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Application of Lactic Acid Bacteria to Produce Bioactive Compounds from Tofu Waste Using Pineapple Bromelain Enzyme

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ABSTRACT

Tofu is one of the main human foodstuffs and has become an alternative substitute for animal protein. Tofu contains relatively high protein, and active compounds, but the waste produced during the tofu processing process is very abundant, namely 3.5 million tons per year in Indonesia. The production of active compounds from tofu waste can use bromelain enzymes. The bromelain enzyme in this study was produced from the fermentation of pineapple leaf waste using *Pediococcus pentosaceus* E7. The production of active compounds from tofu waste has not been widely reported, so this study aimed to produce active compounds from tofu waste. This study began with bromelain production, measurement of bromelain activity, bromelain precipitation, peptide production from tofu waste, Liquid Chromatography-Mass Spectrometry (LC-MS) analysis, and antibacterial testing of active compounds derived from tofu waste degradation. The results showed that the peptide content of tofu liquid waste degraded using the bromelain enzyme was 41.01±0.06 mg/mL. The LCMS results showed that 18 bioactive compounds were contained in the degradation products of tofu liquid waste. The active compounds derived from the degradation of tofu waste are capable of inhibiting the growth of *Salmonella* Typhimurium ATCC 14028, yielding a clear zone index of 2.53±0.12.

1. Introduction

Healthy living is highly dependent on the food consumed daily. Vegetarian diets are becoming increasingly popular. Many meat products are being replaced with plant-based alternatives, such as soy-based foods, leading to a continued increase in soybean production. Soybeans are typically used as a raw material for foods such as tofu. Tofu is one of the main human foods and has become an alternative to animal protein (Tkaczewska *et al.* 2023). Tofu is rich in dietary fibre, minerals, and vitamins and also contains protein, lipids, and other active compounds. The enormous benefits of tofu lead to its production continuing to increase; however, it also has an impact on waste production (Du *et al.* 2023).

The tofu waste produced by tofu factories in Indonesia is extremely abundant, amounting to approximately 3.5 million tons per year. Tofu waste consists of 11.07% carbohydrates, 4.71% protein, 1.94% fat, and 0.08% ash (Sari *et al.* 2019; Ningsih *et al.* 2024). Various active compounds contained in tofu waste have great potential for development, especially on an industrial scale, and can also help overcome environmental problems. One way to produce active compounds from tofu waste is through a degradation process using protease such as bromelain (Netcha *et al.* 2021).

Bromelain enzyme can be obtained from pineapple plants, which are one of the most widely consumed tropical fruits, starting from the skin, leaves, fruit, and stems. The largest waste produced in the pineapple

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industry is leaves. Pineapple leaf waste can be an abundant source of bromelain (Saini *et al.* 2023). The bromelain enzyme in this study was extracted from pineapple leaf waste using *Pediococcus pentosaceus* E7. Previous research has utilized lactic acid bacteria (LAB) as an antihypertensive agent, a source of antioxidants in pineapple juice, and a producer of amino acids from fermented fish waste (Tsaaqifah *et al.* 2023; Amalia *et al.* 2024). However, the application of LAB to produce active compounds from tofu waste using the enzyme bromelain has not been reported. Pineapple leaf fermentation utilizes LAB because these bacteria can maintain nutritional quality, suppress the growth of pathogenic bacteria, and are classified as Generally Recognized As Safe (GRAS) microbes (Quinto *et al.* 2014). *P. pentosaceus* E7 is able to produce xylanase, pectinase, lipase, and laccase enzymes (Silvia *et al.* 2020), so it has the potential to be used in the fermentation of pineapple leaf waste for the production of bromelain enzymes. Bromelain enzyme can be applied to degrade organic waste, such as tofu waste, to produce bioactive compounds, including bioactive peptides. Bioactive compounds can be used in various fields, one of which is as an antimicrobial (Ramli and Munir 2022). The various active compounds contained in tofu waste have not been widely reported. Therefore, this study was conducted to produce active compounds, peptides, and antibacterial compounds from the degradation of tofu waste with pineapple leaf bromelain extracted using *P. pentosaceus* E7.

