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Research Article
Antioxidant and Antimicrobial Potential of Peptide from Rabbit Meat Hydrolysate Prepared by Trypsin and Zingibain

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Abstract

Background and Objective: Rabbit meat is a livestock product potentially viable as a protein source to obtain peptides. Antioxidant and antimicrobial peptides are ingredients extracted from various foods through enzymatic hydrolysis, chemical hydrolysis and fermentation to produce health-promoting foods. This research aims to investigate the potential of rabbit meat as a source of antioxidant and antimicrobial peptides through hydrolysis using trypsin and zingibain enzymes. **Materials and Methods:** This research conducted an explorative-descriptive approach, focusing on antioxidant and antimicrobial activity. Rabbit meat was extracted using trypsin, zingibain and a combination of trypsin and crude extract zingibain. The hydrolyzed rabbit meat extract was tested at intervals of 0, 2, 6, 16, 24, 40 and 48 hrs to determine the degree of hydrolysis and the profile of hydrolyzed proteins with electrophoresis SDS PAGE. The antioxidant activity was tested using the DPPH method and the antimicrobial activity using agar well diffusion method. **Results:** The degree of hydrolysis increased with the hydrolysis time. The highest protein content of rabbit meat extract hydrolyzed with trypsin was 287.65 mg/mL, observed during 12 hrs hydrolysis. The optimum conditions for the hydrolysis of rabbit meat protein were obtained at 24 hrs, with an IC_{50} value of 52.45% hydrolyzed by trypsin. As per antimicrobial activities, *Escherichia coli* and *Salmonella* sp. were more effective in inhibiting rabbit meat hydrolysates compared to *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The inhibition of all pathogen increased until 12 hrs hydrolysis but decreased in 24 hrs hydrolysis. **Conclusion:** The combination zingibain enzyme and trypsin is feasible for hydrolyzing rabbit meat and the optimum hydrolysis time was 24 hrs with IC_{50} 52.45 ppm, although accompanied by reduction in antibacterial activities.

Key words: Antioxidant, antibacterial, peptide, rabbit meat, trypsin, zingibain

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Food industry around the world, especially meat, has changed following public raised awareness about the consumption of functional foods. Functional food products based on bioactive peptides are currently in the spotlight of research and development[1]. Antioxidant activities in peptides are crucial to replace commercial antioxidants for preventing fat oxidation in processed animal products. The use of synthetic antioxidants is limited due to their carcinogenic risks to humans[2].

The bioactive peptide components of meat proteins are not always available in their natural state; some will be active only when hydrolyzed from their natural proteins[3]. Meat protein hydrolysis can be carried out using proteolytic enzymes from various sources, including digestive enzymes or plant-derived proteases. A diverse source of animal protein has been used to produce bioactive peptides including rabbit[4]. Rabbit meat contains high protein with essential amino acids such as histidine, lysine, methionine, threonine, valine, isoleucine, leucine and phenylalanine[5].

Meat protein based bioactive peptides include chicken liver hydrolysates prepared by fermentation (FCLH) and enzymatic hydrolysis (ECLH) contain antibacterial (*Listeria monocytogenes* and *Micrococcus luteus*) and antioxidant activities[6]. Duck meat hydrolyzed using Protamex produced 2 antioxidant activity peptides: LQAEVEELRAALE with the highest DPPH (•) scavenging activity and IED-PFDQDDWGAWKK with the highest (•) OH scavenging activity[7]. Similarly, chicken breast protein was hydrolyzed by papain and produced a hydrolysate with antioxidant activity[8].

Ginger (*Zingiber officinale* var. Officinarum) is a plant that contains a protease enzyme called zingibain. As a part of rhizome group of biopharmaceutical plants, ginger was the main production of Indonesian biopharmaceutical plants in 2021, amounting to 303.53 ton[9]. Therefore, ginger has the potential as a source of plant-derived proteases to produce bioactive peptides. Protease enzymes have different specificities, so meat protein hydrolysis using different enzymes will produce peptide fragments with different activities. Enzymatic hydrolysis methods are easy to scale up and have a shorter reaction time than fermentation[10]. Therefore, the current study was conducted to determine the potential of rabbit meat as a source of peptides through hydrolysis using trypsin and zingibain enzymes as antioxidants for processed livestock products in order to increase functional health values.

MATERIALS AND METHODS

Study area: The study was carried out at Faculty of Animal Husbandry, Padjadjaran University, Sumedang, Indonesia in June, 2023 until the data were fully collected.

