be measured at a wavelength of 570 nm using a multimode reader[17].

Separation and Purification of Anticancer Peptides from Hydrolysates

The hydrolysate with the best anticancer activity was fractionated using a Sephadex G-15 column to separate peptide fractions under 3 kDa. Fractionation was carried out until 40 fractions were obtained. The blue fraction is the fraction that contains peptides. The identified fractions containing peptides were then analyzed for their molecular weights using LCMS/MS Q-TOF[18].

Characterization of Bioactive Peptides with LCMS Q-TOF MS

The peptide fraction which has the highest anticancer activity was dissolved in water/methanol (1:1 v/v) and then injected into the LCMS/MS Q-TOF instrument with the ionization mode in the form of electrospray ionization (ESI) through the C18 column (1.8 µm 2.1 x 100 mm) at 50 °C and 25 °C room temperature, the mobile phase used was a mixture of water and 5 mM ammonium formate (mobile phase 1) and acetonitrile with 0.05% formic acid (mobile phase 2), a flow rate of 0.2 mL/minute worked for 23 minutes and an injection volume of 5 µL. The molecular weight of the peptide was analyzed using mass spectroscopy (M+2H). Deconvulation using ESIProt Online[19].

3. RESULTS AND DISCUSSION

Soy Protein Isolate

Soy protein isolate (SPI) was prepared from fat-free soybean flour using the Soxhlet extraction method with *n*-hexane. The isolation of SPI is carried out through the homogenization technique, which operates on the principle of breaking down the cell membrane of soybean tissues through vibrations and impacts occurring at a high enough speed to cause cell lysis. The soybean protein isolate is freeze-dried to preserve it, achieved by freezing and then drying through evaporation in a vacuum process known as lyophilization. The freeze-dried soy protein isolate has a mass of 20.66 g. The calculated soybean protein yield in this study was 41.32%. This value is derived from comparing the total amount of protein to the weight of the sample, indicating the percentage of protein produced. A higher yield value suggests a more effective and efficient isolation treatment

Bromelain Enzyme Activity Test Results

Optimum Temperature

The highest bromelain enzyme activity in this study was obtained at 65 °C, the optimum temperature, with an enzyme activity value of 0.161 U/mL (Figure 1).

Bromelain enzyme activity increases with rising temperature, reaching its peak at the optimum temperature. However, at temperatures above this optimum, the enzyme activity decreases. This decrease is attributed to the denaturation of bromelain caused by the weakening of hydrogen bonds. Consequently, the enzyme's conformation alters, leading to a reduction in its activity. According to Bhattacharya & Bhattacharya