2. Materials and Methods

2.1. Proximate Analysis

Pineapple leaf composition including air, ash, protein, fat, crude fiber, and total carbohydrate content, was carried out based on AOAC (1990). The measurement of air content was analyzed by the drying method in the oven at 105°C for 60 minutes. Ash content was analyzed using the gravimetric method. Protein content was measured using the Kjeldahl method by converting total nitrogen content with a conversion factor. The fat content measurement used the Soxhlet extraction method. Fiber content measurement was done by dissolving the fiber contained in pineapple leaves with a 1.25% H_2SO_4 concentration solution and NaOH. Total carbohydrate measurement was carried out using the differential carbohydrate method.

2.2. Growth Curve

The *P. pentosaceus* E7 growth curve was made by measuring Optical Density (OD) and Total Plate Count (TPC). A total of 1 mL of LAB with a cell count of 10^8 CFU/mL was grown on a medium containing 100 mL of aquadest and 10 grams of pineapple leaves. Incubation was carried out for 4 days at room temperature, with $OD_{600\ nm}$ and TPC measurements every 12 hours. The total LAB calculation was carried out using De Man Rogosa and Sharpe Agar (MRSA) media. The fermented sample was then diluted in stages (dilution 10^{-1} to 10^{-8}), 1 milliliter of the sample was put into a test tube containing 9 mL of 0.85% NaCl solution. The dilution sample was inoculated into a petri dish containing 0.1 mL of MRSA media. Observations were made by counting the number of colonies in the petri dish with a colony counter. The TPC was calculated based on intervals of 30-300 (Kusumah *et al.* 2023). The bromelain enzyme activity was also measured, as described by Walter (1984). The highest bromelain enzyme activity is used as an indicator of the optimal time to harvest cells for fermentation.

2.3. Pineapple Leaf Fermentation for Bromelain Enzyme Production

A total of 10 grams of pineapple leaves that had been prepared previously were put into a sterile Erlenmeyer flask containing 100 mL of distilled water. A total of 1 mL of LAB with an $OD_{600\ nm}$ of 0.8 was then added to the Erlenmeyer. The fermentation time was determined based on the optimum time of protease production that had been previously carried out. The incubation was carried out at room temperature. The sample was then centrifuged at a speed of 10,000 g for 10 minutes to test the activity of the bromelain enzyme (Tsaaqifah *et al.* 2023).

2.4. Measurement of Bromelain Enzyme Activity

Measurement of enzyme activity based on Walter (1984). A total of 50 µL of enzyme was added to a microtube containing 250 µL of 1% casein and 250 µL of 0.1 M phosphate buffer pH 7.0. Specifically for blank and standard treatments, the enzyme solution was replaced with 50 µL of distilled water and 50 µL of 5 mM tyrosine, respectively. The solution was then incubated at room temperature for 10 minutes. Enzyme activity was stopped by adding 500 µL of 0.3 M trichloroacetic acid

(TCA). Blank and standard were added with 50 µL of enzyme, while 50 µL of distilled water was added to the sample. The solution was incubated at room temperature for 10 minutes, followed by centrifugation at 9,000 g for 10 minutes. 600 µL of supernatant was placed in a tube containing 2 mL of 0.4 M Na_2CO_3 and was added with 400 µL of Folin-Ciocalteau reagent diluted with distilled water (1:2). The solution was incubated for 20 minutes at room temperature, then the absorbance was measured at a wavelength of 578 nm. One unit of protease activity is defined as the amount of enzyme that produces the release of 1 µg/mL of tyrosine per minute under the test conditions. The specific activity of the enzyme was obtained by comparing the enzyme activity with the protein content of the enzyme.

2.5. Precipitation of Bromelain Enzyme with Acetone

Acetone precipitation was performed using the Scopes method (Scopes 1994). Protein extracts were precipitated with cold acetone at a concentration of 0-80% and stored at -20°C overnight. Protein pellets were collected by centrifugation at 10,000 g for 30 minutes at 4°C, then dissolved in 0.1 M phosphate buffer, pH 7.0, for protein analysis.

2.6. Analysis of Bromelain by SDS-PAGE

SDS-PAGE was used to determine the molecular weight of proteins, using commercial bromelain as a comparison. The stacking gel was made with a concentration of 4% acrylamide, while the separating gel was 12.5%. Samples and commercial bromelain at a concentration of 50 µg were placed into the wells, and the Unstained Protein Ladder marker protein was added in amounts of up to 0.005 mL. The electrophoresis process was carried out using a voltage of 80 volts, which lasted for 1 hour and 30 minutes. The polyacrylamide gel that had undergone the previous process was then soaked in a Coomassie Brilliant Blue (CBB) dye solution for 12 hours at room temperature and gently agitated. The presence of the bromelain enzyme is characterized by a molecular weight ranging from 23.8 to 37.0 kDa, as compared with commercial bromelain (Ketnawa *et al.* 2012).