Collection of materials: The raw materials for this study were rabbit meat (New Zealand White crossbreed aged 3 months old) purchased from Tanjung Sari Farm (Tanjungsari, West Java, Indonesia). Fresh ginger (*Zingiber officinale* var. Officinarum) was obtained from a local market in Tanjungsari, West Java, Indonesia). Trypsin (HiMedia); phosphate buffer and trichloroacetic acid (TCA) (Merck); Coomassie brilliant blue and bovine serum albumin (HiMedia); Tris HCl buffer solution (Vivantis Tech). The SDS, pH 8.8, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) (Merck).

Zingibain enzyme extraction and determination protease enzyme activity: The ginger rhizomes were washed and cut into small pieces and dried in the sun for 24 hrs. Dried ginger rhizomes chopped and 100 g were homogenized with 200 mL of 100 mM potassium phosphate buffer using a wiring blender (Waring, Surabaya, Indonesia). The homogenate was then filtered through a piece of cheesecloth following which the filtrate was centrifuged (Siga 1-16K, Sigma-Aldrich, Osterode am Harz, Germany), for 10,500 $\times$ g for 4°C for 30 min.

Determination of protease activity: Determination of activity enzymes was carried out using the Bergmeyer *et al.*[11] method. This determination of activity enzymes was calculated by reacting 250 µL casein (Oxoid, UK) with a concentration of 2% (w/v) with 50 µL enzyme and 250 µL PBS 0.05 M pH 7. The reaction mixture was incubated at 37°C for 10 min and then 500 µL TCA was added (Sigma-Aldrich, USA) 0.2 M. The solution was incubated again at 37°C for 10 min with 2000 g centrifuge (Sigma 1-16K, Sigma-Aldrich, Osterode am Harz, Germany) for 10 min. Supernatant 375 µL add with 1250 µL Na_2CO_3 0.4 M and add even 250 µL of Folin Ciocalteu reagent (Merck, Darmstadt, Germany) with a dilution of 1:2 (v/v) and incubated at 37°C for 20 min. Absorbance was measured with a spectrophotometer (Agilent Cary 60 UV-Vis Spectrophotometer, US) at λ 578 nm. Aquadest was used as a blank and tyrosine solution (Sigma-Aldrich, USA) 5 mM as a standard. One unit of activity is defined as the amount of enzyme that can produce 1 µmol tyrosine per minute under test conditions.

Extraction and hydrolysis of rabbit meat protein: Exactly 20 g of rabbit meat was extracted using the method of Jang and Lee[12] with modifications. The meat was added with 200 mL of 0.02 m phosphate buffer (pH 7.4). The mixture of meat and buffer was homogenized and then centrifuged for 15,770 g for 20 min at 4°C. The obtained supernatant was filtered using a 0.22 µm filter (Biosharp, Beijing, China) under aseptic conditions and the result was rabbit meat extract. The rabbit meat extract was treated with trypsin, zingibain and trypsin+zingibain combination (1:1) with 1:100 (v/v) ratio of extract to enzyme. The mixture was incubated for 8 hrs at 50°C and then 5 mL of rabbit meat hydrolysate was collected at the intervals of 0, 2.6, 16, 24, 40 and 48 hrs to measure the degree of hydrolysis. After completing the incubation process, the hydrolysate was heated at 98°C for 5-10 min to inactivate the enzyme.

Hydrolysis degree test[13]: The degree of hydrolysis was determined using the SN-TCA method. A total of 2 mL of protein hydrolysate was combined with 2 mL of 10% (w/v) trichloroacetic acid (TCA). The mixture was left to stand for 30 min to allow precipitation to occur, then followed by centrifugation (7,800 g speed, 15 min) (Sigma 1-16K, Sigma-Aldrich, Osterode am Harz, Germany). The supernatant was then analyzed for protein content using the Lowry method (1951). Lowry method of protein content measurement used bovine serum albumin (Sigma Chemical, USA) as the protein standard and the result of protein content was expressed in mg/mL[14].