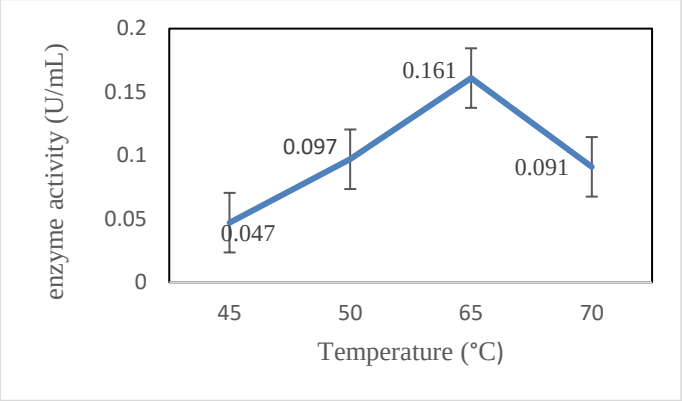

Figure 1. The effect of increasing temperature on bromelain enzyme activity

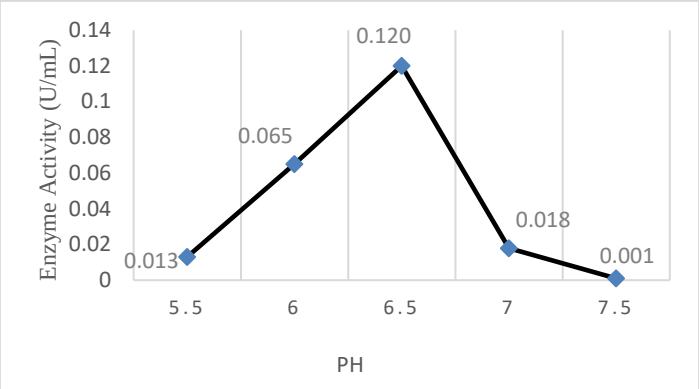

Figure 2. Effect of pH on bromelain enzyme activity

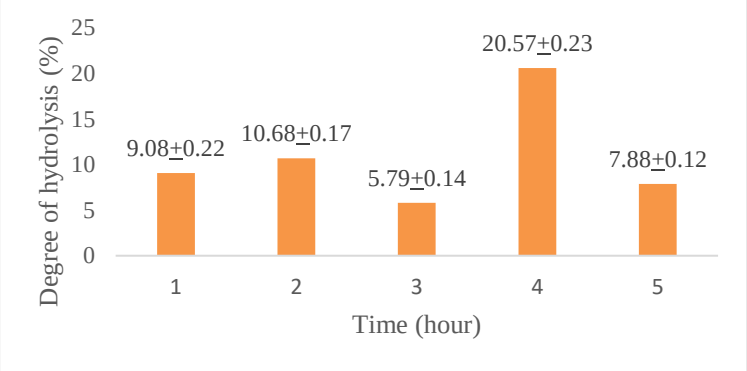

Figure 3. The degree of hydrolysis of soy protein hydrolysate

(2007), temperature increases beyond the enzyme's optimum does not significantly affect its activity but rather impact its structural stability[20]. Denaturation is suspected to occur at 70°C due to the disruption of weak bonds within the enzyme's structure. Exceeding the maximum temperature results in the denaturation of the enzyme, as the protein structure becomes exposed and nonpolar groups within the molecule are revealed. This leads to decreased solubility of polar proteins in water and a subsequent decrease in enzyme activity[21].

Optimum pH

The optimum pH of the bromelain enzyme in this study was pH 7.0 with an enzyme activity of 0.00035 U/mL (Figure 2).

Rabelo et al. (2011) stated that changes in pH affect enzyme activity by altering the enzyme's conformation in solution. pH changes indicate variations in the ionization of the enzyme's ionic groups, both at the active site and elsewhere, thereby affecting enzyme activity through the concentration of H^+ ions present in the solution[22]. The presence of H^+ ions increases the enzyme's positive charge, while OH^- ions increase its negative charge. At the optimum pH (7.0), the number of H^+ ions does not significantly impact the conformation of the bromelain enzyme, allowing it to maintain the proper conformation for substrate binding and the formation of the enzyme-substrate complex.

Degree of Hydrolysis (DH) of Soybean Protein Isolate

Hydrolysis of SPI was carried out with variations in hydrolysis time of 1 to 5 hours. The optimum hydrolysis time was obtained at 4 hours with a DH value of 20.57% (Figure 3).

The DH value increased linearly from 1 to 2 hours and then decreased at 3 hours. This decrease in DH value also occurred after surpassing the optimum hydrolysis time of 5 hours. The decline in DH value at 3 hours of hydrolysis is affected by inadequate mixing or agitation of the reaction mixture which can lead to uneven distribution of enzymes and substrates. Impurities in the bromelain enzyme are believed to influence the cleavage pattern, leading to improper cutting of lysine, phenylalanine, arginine, and tyrosine residues. Optimal condition during hydrolysis enhances kinetic energy, accelerating molecular collisions and facilitating the formation of enzyme-substrate complexes, thus yielding more peptide products. Extending the reaction time beyond the optimal duration may lead to substrate depletion or enzyme inactivation, resulting in a decrease in hydrolysis efficiency[23].

Enzymes work to break peptide bonds. As the reaction progresses, the amount of protein hydrolyzed into peptides, oligopeptides, and amino acids continues to increase, resulting in a degree of hydrolysis at optimum conditions. The cleavage process continues until the enzyme is unable to further cleave the peptides, indicating that the rate of hydrolysis has reached a stationary state. Hasnaliza et al. (2010) stated that the increase in DH value was caused by an increase in peptides and amino acids dissolved in TCA as a result of peptide bond breakage during protein hydrolysis[23].

Soybean Protein Profile Results of SDS-PAGE Analysis

Electrophoresis was performed on soy protein isolates and soy protein hydrolysates over a period of 1 to 5 hours of hydrolysis. The results of the SDS-PAGE analysis in Figure 4 show that 10 protein bands were detected in soybean protein isolate before hydrolysis (0 hour), ranging in molecular weight from 10 to 115 kDa. Among these bands, several were notably thick at 18, 22, 26, and 31 kDa, indicating the presence of two 11S (glycinin) protein subunits: acid A polypeptide and base B polypeptide. Additionally, a thin band was observed at a molecular weight of 66 kDa, thought to be a β-conglycinin protein subunit, and at a molecular weight of 115 kDa, thought to be a hemagglutinin[24].