2.7. Peptide Production from Tofu Waste with Bromelain

A total of 10 mL of tofu liquid waste was added to 2 U/mg of bromelain enzyme. The sample was then incubated at room temperature for 2 hours at 120 rpm. The sample was then added with 1 mL of Na_2CO_3 and then centrifuged at 10,000 rpm for 15 minutes at a temperature of 4°C. The filtrate obtained was analyzed using Liquid Chromatography-Mass Spectrometry (LC-MS) to determine the profile of active compounds and the biuret test to assess the concentration of the peptides successfully obtained (Sulfitri *et al.* 2020).

2.8. Active Compound Profile from Tofu Waste Degradation Results using Liquid Chromatography-Mass Spectrometry (LC-MS)

The filtrate obtained from the degradation of tofu waste with bromelain enzyme was dissolved in 2% acetonitrile/0.1% formic acid (v/v), then analyzed using Liquid Chromatography-Mass Spectrometry (LC-MS). Analysis of bioactive compound profiles using Liquid Chromatography (LC) AQCUITY Ultra Performance Liquid Chromatography (UPLC) instrument, AQCUITY UPLC® High Strength Silica (HSS) C18 column system (1.8 µm, 2.1 × 100 mm), and Mass Spectrometer (MS) Xevo G2-S Two Generation Quadrupole time-of-flight QToF system (Waters, USA). LC-MS was set with chromatographic separation settings, namely, temperature: 50°C (Column) 25°C (room); mobile phase: water + 5 µM Methanol; flow rate: 0.2 mL/min (linear step) running 22.50 minutes; injection volume: 5 µL (filtering sample using a 0.2 µm filter). Mass spectrophotometry settings as follows, system: ES (electrospray ionization); Mode: positive mode; mass analysis range: 50-1200 m/z; source temperature: 100°C; cone gas flow: 0 L/h; desolvation gas flow: 793 L/h; collision energy: 4 volts (low energy); rampt collision energy: 25-60 volts (high energy). A 5 µL sample is injected into the column using an autosampler, then the sample is carried by the mobile phase and separated by the column. The analysis results were presented in a chromatogram and molecular weight, which serve as the basis for compound identification (Hanifati *et al.* 2024).

2.9. Test of Active Compounds from Degradation of Tofu as Antibacterial Agents

Antibacterial testing was conducted using the well method, as described by Hood *et al.* (2003), with slight modifications. A total of 100 µL of *Salmonella* Typhimurium ATCC 14028 with a cell count of 10^8 CFU/mL was inoculated onto a plate containing Nutrient Agar (NA) media. Each Petri dish was divided into 4 wells. Then, supernatants from the degradation of tofu waste at various concentrations, a positive control (Chloramphenicol), and a negative control (Aquadest)

were added. The incubation process was carried out for 24 hours at room temperature.

3. Results

3.1. Proximate Analysis

The parameters in the proximate analysis of pineapple leaves consist of air, ash, fat, protein, crude fiber, and total carbohydrate. The results of the proximate analysis of pineapple leaves are presented in Table 1.

3.2. LAB Growth on Pineapple Leaf Substrate and Fermentation for Bromelain Production

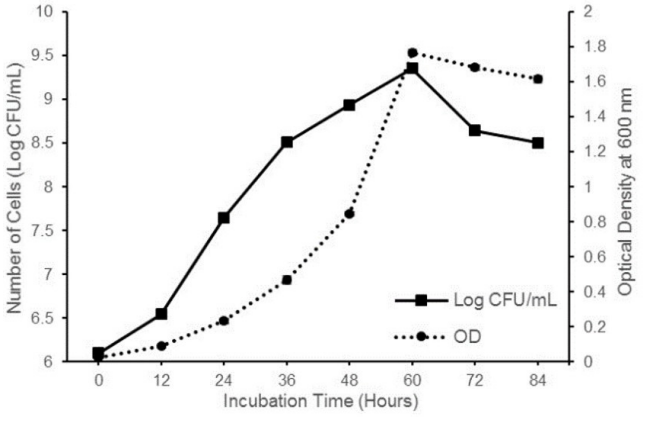
The growth curve was used to determine the ability of lactic acid bacteria (LAB) to live on pineapple leaf substrate. The growth curve of *P. pentosaceus* E7 showed that it was in a logarithmic phase from 0 to 48 hours and in a death phase from 60 to 84 hours. Based on these results, *P. pentosaceus* E7 was able to grow on pineapple leaf substrate (Figure 1A). Pineapple leaf fermentation exhibits changes in pH over incubation time, indicating the presence of specific organic compounds produced. The pH value during pineapple leaf fermentation using *P. pentosaceus* E7 isolate continued to decrease from normal pH to acidic pH (Figure 1B).