The degree of hydrolysis can be calculated by the following formula:

$$\text{DH (\%)} = \frac{\text{TCA soluble protein concentration}}{\text{Total protein concentration of sample}} \times 100$$

Hydrolysates molecular weight test: The degree of hydrolysates molecular weight was determined using SDS-PAGE electrophoresis (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis). The SDS-PAGE electrophoresis (Cleaver scientific, UK) was conducted using the modified Laemmli[15] method with a 4% stacking gel solution and a 12% separating gel in 3M Tris HCl buffer solution containing 0.3% SDS at pH 8.8. The rabbit meat hydrolysate was denatured using sample buffer (Tris-Cl 1M pH 6.8, SDS 20%, Coomassie brilliant blue) with a protein to buffer ratio of 2:1. The denaturation sample was then boiled at 90°C for 10 mins and subsequently centrifuged for 5 min[15]. The electrophoresis was performed at 150 volts for 60 mins and protein was carried out using Coomassie brilliant blue dye. This study used the estimation of the molecular weight (MW) of hydrolysates using SDS PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis)[16]. Sigma Marker in the molecular range of 14-96 kDa was employed and the Quantity One® 1-D analysis Software (Bio-Rad Laboratories, Italy) was employed to determine the MW and intensity of protein bands.

Antimicrobial activity: The antimicrobial activity was assessed through the agar well diffusion assay method[17]. The pathogens in this study were Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538; *Listeria monocytogenes* ATCC 7644) and Gram-negative bacteria (*Escherichia coli* ATCC 11229; *Salmonella typhimurium* ATCC 14088) sourced from the Central Laboratory of Padjadjaran University in Jatinangor, Indonesia. A standard chloramphenicol solution at a concentration of 30 mg/mL (HiMedia) served as the positive control. The pathogens, previously cultured in nutrient broth (HiMedia), were spread onto NA plates. Wells with a diameter of 5 mm were created on the plate, into which 40 µL of LAB isolate was poured. The plates were then incubated at 37°C for 24 hrs. The diameter of the clear zone formed around each well post-incubation was measured to confirm the antimicrobial activity.

Antioxidant activity test[18]: A total of 20 mL of each hydrolysate was collected as the mother liquor, from which sample solutions were prepared with concentrations of 1000, 640, 320, 160, 80, 40, 20 and 5 ppm. Subsequently, each sample solution was transferred into a test tube and 2 mL of 0.002% DPPH (2,2-difenil-1-pikrilhidrazil) was added. The sample solution was vortexed until the solutions were homogeneous and then incubated for 30 min in a dark room. After the incubation period, the absorbance value of each sample solution was measured using a UV-vis spectrophotometer (Agilent Cary 60 UV-Vis Spectrophotometer, US) at a wavelength of 512-520 nm. The blank solution, used as a reference, consisted of 2 mL of methanol and 2 mL of 0.002% DPPH.

The percentage inhibition value is calculated by the following formula:

$$\text{Inhibition (\%)} = \frac{A_{\text{blanko}} - A_{\text{sample}}}{A_{\text{blanko}}} \times 100$$

The IC_{50} value is determined using the linear regression equation y = ax+b, where y is the percent inhibition with a value of 50 and x is the concentration of the sample from which the IC_{50} value would be determined.

Statistical analysis: All experiments were carried out in triplicate. Data were analyzed using Analysis of Variance (ANOVA) and mean comparisons were performed using Duncan's multiple range t-test with $p<0.05$ significance level.

RESULTS

Chemical composition: The mean values of the chemical composition of rabbit meat were presented in Table 1. The results showed that rabbit meat had a high nutritional content. Rabbit meat was a good source of protein.

Protein content of rabbit meat hydrolysate: The hydrolysis process of rabbit meat protein was conducted for 24 hrs and sampling was performed at 0, 4, 8, 12 and 24 hrs. Afterwards, the protein content of rabbit meat hydrolysate after hydrolysis using trypsin, zingabin and trypsin+zingibain enzymes was measured using the Lowly Method and the results were presented in Table 2.

Protein profile of rabbit meat protein hydrolysate: The rabbit meat hydrolysate was tested for its profile using SDS PAGE shown in Fig. 1. Based on the protein profile, it was observed that the longer the hydrolysis time, the shorter the protein molecular structure. This indicates that the enzymatic hydrolysis with pepsin enzyme has been effective in producing peptides with lower molecular weights.

The comparison between rabbit meat before hydrolysis and after hydrolysis showed that protein substrate molecules degraded into short peptide fragments characterized by the appearance of protein bands with low molecular weight (<6kDa).