The hydrolysate shows bands at lower molecular weights. After 1 hour of each enzyme concentration variant, the subunits of β-conglysinin had been completely degraded, as well as the protein component at a molecular weight >100 kDa. Gel bands indicating the presence of glycinin subunits (15 kDa – 25 kDa) were still visible in some of the hydrolysate variants[25]. Based on this it is known that glycinin shows a higher resistance to the hydrolysis process. This could be due to the presence of disulfide bonds in glycinin[26]. Each constituent of the glycinin subunit is connected by a chain of disulfide bonds, while the β-conglysinin subunit does not contain disulfide bonds. Electrostatic and hydrophobic interactions accompanied by the presence of disulfide bonds make the quaternary structure of glycinin more stable against denaturation[27].

Anticancer Activity of Soybean Protein Hydrolysate

Soy protein isolates and soy protein hydrolysate with the highest DH value (4 hours) were tested for anticancer activity against MCF-7 breast cancer cells. The positive control used was cis-diammineplatinum (II) dichloride (cisplatin). Samples were prepared in eight

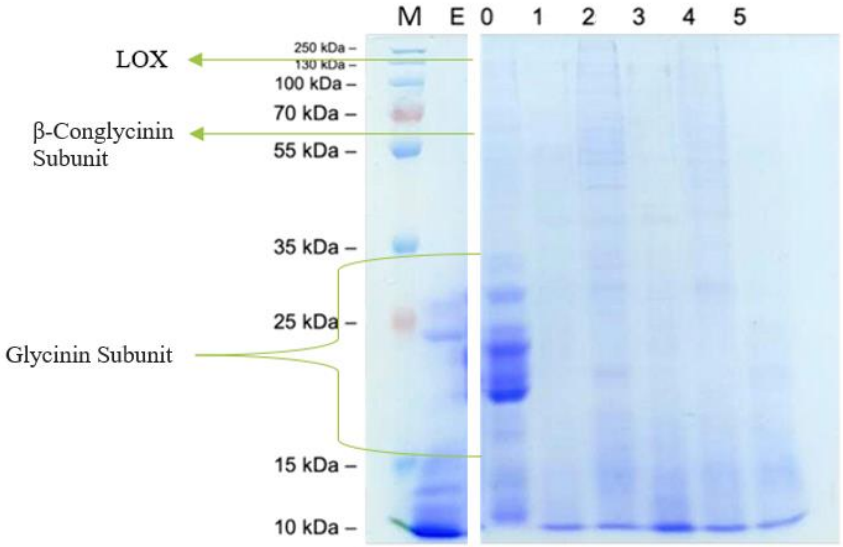

Figure 4. Molecular weight profile of soybean protein hydrolysate. M = protein marker, E = bromelain enzyme, 0 = soy protein isolate, 1-5 = 0.5% hydrolysate 1-5 hours

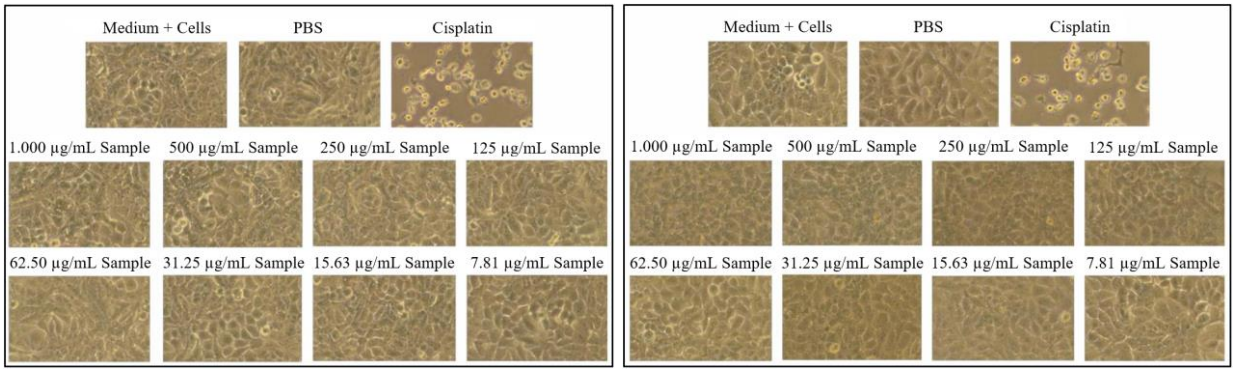

Figure 5. (a) Morphology of MCF-7 cells from soy protein isolate sample (b) Morphology of MCF-7 cells from the 4 hours hydrolysate sample