3.3. Specific activity, Precipitation, and Analysis of Bromelain Enzyme by SDS-PAGE Electrophoresis

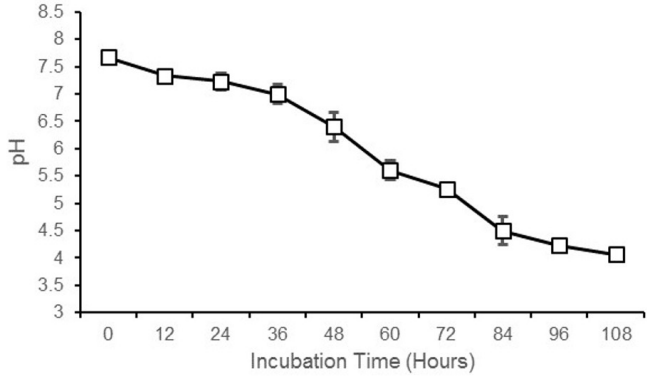
The specific activity of bromelain extracted with *P. pentosaceus* E7 towards the end of the incubation period obtained the most optimal activity. Bromelain had the highest specific activity value at 84 hours of 0.56±0.02 U/mg (Figure 2A). The crude extract of bromelain enzyme was concentrated using acetone to increase its activity and purity. The concentration of 70% saturated acetone obtained the highest bromelain activity (1.56±0.01 U/mg) (Figure 2B). Bromelain concentrated with acetone at 70% saturation obtained a purity level of 21.47 and a yield of 19.39% (Table 2). The extraction of bromelain during pineapple leaf fermentation was confirmed using SDS-PAGE by comparing the commercial bromelain band. Commercial bromelain and bromelain from pineapple leaves extracted with *P. pentosaceus* E7 had the same molecular weight (25.94 kDa) (Figure 2C).

Table 1. Proximate results of pineapple leaves

Sample	Level testing	Total (%)
Pineapple leaves	Water	83.48±0.02
	Ash	0.93±0.04
	Fat	0.70±0.00
	Protein	1.73±0.00
	Crude fiber	3.56±0.09
	Carbohydrate	13.17±0.00



A



B

Figure 1. (A) Growth curve of *P. pentosaceus* E7 on pineapple leaf substrate, (B) pH value during pineapple leaf fermentation

3.4. Peptide Production from Tofu Degradation Results using Bromelain

A peptide can be produced from tofu waste by using protease. The peptide content in tofu liquid waste degraded using bromelain extracted using *P. pentosaceus* E7 was 41.01±0.062 mg/mL. The results obtained were more optimal when compared to those of tofu liquid waste degraded using bromelain extracted from *L. plantarum* NHC7 at 29.89±0.096 mg/mL (Figure 3).

