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Effect of earthworm and papain enzyme concentration on antioxidant activity of earthworm (*Eudrilus eugeniae*) protein hydrolysate

Dianah Salsabila*, Nur Hidayat and Suprayogi

Department of Agro-industrial Technology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia

KEYWORDS

Antioxidant activity

Earthworm *Eudrilus eugeniae*

Papain enzyme

Soluble protein

Total phenolic content

ABSTRACT

The earthworm *Eudrilus eugeniae* (African Night Crawler) can be used as a raw material for making protein hydrolysates. Hydrolysis of earthworms using papain enzyme produces peptides that have bioactivity as antioxidants. Antioxidant activity in earthworm hydrolysate is caused by phenolic compounds that have an important role in neutralizing free radicals. This study aims to determine the effect of earthworm concentration and papain enzyme concentration on soluble protein, total phenolic content, and antioxidant activity. This study used the Randomized Block Design (RBD) method arranged with two factors, namely earthworm concentration (20% 30%, and 40% (w/v)) and papain enzyme concentration (8%, 10%, and 12% (w/v)) with three replications. Data analysis used *Analysis of Variance* (ANOVA) and continued with the DMRT test. The results showed that earthworm concentration, papain enzyme concentration, and the interaction between the two factors had a significant effect ($p<0.05$) on the test results of soluble protein, total phenolic content, and antioxidant activity. The treatment with earthworm concentration (40% (w/v)) and papain enzyme concentration (12% (w/v)) produced the highest mean soluble protein, total phenolic content, and antioxidant activity of 11.47%, 23.07 mg GAE/mL hydrolysate, and 3301.42 ppm.

Introduction

Eudrilus eugeniae (African Night Crawler) is an earthworm widely cultivated in Indonesia. Earthworms have various benefits and potential to be developed because they have a relatively high protein content, 58-78% of their dry weight, higher than the protein content found in fish (49.81%) and mammals (65%) (Herawati et al., 2019; Rusmini et al., 2016). The high protein content in the body of earthworms can be utilized as an alternative feed for livestock (Ernawati et al., 2019). In addition, *Eudrilus eugeniae earthworm* extract can also be used to treat several diseases because it has many therapeutic properties such as antibacterial activities, anticancer, antioxidant, and anti-inflammatory (Sarita and Kuswanti, 2022).

Antioxidants found in earthworms are of great interest as protective substances to help the human body reduce oxidative damage without side effects (Mustafa et al., 2022). The antioxidant properties of earthworm extract can be caused by phenolic compounds essential in neutralizing free radicals (Deswal et al., 2020). Earthworm extract has a phenolic compound content of 247.3 mg/L with an antioxidant activity of 38.26% dissolved in 80% ethanol (Aldarraji et al., 2013). In addition, research by Dewi et al. (2017) also showed that earthworm powder extracted using ethanol has a phenolic compound content of 1016.31 mg GAE/g and IC50 of 12.33 mg/mL.

Efforts can be made to utilize the protein content, total phenolic content, and antioxidant activity in earthworms *Eudrilus eugeniae* by manufacturing earthworm protein hydrolysate. Earthworm protein hydrolysate is a product resulting from the decomposition of earthworm protein into simple peptides and amino acids through enzymatic or chemical protein (Nurjanah

*Corresponding author
E-mail address: dianahsalsa@student.ub.ac.id

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et al., 2021; Sadiyah et al., 2018). However, enzymatic protein hydrolysis has advantages over chemical protein hydrolysis using acids or bases because it can be carried out under mild conditions, so it does not damage the amino acids of the protein source. Protease enzymes are one type that is generally widely used to carry out enzymatic protein hydrolysis procedures (Witono et al., 2020). Based on research by Hidayat et al. (2021), papain enzyme with the brand "PAYA" is one type of protease enzyme that can be used for enzymatic protein hydrolysis. Papain enzyme has also been widely traded commercially so that it is easy to obtain, available in large quantities, and relatively cheaper than other types of proteolytic enzymes or protein-breaking enzymes (Hidayat et al., 2021; Prihatini et al., 2021).

The concentration of papain enzyme is one of the factors that significantly influences the enzymatic hydrolysis process (Anggreini et al., 2017). This is because the higher the concentration of papain enzyme used, the more peptide bonds can be hydrolyzed so that the resulting protein content will also be higher (Fadilah et al., 2021; Hidayat et al., 2021). In addition, the percentage of earthworm raw materials used is another factor that can affect the number of components in the solution, so the higher the percentage of earthworm material used, the higher the protein that accumulates and decomposes (Hidayat et al., 2018). Based on this description, this study aims to determine the effect of adding earthworm concentration and papain enzyme concentration on soluble protein content, total phenolic content, and antioxidant activity in earthworms *Eudrilus eugeniae*.

Research and Methods

Materials

Fresh earthworms *Eudrilus eugeniae* was obtained from CV. RAJ Organik in Sukun District, Malang City, Indonesia. The earthworms used have an average length of 140-145 mm, a body diameter between 5-7 mm, an average body weight of 12 mg, and a brownish-red color.

Preparation of earthworm (Eudrilus eugeniae) powder

Fresh *Eudrilus eugeniae* earthworms were washed using running water for five repetitions, then boiled using boiling water at approximately 100°C for 1 minute. Afterward, the samples were dried in the sun for 12 hours and dried again using a *microwave* for 3 minutes. The earthworms were then crushed using a powder grinder for 3 minutes and then sieved using a 150 mesh, resulting in earthworm powder with a size of 150 mesh.