Table 1: Chemical analysis of rabbit meat

Parameter	Percentage
Moisture	74.15±0.51
Protein	19.32±0.13
Fat	3.03±0.24
Ash	1.93±0.12
Carbohydrate	1.57±0.17

Mean±Standard Deviation

Table 2: Protein content hydrolyzed rabbit meat during hydrolysis

	Protein content (mg/mL)		
Hydrolysis time (hrs)	Trypsin	Zingibain	Trypsin+Zingibain
1	148.51±0.05^{a,3}	125.66±0.07^{a,1}	135.08±0.13^{a,1}
4	157.65±0.12^{a,2}	132.54±0.15^{a,1}	150.55±0.08^{a,2}
8	225.67±0.10^{b,2}	150.64±0.07^{b,1}	200.21±0.18^{b,2}
12	287.65±0.04^{b,3}	170.43±0.12^{c,1}	235.57±0.07^{b,2}
24	256.55±0.13^{b,3}	165.79±0.21^{b,1}	247.78±0.13^{b,2}

The values represent means of three experiments±SD. $^{a-c}$Different superscripts in the same column and ^{1,3}Different superscripts in the same row represent significant differences (p<0.05)

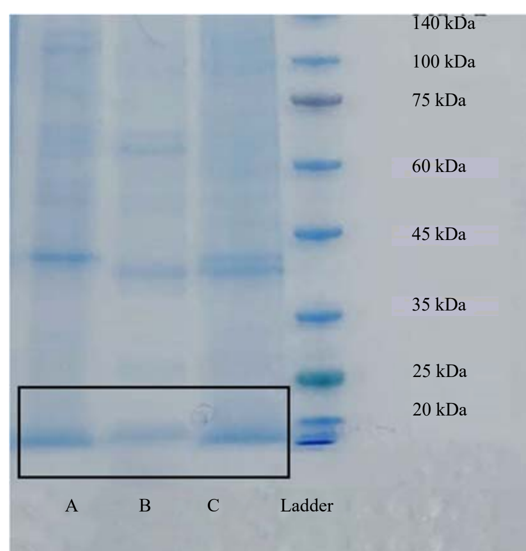

Fig. 1: SDS-PAGE profile of crude peptide in rabbit meat, (A) Rabbit meat extract hydrolyzed using trypsin, (B) Rabbit meat extract hydrolyzed using zingibain and (C) Rabbit meat extract hydrolyzed using trypsin+zingibain

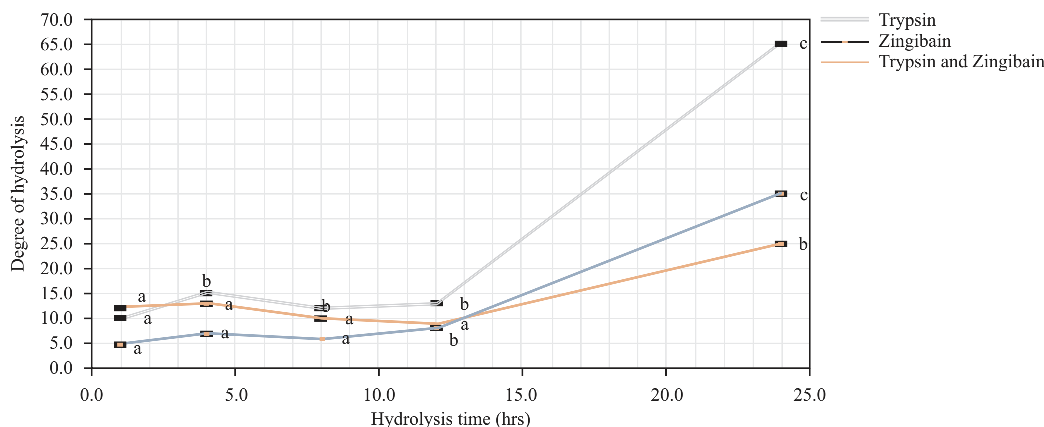

Fig. 2: Degree of hydrolysis of rabbit meat
Different letters indicate that the samples were considered significantly different at the 5% level (p<0.05)

Table 3: IC_{50} value of rabbit meat hydrolysate

	IC_{50} (ppm)		
Hydrolysis time (hrs)	Trypsin	Zingibain	Trypsin+Zingibain
1	$146.11\pm0.18^{c,1}$	$210.05\pm0.15^{d,3}$	$170.32\pm0.15^{c,2}$
4	$76.48\pm0.15^{b,1}$	$90.55\pm0.23^{c,3}$	$87.56\pm0.12^{b,2}$
8	$57.65\pm0.16^{a,1}$	$85.78\pm0.18^{b,3}$	$69.42\pm0.21^{a,2}$
12	$52.45\pm0.23^{a,1}$	$80.57\pm0.25^{b,3}$	$60.50\pm0.20^{a,1}$
24	$71.22\pm0.23^{a,1}$	$102.22\pm0.17^{a,2}$	$65.78\pm0.15^{a,2}$