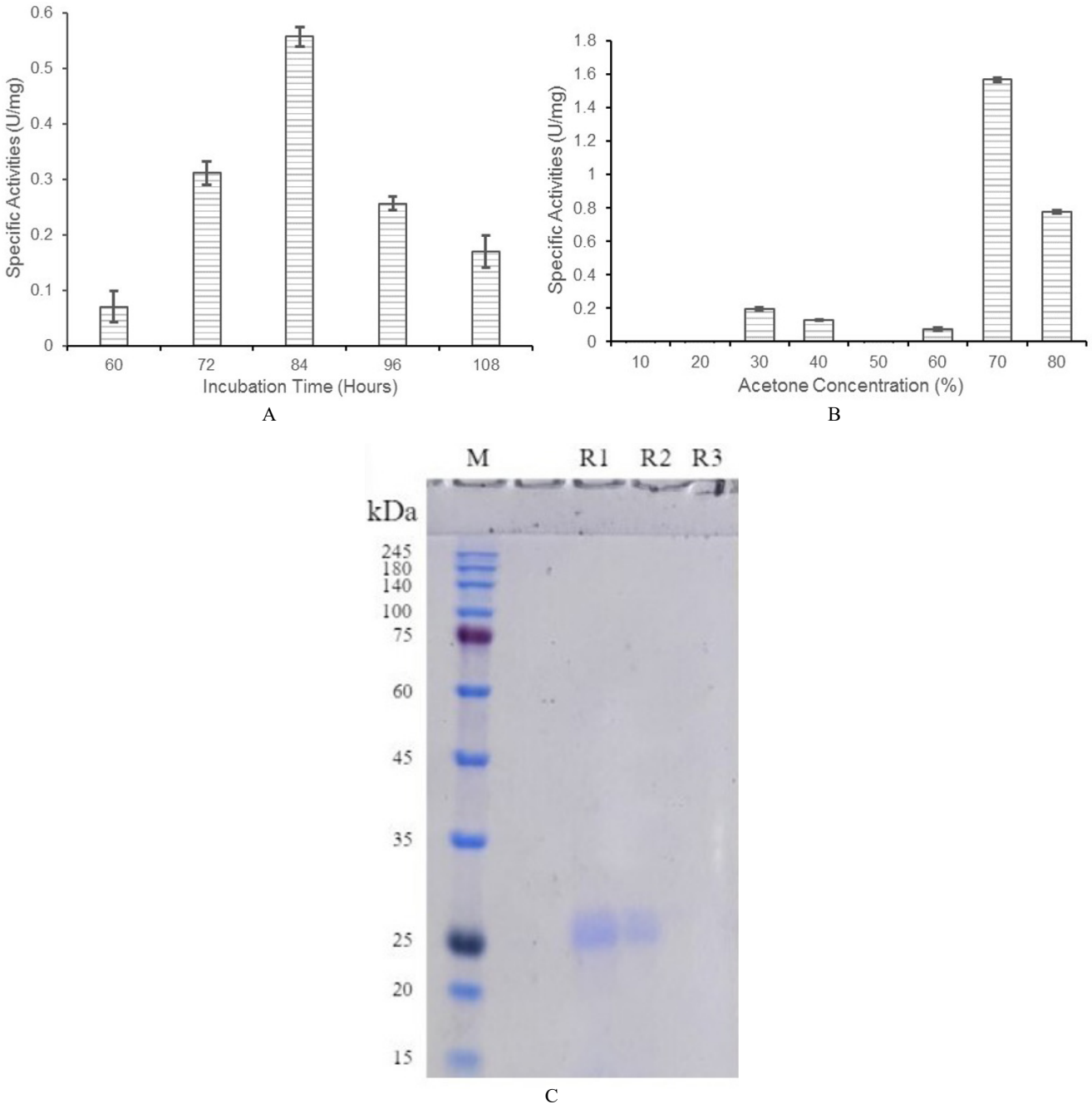


Figure 2. (A) Specific activities of bromelain, (B) precipitation of bromelain using acetone, (C) SDS-PAGE electrophoresis, M: protein molecular weight marker (kDa), R1: commercial bromelain, R2 and R3: bromelain from pineapple leaves

Table 2. The results of precipitation of bromelain enzyme from pineapple leaves

Purification stages	Volume (mL)	Total activity (U/mL)	Total activity (U/mL)	Specific activities (U/mg)	Purification (fold)	Yield (%)
Crude extract enzyme	100	5.46	5.46	100	5.46	100
Acetone 70%	2	1.06	1.06	2	1.06	19.39

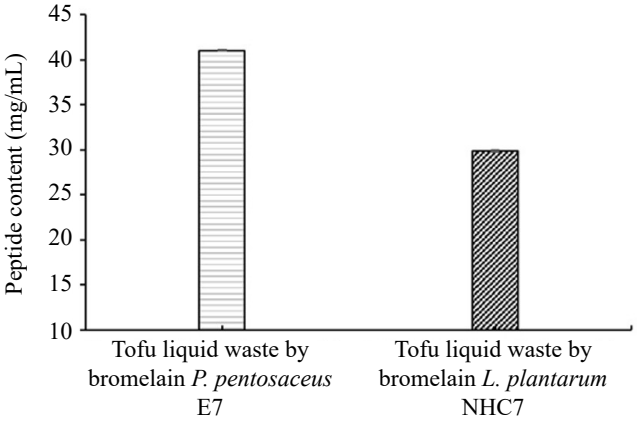


Figure 3. Peptide production from tofu liquid waste degradation using bromelain enzyme extracted with *P. pentosaceus* E7 and *L. plantarum* NHC7