Table 1. IC_{50} value of each test sample

Sample	**IC_{50} (mg/mL)**
Cisplatin	0.053
Soy protein isolate	64.725
Hydrolysate 4 H	70.375

concentration variants, which showed a pattern of relationship with cell activity and represented each toxicity parameter, i.e., 1,000 µg/mL as less toxic; 500, 250, and 125 µg/mL as moderately toxic; 62.50 and 31.25 µg/mL as toxic; and 15.63 and 7.81 µg/mL as very toxic. The difference in the concentration of the isolated sample and the protein hydrolysate can affect the inhibition of MCF-7 breast cancer cells. The test results in this study showed that the inhibitory abilities of isolate and hydrolysate samples were still less active than cisplatin (Figures 5a and 5b).

Compared to the cell density produced by the addition of cisplatin, the ability of each sample to inhibit the growth of MCF-7 breast cancer cells is still less active. The morphology of the MCF-7 cells, which had a long and dense shape when cisplatin was added, changed to become rounder and hollower, whereas, in the cells added to the sample, there was no similar change.

The cells treated with cisplatin showed very clear signs of cell reduction. A reduction in the number of cells can be an indication of apoptosis. Apoptosis indicates cell death that begins with the formation of indentations in the cell membrane and fragmented DNA. Cells undergoing apoptosis can be observed through their morphological characteristics, specifically the change to a spherical shape because the protein structures that make up the cytoskeleton are digested by specific peptidase enzymes activated in the cells.

The lower anticancer activity of soy protein isolate and soy protein hydrolysate compared to positive control (cisplatin) can be attributed to several factors. The lower anticancer activity of protein extracts compared to the positive control cisplatin can be attributed to several reasons, such as potency and mechanism of action: cisplatin is a highly potent chemotherapy drug with a well-established mechanism of action. It forms cross-links with DNA, which disrupts DNA replication and transcription, ultimately leading to cell death. Soy protein isolate, on the other hand, may not have such strong and direct mechanisms for inducing apoptosis or inhibiting cell growth. Cisplatin is a pure compound with a known effective dose. Soy protein isolates and hydrolysate are mixtures containing various proteins, peptides, and possibly other compounds, which might not be present in concentrations high enough to achieve similar levels of anticancer activity. Specificity and efficacy of cisplatin, which specifically targets rapidly dividing cancer cells and is very effective in its action. Protein extracts may contain a variety of molecules, some of which might have anticancer properties, but their collective efficacy is generally lower than that of a single, highly potent chemotherapeutic agent like cisplatin. On the other hand, the bioavailability and stability of cisplatin in biological systems are well characterized and optimized for maximum therapeutic effect. Protein extracts might have components that are less stable or less bioavailable, reducing their overall effectiveness.

Protein extracts might lack such synergistic interactions and therefore show reduced activity when used alone. Cisplatin efficiently enters cancer cells and exerts its cytotoxic effects. Protein extracts may face challenges in terms of cellular uptake and penetration, limiting their ability to reach intracellular targets and exert their anticancer effects. Cisplatin is active in its administered form. In contrast, the active components