[a-c]Different superscripts in the same column represent significant differences (p<0.05) and [1,3]Different superscripts in the same row represent significant differences (p<0.05)

Table 4: Analysis of inhabitation zone of selected bacteria with various hydrolysis times

		Hydrolysis time (hrs)				
Types of bacteria	Enzyme	0	4	8	12	24
Escherichia coli	Trypsin	10.0^{a}	12.0^{b}	12.0^{b}	14.0^{c}	11.0^{a}
	Zingibain	6.0^{a}	6.2^{b}	6.5^{c}	6.0^{a}	6.2^{b}
	Trypsin+Zingibain	8.0^{a}	9.5^{a}	10.0^{b}	10.5^{b}	$9,0^{a}$
Salmonella typhimurium	Trypsin	11.0^{a}	12.5^{b}	12.5^{b}	13.3^{c}	12.5^{b}
	Zingibain	5.5^{a}	6.0^{a}	6.5^{a}	6.0^{a}	6.2^{a}
	Trypsin+Zingibain	6.5^{a}	8.7^{b}	8.5^{b}	10.5^{c}	9.0^{b}
Staphylococcus aureus	Trypsin	11.3^{a}	11.2^{a}	11.5^{a}	12.2^{b}	11.0^{a}
	Zingibain	6.7^{a}	7.2^{a}	7.8^{b}	8.4^{c}	7.3^{a}
	Trypsin+Zingibain	5.7^{a}	8.2^{b}	8.8^{b}	9.4^{c}	8.2^{b}
Pseudomonas aeruginosa	Trypsin	9.0^{a}	10.3^{b}	10.8^{b}	11.3^{b}	10.1^{b}
	Zingibain	5.8^{b}	6.2^{b}	6.7^{c}	6.0^{b}	5.0^{a}
	Trypsin+Zingibain	6.6^{a}	9.3^{c}	9.6^{c}	10.6^{c}	8.0^{b}

[a-c]Different superscripts in the same column represent significant differences (p<0.05)

Degree of hydrolysis of rabbit meat: The degree of hydrolysis indicates the number of peptide bonds of protein in the hydrolysate that were cleaved by protease activity. The degree of hydrolysis of rabbit meat hydrolysate with various enzymes and hydrolysis times was measured using the SN-TCA method shown in Fig. 2.

Antioxidant activity of rabbit meat hydrolysate: Antioxidant activity test was carried out using the DPPH method (2,2-Diphenyl-1-Picrylhydrazyl) and expressed as the IC_{50} value, representing the concentration needed to inhibit 50% of free radicals (DPPH). Rabbit meat hydrolysate obtained at different hydrolysis times resulted in varying antioxidant activity values shown in Table 3.

Antimicrobial activity of rabbit meat hydrolysate towards several pathogenic bacteria: This study evaluated the antibacterial activities of all enzymatic hydrolysates against four selected bacteria, namely *S. aureus*, *E. coli*, *S. typhimurium* and *P. aeruginosa*. The antibacterial activities were assessed by evaluating the inhibition zones shown in Table 4.

DISCUSSION

The extraction and optimization of the zingibain enzyme from ginger was performed, followed by the hydrolysis of crude peptides from rabbit meat using both zingibain and trypsin enzymes. A previous study regarding the characterization of protease extracted from common ginger (*Zingiber officinale* Roscoe) showed optimum activity at 60°C with pH ranging from 6 to 8[19]. Phenolic compounds are major secondary metabolites found in plants and in various foods and nutraceuticals. Phytochemicals have multiple functions, namely, acting as biological agents and antioxidants, reducing inflammation, modulating enzyme activity and regulating gene expression. The ginger extract contained 181.41 mg/g of polyphenols and 14.15 mg/g flavonoids[20]. Protease activity of ginger extract was 1.02 U/mg.

Based on Table 2, the hydrolyzed protein content of rabbit meat treated with trypsin, zingibain and trypsin+zingibain increased until 12 hrs but decreased at 24 hrs. Protein is the most crucial component in hydrolysate products. Meat protein hydrolysis occurs with the assistance of trypsin and crude zingibain to convert insoluble proteins into soluble proteins which are further hydrolyzed into peptides or amino acids. The degree of hydrolysis (DH), may be understood as the number of broken peptide bonds in relation to the original protein. As a result, the total dissolved protein increases in final product[21]. The process of protein hydrolysis leads to an increase in antioxidant activity. Proteins are hydrolyzed into amino acids, thus increasing the amount of hydrophobic free amino acids (valine, isoleucine, phenylalanine and methionine) which significantly contribute to enhancing the antioxidant activity of protein hydrolysates[22].