3.5. Analysis of Active Compounds from Tofu Waste Degradation Using LC-MS

Analysis using LC-MS revealed the presence of 18 compounds (Table 3) that were successfully confirmed. The marker compound (daidzein) was also successfully confirmed, which is commonly found in soybean degradation products. The highest relative abundance was obtained at RT (retention time) 11.12, namely, which is N~6~-[4-(diethylamino)-2-methylphenyl]-N~2~-ethyl-1,3,5-triazine-2,4,6-triamine of 41.68% and at RT 12.00, namely which is Scorodocarpine A of 41.68%.

Table 3. Active compounds resulting from the degradation of tofu waste using bromelain

Compound name	Formula	RT	Relative abundance (%)	Mass (m/z)	Bioactivity	Reference
(8E,10E)-7,11-Bis(4-methoxyphenyl)-8,10-bis(4-nitrobenzylidene)-3-thioxo-2,4-diazaspiro[5.5] undecane-1,5,9-trione	$C_{37}H_{28}N_4O_9S$	1.39	4.94	705.16	immunomodulator	(Bavo *et al.* 2021)
4,6-diamino-1,3,5-triazine-2-carbohydrazide	$C_4H_7N_7O$	1.89	0.05	170.07	breast cancer agent	(Lim and Dolzhenko 2024)
sarcosine ammonium nitrate	$C_3H_{11}N_3O_5$	1.91	0.05	170.07	fertilizer	(Palupi *et al.* 2020)
2,4,6-triamino-1,3,5-triazine-1,3-dioxide dihydrate	$C_3H_{10}N_6O_4$	2.36	0.07	195.08	antimicrobial, antimalarial, and anticancer	(Shah *et al.* 2014)
3,3'-diamino-4,4'-azo-1,2,4-triazole	$C_4H_6N_{10}$	2.11	0.07	195.08	biocidal agent	(Yu *et al.* 2023)
D-Pinitol	$C_7H_{14}O_6$	2.20	0.07	194.18	antidiabetic	(Pandi *et al.* 2022)
S-butyl benzenecarbothioate	$C_{11}H_{14}OS$	2.27	0.38	195.08	delocalization of π electrons	(Chagas *et al.* 2024)
Indoline	C_8H_9N	3.47	0.27	120.07	antibacterial agent	(Wei *et al.* 2023)
Triazolylpurine	$C_7H_5N_7$	4.29	0.61	188.06	antituberculosis and antimicrobial	(Hamdi *et al.* 2024)
1-(4-amino-1,2,5-oxadiazol-3-yl)-5-(morpholin-4-ylmethyl) triazole-4-carboxamide	$C_{10}H_{14}N_8O_3$	4.62	0.72	295.12	biorifenery	(Murali *et al.* 2017)
Dimethyl 4,4′-[(2E)-1,4-bis(methylsulfanyl)-2-butene-2,3-diyl]dibenzoate	$C_{22}H_{24}O_4S_2$	4.99	0.12	417.11	antibiotics	(Antoszczak *et al.* 2014)
2-[3-(3-ethyl-5-piperazin-1-ylphenyl)-1,2,4-triazol-1-yl] pyrazine;1-pyrazin-2-yl-1,2,4-triazol-3-amine	$C_{24}H_{27}N_{13}$	5.65	1.40	498.25	antibacterial, antifungal, anticancer, and antituberculosis	(Matin *et al.* 2022)
5-amino-1-(4-nitrofenil)pirazol-3,4-dikarbonitril	$C_{11}H_6N_6O_2$	7.10	2.11	254.20	antimicrobial	(Abunada *et al.* 2009)
Daidzein	$C_{15}H_{10}O_4$	7.12	2.11	255.06	anticancer, antimicrobial, antiallergic, antiaging, and antidiabetic	(Alshehri *et al.* 2021)
2-aminoguanidine;3,6-dinitropyrazolo[4,3-c] pyrazole	$C_5H_6N_{10}O_4$	8.12	0.68	271.06	antifungal	(Teixeira *et al.* 2022)
N~2~-Butyl-N~4~-[4-(diethylamino)butyl]-N~6~-[4-(diethylamino)-2-methylphenyl]-N~2~-ethyl-1,3,5-triazine-2,4,6-triamine	$C_{28}H_{50}N_8$	11.12	41.68	499.42	antimicrobial, antimalarial, and anticancer	(Shah *et al.* 2014)
Scorodocarpine A	$C_{32}H_{54}N_2O_2$	12.00	41.68	499.42	antibiotics	(Wiart *et al.* 2023)
N-[2-(5-Hydroxy-1H-indol-3-yl)ethyl]tetracosan	$C_{34}H_{58}N_2O_2$	17.32	2.99	527.45	antibacterial and anti-inflammatory agents	(Wei *et al.* 2023)