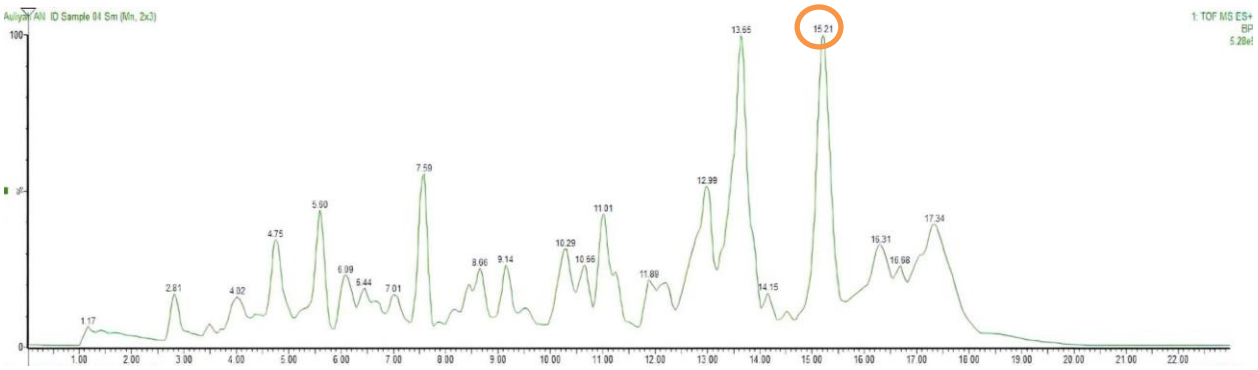

Figure 7. Chromatogram of fraction 4 separated by Sephadex G-15

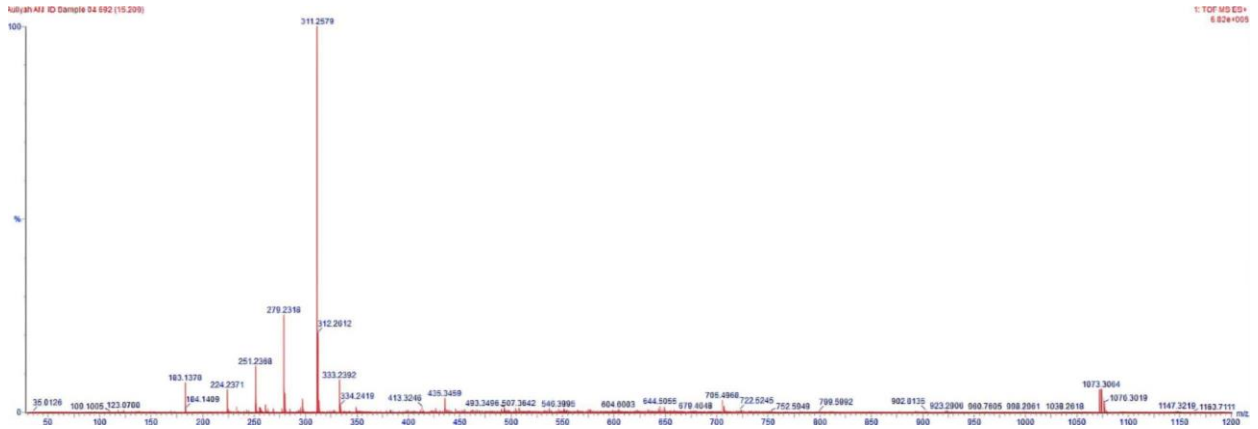

Figure 8. The mass spectrum of the peptide at a retention time of 15.21 minutes

within protein extracts might require metabolic activation or specific conditions to become effective, which might not be fully achieved in the experimental conditions. In summary, the higher anticancer activity of cisplatin compared to protein extracts is due to its high potency, specific and well-understood mechanism of action, optimized bioavailability and stability, and efficient cellular uptake[28].

Fractionation Results of Anticancer Bioactive Peptides

Soybean protein hydrolysate was separated for 4 hours using gel filtration chromatography. The stationary phase used was Sephadex G-15 gel (Separation Pharmicia Dextran G-15). The 40 fractions resulting from separation were tested qualitatively with Bradford reagent to determine the fraction with the most dominant bioactive peptides. The selected fractions were then characterized using LCMS/MS Q-TOF to determine the molecular weight of the peptides when data analysis was performed with MassLynx software. The chromatogram results from fraction number 4 produced many peaks with the main peak at a retention time of 15.21 minutes (Figure 7).

The chromatogram peak at a retention time of 15.21 minutes was chosen because it is the highest peak and further identification of the mass spectrum was carried out based on m/z data to determine the molecular weight of the peptide. Based on the results of this analysis, it is known that the peak that appears at a retention time of 15.21 minutes is a peptide fragment with a molecular weight of 5.133 kDa (Figure 8).