The highest protein content was in the hydrolysate of rabbit meat, which was hydrolyzed using trypsin for 24 hrs, resulting in a protein content of 276.55 mg/mL. The protein content of rabbit meat extract hydrolyzed by trypsin reached its peak during the 12 hrs hydrolysis, with a protein content of 287.65 mg/mL. Meanwhile, the protein content of rabbit meat extract hydrolyzed with zingibain showed the highest value during the 12 hrs hydrolysis, with a protein content of 170.43 mg/mL.

Based on Fig. 1 showed that the degree of hydrolysis for the various hydrolysates ranged from 5.25 to 75.29%. It was observed that the degree of hydrolysis increased with the prolongation of the hydrolysis time. This finding aligned with a previous study that used papain, to hydrolyze he muscles of Chinese sturgeon to produce functional properties of fish protein hydrolysate, where a higher degree of hydrolysis was achieved with longer hydrolysis times and the higher enzyme concentration[23]. Similar results were observed in another study that used papain to hydrolyze goat milk and cow milk, revealing that a longer hydrolysis time led to a higher DH level of hydrolysates[24].

However, it is worth nothing that the degree of hydrolysis did not show a significant increase beyond 12 hrs hydrolysis time. Trypsin specifically cleaves peptide bonds at the C-terminal side of lysine and arginine residues[25]. The research on assessing the characteristics of ginger protease-degraded collagen hydrolysate revealed that LC-MS findings indicated the distinctive substrate specificity of ginger protease, which recognizes Pro and Hyp at the P2 position. This recognition was based on the amino acids observed at the P2 position across three types of tripeptides (Gly-Pro-Y, X-Hyp-Gly and Z-Pro-Gly) and 136 identified peptides (>4 amino acids)[26]. The degree of hydrolysis markedly increased from 12 to 24 hrs of hydrolysis time and therefore, the optimum hydrolysis time for all enzyme was 24 hrs. Among the enzymes tested, trypsin exhibited the highest degree of hydrolysis (75%), while the combined trypsin+zingibain showed a better degree of hydrolysis (45%), compared to crude zingibain (35%) as zingibain enzyme was present only the crude extract. Zingibain is a protease enzyme sourced from ginger and is capable of hydrolyzing meat peptide bonds and lipase enzymes that can break down fat. Ginger contains active substance found in volatile oil that can reduce oxidation level and prevent odor (off-flavor). Ginger also comprises of essential oils and antioxidant compounds, such as gingerol, shogaol, zingerone and parasols[27].

Based on Table 3, it can be observed that the highest antioxidant hydrolysis activity of rabbit meat hydrolysate is achieved at a hydrolysis time of 24 hrs with an IC_{50} value of 49.22 ppm. Different hydrolysis time produces different antioxidant activity; the longer the hydrolysis, the higher the antioxidant activity as indicated by smaller IC_{50} value. Differences in antioxidant activity hydrolysates with similar DH may be caused by differences in the catalytic action and enzyme specificity, which may affect the number and location of peptide bonds hydrolyzed. The antioxidant activity of rabbit meat hydrolysate was lower than that of porcine liver protein hydrolysates using alcalase, bromelain and papain[28]. The antioxidant activity of bioactive peptides is strongly influenced by the protein structure and its constituent amino acids. Longer hydrolysis processes degrade protein into smaller peptide fragments, thereby enhancing the antioxidant activity. Several amino acids, such as leucine, tyrosine, methionine and cysteine, generally act as antioxidants by donating electrons to free radicals.

Data analysis from Table 4 indicates that rabbit meat hydrolysates inhibited *E. coli* and *Salmonella* sp. more effectively than *P. aeruginosa* and *S. aureus*. The inhibition of all pathogens increased until 12 hrs hydrolysis but decreased in 24 hrs hydrolysis. Antibacterial activities were not in line with the degree of hydrolysis, which was still increased up to 24 hrs. The higher DH did not necessarily correlate with a higher zone of inhibition of the antibacterial activities of rabbit meat hydrolyzed with trypsin was higher than that with zingibain and trypsin+zingibain[29]. Overall, the zone of inhibition of rabbit meat hydrolysate against Gram-negative bacteria was greater than the Gram-positive bacteria. Different cell wall structures between Gram-positive and Gram-positive bacteria are believed to cause differences in response to various treatments. Rabbit meat hydrolyzed with trypsin for 12 hrs was the most efficient in inhibiting *E. coli*, *S. typhimurium*, *S. aureus* and *P. aeruginosa* had the largest clear zone at 12.0; 12.3; 12.2; 11.3, respectively.