3.6. Potential of Active Compounds from Tofu Waste Degradation as Antibacterial Agents

Active compounds resulting from the degradation of tofu waste can be used as anti-Salmonella agents. The test obtained a clear zone index of 2.53±0.12 mm. The positive control with chloramphenicol yielded a clear zone index of 5.86±0.15 mm, whereas the negative control did not exhibit a clear zone (Figure 4).

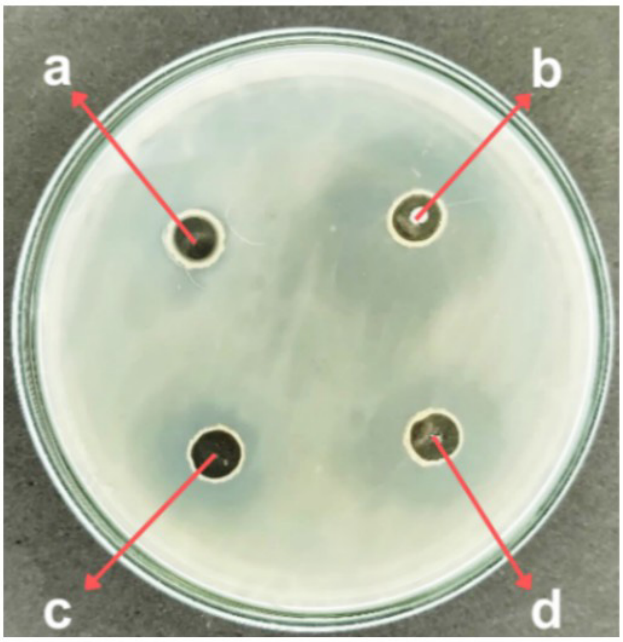


Figure 4. Antimicrobial test of active compounds from tofu waste hydrolysis at a concentration of 100 µL, a: negative control (aquadest), b: positive control (Chloramphenicol), c and d: tofu waste hydrolysis results

4. Discussion

Protein is the component with the fourth-highest percentage in pineapple leaves. The results obtained are in line with research by Nisha *et al.* (2020) the protein content in pineapple leaves is 0.72±0.02%. According to (Ramli and Munir 2022) bromelain is a typical protein found in pineapple.

The growth curve of *P. pentosaceus* E7 shows four different growth phases. The maximum growth rate (µmax) of *P. pentosaceus* E7 bacteria occurred at 12 to 24 hours, which was 0.09 log CFU/hour. Each phase represents a different growth period related to physiological changes in cell culture and changes in the metabolites produced (Maier and Pepper 2015). Pineapple leaf fermentation shows changes in pH over incubation time. This decrease in pH is caused by the accumulation of lactic acid and organic acids produced during the fermentation process. LAB growth converts carbohydrates into lactic, acetate, formate, caproate, propionate, butyrate, and valerate acids (Wulandari *et al.* 2020).

Protein levels during the fermentation process showed fluctuating values. This is because the extracted enzyme is not pure so that there is a possibility of other proteins. Pineapple leaf fermentation using P. pentosaceus E7 showed the highest protein levels at 48 and 60 hours, these results are in line with the growth curve, where at that hour *P. pentosaceus* experienced exponential growth. The enzymes produced by bacteria can be used to damage the cell walls of pineapple leaves consisting of 70-82% holocellulose and 5-12% lignin, causing the bromelain enzyme to be released out of the cell (Chittappa *et al.* 2023). These results are in line with research by Neta *et al.* (2012) the bromelain content in pineapple leaves was 16,336 mg/mL.