Peptides that are shorter than protein hydrolysates can increase their bioactivity, as in the study of Rayaprolu et al. (2017) who reported that the 5-10 kDa peptide fraction from yellow soybeans had a higher antiproliferative effect on MCF-7 cells than the 10-50 kDa fraction[29]. Chen et al.[4] revealed that black soybean protein hydrolysate with peptide fraction <4 kDa had the highest anticancer activity compared to fractions 4-6 kDa and >6 kDa against MCF-7 cells. Based on the results of this study, it is necessary to conduct further research using the soy protein fraction resulting from the hydrolysis of the bromelain enzyme under different hydrolysis conditions on MCF-7 cells and other cancer cells such as colon cancer (WiDr) and cervical cancer (HeLa).

Generally, peptides with anticancer properties contain 21 to 30 amino acids and include glycinin, lysine, and leucine. Amino acid residues in peptides can influence their anticancer activity depending on the cationic, hydrophobic, and amphiphilic properties associated with the formation of the helical structure. Chiangjong et al. (2020) reported that amino acid residues that are acidic or neutral and have low hydrophobicity, when substituted with positively

charged amino acid residues (such as lysine and leucine) on the polar and nonpolar sides of the alpha helix, can produce cationic peptides with moderate hydrophobicity and increase anticancer activity[30].

4. CONCLUSIONS

Based on the research results, the following conclusions were drawn: the optimum conditions for hydrolysis of soy protein isolate using 0.5% (w/v) bromelain enzyme were obtained at 65 °C and pH 7.0 for 4 hours, resulting in a DH value of 20.57% and anticancer activity against MCF-7 breast cancer cells lower than that of cisplatin. After fractionation, bioactive peptides from the protein hydrolysates were found to have a molecular weight of 5.133 kDa. In the future, further research is needed to isolate and test the bioactive peptide for its anticancer activity.

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REFERENCES

1. Baradero, M., Dayrit, M. W., & Siswadi, Y. (2008). *Seri Asuhan Keperawatan Klien Kanker.* Cetakan Pertama. Jakarta: EGC.
2. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209-249.
2. Ihnat, P., Vavra, P., & Zonca, P. (2015). Treatment Strategies For Colorectal Carcinoma With Synchronous Liver Metastases: Which Way To Go. *World J. Gastroenterol*, *21*, 7014-7021.
3. Mazalovska, M., & Kouokam, J. C. (2020). Plant-Derived Lectins as Potential Cancer Therapeutics and Diagnostic Tool. *Biomed. Res. Int.*
4. Schirrmacher, V. (2019). From Chemotherapy To Biological Therapy: A Review Of Novel Concepts To Reduce The Side Effects Of Systemic Cancer Treatment (Review). *Int J Oncol*, *54*(2), 407-419.
5. Singh, B. P., Vij, S., & Hati, S. (2014). Functional Significance Of Bioactive Peptides Derived From Soybean. *Peptides*, *54*, 171–179.
6. Odani, S., Koide, T., & Ono, T. (1987). Amino Acid Sequence of a Soybean (*Glycine Max*) Seed Polypeptide Having a Poly (L-Aspartic Acid) Structure. *Journal Biol Chem*, *262*, 10502-10505.
7. Ďúranová, H., Fialková, V., Bilčíková, J., Lukáč, N., & Kňažická, Z. (2019). Lunasin and Its Versatile Health-Promoting Actions. *J Microbiol Biotech Food Sci*, *8*(4), 1106-1110.
8. Vuyyuri, S. B., Shidal, C., & Davis, K. R. (2018). Development of The Plant-Derived Peptide Lunasin as An Anticancer Agent. *Curr Opin Pharmacol*, *41*, 27-33.
9. Galvez, A. F., & de Lumen, B. O. (1999). A Soybean cDna Encoding a Chromatin-Binding Peptide Inhibits Mitosis of Mammalian Cells. *Nat Biotechnol*, *17*, 495-500.
10. Kristinsson, H. G. (2007). Aquatic Food Protein Hydrolysates. *Woodhead Publishing Series in Food Science, Technology and Nutrition*, 229-248
11. Rayaprolu, S. J., Hettiarachchy, N. S., Horax, R., Phillips, G. K., Mahendra, M., & Chen, P. (2017). Soybean Peptide Fractions Inhibit Human Blood, Breast and Prostate Cancer Cell Proliferation. *Journal of Food Science and Technology*, *54*(1), 38–44.
12. Chen, Z., Li, W., Santhanam, R. K., Wang, C., Gao, X., Chen, Y., Wang, C., Xu, L., & Chen, H. (2018). Bioactive Peptide with Antioxidant and Anticancer Activities from Black Soybean [*Glycine Max* (L.) Merr.] Byproduct: Isolation, Identification and Molecular Docking Study. *European Food Research and Technology*, *245*(3), 677-689.
13. Pavan, R., Jain, S., Shraddha, & Kumar, A. (2012). Properties and Therapeutic Application of Bromelain: A Review. *Biotechnol. Res. Int*, 976203.
14. Alder-Nissen, J. (1986). *Enzymic Hydrolysis of Food Proteins.* New York: Elsevier Applied Science Publisher.
15. Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*, *72*, 248-254.
16. Laemmli, U. K. (1970). Cleavage of Structural Proteins during The Assembly of The Head of Bacteriophage T4. *Nature*, *227*(5259), 680–685.
17. Tan, M. C. S., Oyong, G. G., Shen, C., & Ragasa, C. Y. (2017). Cytotoxic labdane diterpenoids from Andrographis paniculata (Burm.F.) Nees. *Asian Journal of Pharmaceutical and Clinical Research*, *10*(12), 99–104.
18. ChuHua, L. I., Mei, L., Wenrui, C., & Baojiang, G. (2008). Purification and Characterization of a Novel Cholesterol-Lowering Protein from the Seeds of Senna obtusifolia. *Science in China Series C: Life Science*, *5*(11), 1020–1024.
19. Li, Y., Zhang, Y., Li, H., Zhao, W., Guo, W., & Wang, S. (2018). Simultaneous determination of heat stable peptides for eight animal and plant species in meat products using UPLC-MS/MS method. *Food Chemistry*, 245, 125–131. https://doi.org/10.1016/j.foodchem.2017.09.066
20. Bhattacharya, R. and D. Bhattacharya. (2007). Preservation of natural, stability of fruit bromelain from Ananas comosus (pineapple). *J. Food Biochem*. 33: 1-19.
21. Hale, L.P., P.K. Greer., C.T. Trinh and C.J. James. (2005). Proteinase activity and stability of natural bromelain preparations. *Int. Immunopharmacol*. 5: 783-793.
22. Rabelo, M. C., Fontes, C. M. L., & Rodrigues, S. (2011).