From this research, it was revealed that the use of the zingibain enzyme has the potential to be used to hydrolyze rabbit meat to produce peptides with antioxidant and antibacterial activity although using the trypsin enzyme and a combination of trypsin and the zingibain enzyme produces peptides with better antioxidant and antibacterial activity. Enzymes derived from plant materials have the potential to replace synthetic antioxidants which will inhibit fat oxidation and also have the potential to produce antibacterials to replace chemical preservatives to inhibit pathogenic bacteria.

CONCLUSION

The enzymes trypsin, zingibain and a combination of these two enzymes can be effectively used to hydrolyze rabbit meat produced rabbit meat hydrolysate. The optimum conditions for hydrolysis of rabbit meat were 24 hrs using the trypsin enzyme to produce peptides with an IC_{50} of 52.45% and effectively inhibit the growth of *Escherichia coli* and *Salmonella* sp. The peptides can be processed for purification, until the sequence of the peptide is obtained to be used as functional food.

SIGNIFICANCE STATEMENT

Rabbit meat contains high protein so it has the potential to obtain peptides with antioxidant and antimicrobial activity. This research aims to study the potential of antioxidant and antimicrobial peptides from the hydrolysis of rabbit meat using the commercial enzyme trypsin and enzymes from the raw material plant zingibain. In this research, hydrolysis was carried out using trypsin, zingibain and a combination of both. The use of various enzymes is expected to obtain peptide cutting sites that have antioxidant and antimicrobial activity. The research results showed that the optimum conditions for hydrolysis of rabbit meat used trypsin with an IC_{50} of 52.45% and effective inhibition of *Escherichia coli* and *Salmonella* sp.

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REFERENCES

1. Zaky, A.A., J. Simal-Gandara, J.B. Eun, J.H. Shim and A.M. Abd El-Aty, 2021. Bioactivities, applications, safety, and health benefits of bioactive peptides from food and by-products: A review. Front. Nutr., Vol. 8. 10.3389/fnut.2021.815640.
2. Qian, Z.J., W.K. Jung, H.G. Byun and S.K. Kim, 2008. Protective effect of an antioxidative peptide purified from gastrointestinal digests of oyster, *Crassostrea gigas* against free radical induced DNA damage. Bioresour. Technol., 99: 3365-3371.
3. Sánchez, A. and A. Vázquez, 2017. Bioactive peptides: A review. Food Qual. Saf., 1: 29-46.
4. Chakrabarti, S., S. Guha and K. Majumder, 2018. Food-derived bioactive peptides in human health: Challenges and opportunities. Nutrients, Vol. 10. 10.3390/nu10111738.
5. Morshdy, A.E.M.A., W.S. Darwish, E.S.S. El Sebay and E.S.M. Ali, 2022. Amino acid profile of rabbit meat: Dietary intake and the effect of freezing on the amino acid composition. Thai J. Vet. Med., 52: 251-258.
6. Chakka, A.K., M. Elias, R. Jini, P.Z. Sakhare and N. Bhaskar, 2015. *In-vitro* antioxidant and antibacterial properties of fermentatively and enzymatically prepared chicken liver protein hydrolysates. J. Food Sci. Technol., 52: 8059-8067.
7. Wang, L.S., J.C. Huang, Y.L. Chen, M. Huang and G.H. Zhou, 2015. Identification and characterization of antioxidant peptides from enzymatic hydrolysates of duck meat. J. Agric. Food Chem., 63: 3437-3444.
8. Sun, Y., D. Pan, Y. Guo and J. Li, 2012. Purification of chicken breast protein hydrolysate and analysis of its antioxidant activity. Food Chem. Toxicol., 50: 3397-3404.
9. BPS, 2019. Indonesian Biopharmaceutical Plant Statistics 2018. BPS Indonesia, Jakarta, Indonesia, ISBN: 2339-0956, Pages: 71.