The specific activity of bromelain extracted from leaves with P. pentosaceus E7 towards the end of the incubation period was very high and is in line with the research of Ketnawa *et al.* (2012), which reported a specific activity of 3.93 U/mg. Bromelain production carried out by Fissore *et al.* (2023) from pineapple fruit by mechanical blending obtained a specific bromelain enzyme activity of 3.38 U/mg. The increase in the specific activity of pineapple leaf bromelain at the end of incubation was due to the accumulation of organic acids, which damaged the leaf cell walls and facilitated the more optimal extraction of the bromelain enzyme. The longer the incubation time, the more metabolites are formed, including organic acids, resulting in a decrease in pH. Organic acids are the end products of carbohydrate metabolism by lactic acid bacteria (Noor *et al.* 2018).

Pineapple leaf bromelain concentrated with acetone obtained a purity level of 21.47 and a yield of 19.39%. Research conducted by Sripirom *et al.* (2025) using ammonium sulfate for bromelain enzyme precipitation, obtained an enzyme purity level of 0.89 and a yield of 12.61%. Acetone can precipitate protein up to 97.7% because it is an organic solvent that reduces the polarity of water, thereby increasing the interaction between protein molecules, which causes the protein to precipitate (Wang *et al.* 2022). The increase in bromelain activity observed in this study was attributed to acetone, which minimized protein degradation and reduced contaminants such as salt and polyphenols (Niu *et al.* 2018).

The presence of bromelain was successfully detected by comparing the commercial bromelain band. Bromelain, found in pineapple leaf tissue cells, was successfully extracted using *P. pentosaceus* E7. The results obtained are in line with research by Agrawal *et al.* (2022), which found that the molecular weight of the bromelain enzyme ranges from 23.8 to 37.0 kDa. Peptide production from the degradation of tofu liquid waste using bromelain extracted with *P. pentosaceus* E7 was successfully carried out. According to (Setyawati and Herdyastuti 2019), tofu waste contains quite a high crude protein of 27.55% so it can be used for the production of peptides and amino acids through enzymatic degradation using bromelain. Bioactive peptides can be produced through various processes, such as chemical or enzymatic hydrolysis, microbial fermentation, and physical methods, including Surface Active Maghemite Nanoparticles (SAMNs) (Naeem *et al.* 2022).

The active compounds resulting from the degradation of tofu waste using bromelain were analyzed using LC-MS, and 18 compounds were successfully confirmed. One of the confirmed compounds is daidzein, a marker compound commonly found in soybean degradation, which belongs to the isoflavone group. According to Grun *et al.* (2001), the daidzein content from tofu degradation is 0.016 mg/g. Soybeans are an abundant source of isoflavones. The main isoflavones found in soybeans are genistein, daidzein, and glycitein. Isoflavones are the most common estrogenic compounds found in plants and have been shown to possess antimicrobial and insecticidal properties, as well as prevent and reduce the risk of various cancers (Chen and Chen 2021).

Antibacterial tests of active compounds resulting from the degradation of tofu waste with bromelain showed the formation of inhibition zones. Based on the LCMS results, 11 antimicrobial compounds were confirmed in the tofu degradation sample (Table 3). This study showed that the highest relative abundance was obtained at retention time (RT) 11.12, namely the compound N~6~-[4-(diethylamino)-2-methylphenyl]-N~2~-ethyl-1,3,5-triazine-2,4,6-triamine, of 41.68%, and at RT 12.00, namely the compound Scorodocarpine A, also of 41.68%. The compound N~6~-[4-(diethylamino)-2-methylphenyl]-N~2~-ethyl-1,3,5-triazine-2,4,6-triamine from the triazines class has been reported by Shah *et al.* (2014) to have antimicrobial, antimalarial, and anticancer activities and Scorodocarpine A from the indoles class has been reported by Wiart *et al.* (2023) to have antibiotic activity. The results obtained are in line with the research of Araujo *et al.* (2021), Ramirez and Robles (2017), and Silva *et al.* (2018) organic waste such as pomegranate peel contains polyphenols (0.361 g) which can inhibit *Penicillium digitatum*. Organic waste from broccoli, cabbage, and kale contains polyphenols and flavonoids which can inhibit *Alternaria* spp. with an occupancy percentage of 24.14±0.58%. Waste from soybean processing contains phenolic acids and flavonoids which can inhibit *Colletotrichum gloeosporioides* with an inhibition zone diameter of 4.15 mm.

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