Stability Study of Crude Dextransucrase from Leuconostoc citreum NRRL B-742. *Indian J Microbiol*, *51*(2), 164-170.
23. Hasnaliza, H., Maskat, M. Y., & Wan, A. W. M. (2010). The Effect Of Enzyme Concetration, Temperature And Incubation Time On Nitrogen Content And Degree Of Hydrolysis Of Protein Precipate From Cockle (Anadara Granosa) Meat Wash Water. *Int Food Res J*, *17*, 147-152.
24. Mujoo, R., Trinh, D. T., & Ng, P. K. W. (2003). Characterization of storage proteins in different soybean varieties and their relationship to tofu yield and texture. *J Food Chem*, *82*, 265-273.
25. Liu, C., Wang, H., Cui, Z., He, X., & Wang, X. (2007). Optimization of Extraction And Isolation For 11S And 7S Globulins Of Soybean Seed Storage Protein. *Food Chem*, *102*, 1310-1316.
26. Chen, L., Chen, J., Ren, J., & Zhao, M. (2011). Modifications Of Soy Protein Isolates Using Combined Extrusion Pretreatment and Controlled Enzymatic Hydrolysis For Improved Emulsifying Properties. *Food Hydrocolloids*, *25*(5), 887-897.
27. Palupi, N. S., Sitorus, S. R., & Kusnandar, F. (2015). Perubahan Alergenisitas Protein Kacang Kedelai dan Kacang Bogor Akibat Pengolahan Dengan Panas. *J Teknol Industri Pangan, 26*, 222–231
28. Ramesh, G., & Reeves, W. (2010). Mechanisms of Cisplatin Nephrotoxicity. *Journal Toxins*, *2*, 2490-2518.
29. Rayaprolu, S. J., Hettiarachchy, N. S., Horax, R., Phillips, G. K., Mahendra, M., & Chen, P. (2017). Soybean Peptide Fractions Inhibit Human Blood, Breast and Prostate Cancer Cell Proliferation. *Journal of Food Science and Technology*, *54*(1), 38–44.
30. Chiangjong, W., Chutipongtanate, S., & Hongeng, S. (2020). Anticancer Peptide: Physicochemical Property, Functional Aspect And Trend In Clinical Application. *Int J Oncol*, *57*(3), 678-696.