10. Daliri, E.B.M., D.H. Oh and B.H. Lee, 2017. Bioactive peptides. Foods, Vol. 6. 10.3390/foods6050032.
11. Bergmeyer, H.U., J. Bergmeyer and M. Grassl, 1983. Methods of Enzymatic Analysis: Enzymes 3, Peptidases, Proteinases, and their Inhibitors. 3rd Edn., Verlag Chemie, Hoboken, New Jersey, ISBN: 9783527260454.
12. Jang, A. and M. Lee, 2005. Purification and identification of angiotensin converting enzyme inhibitory peptides from beef hydrolysates. Meat Sci., 69: 653-661.
13. Hoyle, N.T. and J.H. Merrit, 1994. Quality of fish protein hydrolysate from Herring (*Clupea harengus*). J. Food Sci., 59: 76-79.
14. Satpathy, L., D. Dash, P. Sahoo, T.N. Anwar and S.P. Parida, 2020. Quantitation of total protein content in some common edible food sources by lowry protein assay. Lett. Appl. NanoBioSci., 9: 1275-1283.
15. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.
16. Alimohammadi, M., N. Ali and M. Khodakovskaya, 2013. Quantitative detection of inositol hexakisphosphate (InsP6) in crop plants using polyacrylamide gel electrophoresis (PAGE). Am. J. Plant Sci., 4: 1-6.
17. Atipairin, A., N. Songnaka, S. Krobthong, Y. Yingchutrakul, T. Chinnawong and T. Wanganuttara, 2022. Identification and characterization of a potential antimicrobial peptide isolated from soil *Brevibacillus* sp. WUL10 and its activity against MRSA pathogens. TropicalMed, Vol. 7. 10.3390/tropicalmed7060093.
18. White, P.A.S., R.C.M. Oliveira, A.P. Oliveira, M.R. Serafini and A.A.S. Araujo *et al*., 2014. Antioxidant activity and mechanisms of action of natural compounds isolated from lichens: A systematic review. Molecules, 19: 14496-14527.
19. Nafi', A., H.L. Foo, B. Jamilah and H.M. Ghazali, 2013. Properties of proteolytic enzyme from ginger (*Zingiber officinale* Roscoe). Int. Food Res. J., 20: 363-368.
20. Syafitri, D.M., J. Levita, Mutakin and A. Diantini, 2018. A review: Is ginger (*Zingiber officinale* var. Roscoe) potential for future phytomedicine? Indones. J. Appl. Sci., 8: 1-6.
21. Hou, Y., Z. Wu, Z. Dai, G. Wang and G. Wu, 2017. Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance. J. Anim. Sci. Biotechnol., Vol. 8. 10.1186/s40104-017-0153-9.
22. Jamil, N.H., N.R.A. Halim and N.M. Sarbon, 2016. Optimization of enzymatic hydrolysis condition and functional properties of eel (*Monopterus* sp.) protein using response surface methodology (RSM). Int. Food Res. J., 23: 1-9.
23. Noman, A., Y. Xu, W.Q. Al-Bukhaiti, S.M. Abeda, A.H. Alia, A.H. Ramadhana and W. Xia, 2018. Influence of enzymatic hydrolysis conditions on the degree of hydrolysis and functional properties of protein hydrolysate obtained from Chinese sturgeon (*Acipenser sinensis*) by using papain enzyme. Process Biochem., 67: 19-28.
24. Shu, G., J. Huang, C. Bao, J. Meng, H. Chen and J. Cao, 2018. Effect of different proteases on the degree of hydrolysis and angiotensin I-converting enzyme-inhibitory activity in goat and cow milk. Biomolecules, Vol. 8. 10.3390/biom8040101.
25. Li, B., F. Chen, X. Wang, B. Ji and Y. Wu, 2007. Isolation and identification of antioxidative peptides from porcine collagen hydrolysate by consecutive chromatography and electrospray ionization-mass spectrometry. Food Chem., 102: 1135-1143.
26. Liu, Q., B.H. Kong, Y.L. Xiong and X.F. Xia, 2010. Antioxidant activity and functional properties of porcine plasma protein hydrolysate as influenced by the degree of hydrolysis. Food Chem., 118: 403-410.
27. Mao, Q.Q., X.Y. Xu, S.Y. Cao, R.Y. Gan, H. Corke, T. Beta and H.B. Li, 2019. Bioactive compounds and bioactivities of ginger (*Zingiber officinale* Roscoe). Foods, Vol. 8. 10.3390/foods8060185.
28. Borrajo, P., M. Pateiro, M. Gagaoua, D. Franco, W. Zhang and J.M. Lorenzo, 2020. Evaluation of the antioxidant and antimicrobial activities of porcine liver protein hydrolysates obtained using alcalase, bromelain, and papain. Appl. Sci., Vol. 10. 10.3390/app10072290.
29. Thamamsena, R. and D.C. Liu, 2020. Antioxidant and antimicrobial activities of different enzymatic hydrolysates from desalted duck egg white. Asian-Australas. J. Anim. Sci., 33: 1487-1496.