Dissolving papain enzyme "PAYA"

"PAYA" papain enzyme was weighed as much as 4 g, 5 g, and 6 g, then added 50 mL of distilled water, then stirred at 200 rpm for 10 minutes until homogeneous so that the resulting concentration of papain enzyme "PAYA" was 8%, 10%, and 12% (w/v).

Preparation of earthworm (Eudrilus eugeniae) protein hydrolysate

Earthworms with concentrations of 20%, 30%, and 40% (w/v) were added to papain enzyme with concentrations of 8%, 10%, and 12% (w/v) in a ratio of 1:2 (papain enzyme: earthworms) and stirred with a *hot magnetic stirrer* (10 minutes, 200 rpm) and adjusted the pH to close to pH 7 (HCL 1M or NaOH 1 M ~ 1mL). The hydrolysis process was then carried out at 55°C for 6 hours using a water bath shaker (110 rpm) and allowed to stand for 5 minutes. Then, the centrifugation process (6000 rpm for 60 minutes at 10°C) to separate the residue and supernatant. The resulting supernatant will then be analyzed for soluble protein, total phenolic content, and antioxidant activity. The preparation of earthworm protein hydrolysate was repeated in triplicate.

Parameter analysis

Soluble protein testing through formol titration was carried out based on the standard method (AOAC, 2005). The formol titration process was carried out in duplo (2 repetitions). The total phenolic content of earthworm hydrolysate was determined by the Folin-Ciocalteu method (Aldarraji et al., 2013; Marjoni et al., 2015). The amount of phenolic compounds was measured based on the *Gallic Acid Equivalent* (GAE)/mL hydrolysate standard curve. Antioxidant activity measurement on earthworm hydrolysate was carried out using the DPPH method based on a modification of previous research (Aldarraji et al., 2013; Dewi et al., 2017).

Statistical analysis

Analysis of variance (ANOVA) with the SPSS 25.0 programme was used in this study to determine the effect of each treatment, namely the addition of earthworm concentration and papain enzyme concentration on soluble protein, total phenolic content, and antioxidant activity produced from *Eudrilus eugeniae* earthworms. If there is a significant difference in the interaction of the two treatments, the DMRT (*Duncan's Multiple Range Test*) further test was carried out at the p<0.05.

Results and Discussion

Soluble protein

The earthworm and papain enzyme concentration affect the soluble protein of earthworm (*Eudrilus eugeniae*) protein hydrolysate (Table 1). The soluble protein hydrolysate ranged from 4.74-11.47%. The highest soluble protein test results in earthworm hydrolysate of 11.47% were shown in the treatment of 40% (w/v) earthworm concentration and 12% (w/v) papain enzyme concentration. The lowest soluble protein test results in earthworm hydrolysate of 4.74% can be seen in the treatment of earthworm concentration of 20% (w/v) and papain enzyme concentration of 0% (w/v). The results of the soluble protein test show that the higher concentration of earthworms and papain enzyme concentration used, the higher the test results of soluble protein produced in earthworm hydrolysate. This is in line with a research by Hidayat et al. (2021), which showed that the optimum solution was produced at the highest earthworm concentration (30% (w/v)) and papain enzyme concentration (10% (w/v)), giving the highest N-Amino response of 7.2%. This is related to the papain enzyme used by the "PAYA", which is one of the protein-catalyzing enzymes that can break down peptide bonds in protein substrates through its active site, which acts as a catalyst through covalent interactions with the substrate (Nguyen et al., 2022). Therefore, an increase in enzyme concentration can accelerate the hydrolysis reaction with sufficient substrate available to bind. This is supported by the statement of Firdha et al. (2021) that substrate concentration is also an essential factor affecting enzyme activity. This is because the addition of substrate concentration can increase enzyme activity to the maximum limit, producing an increase in soluble protein levels (Karnila et al., 2020).

Figure 1 shows differences in the average results of soluble protein in earthworm hydrolysate caused by differences in earthworm concentration. The concentration of earthworms at 20% (w/v) has significantly different results from that of at 30% (w/v) and 40% (w/v), respectively. The highest soluble protein value (10.06%) was found in the treatment with the addition of 40% (w/v) earthworm concentration. The lowest soluble protein test results value of 5.16% was found in the treatment with the addition of 20% (w/v) earthworm concentration. These results showed that increasing the concentration of earthworms was parallel to an increase in the soluble protein produced, similar to the results reported by Hidayat et al. (2021). Their study found the highest soluble protein value (7.2%) obtained from adding the earthworm concentration of 30% (w/v). Increasing the level of raw materials used can increase hydrolyzed protein content. Hydrolysis breaks down the protein bonds and decreases the molecular weight, increasing the protein's solubility (Baehaki et al., 2020; Hidayat, et al., 2018). This is supported by the statement by Hidayat et al. (2021) that the amount of material used plays a vital role in the solution, because the higher concentration of earthworms used, the higher protein content will accumulate and then decompose.

Based on Figure 2, treatment without and with addition of papain enzyme concentration has significantly different results. Adding 12% (w/v) of papain enzyme produced the highest soluble protein content of 8.41%, while the lowest soluble protein content of 6.75% was found at control treatment (without or 0% (w/v) papain enzyme addition). This shows that the higher papain enzyme concentration used, the higher the soluble protein content produced in earthworm hydrolysate. The research results obtained are in line with the results of research from Hermaya et al. (2021), which also showed that the highest soluble protein content was produced in the fish hydrolysate when added with the highest enzyme concentration of 6%, giving the value of 11.64%. This is because the higher concentration of papain enzyme used can accelerate the hydrolysis reaction to increase the number of amino acids and polypeptides dissolved (Saputra and Nurhayati, 2016). In addition, the higher the soluble protein content in the hydrolysate product, the higher the nutritional value that can be digested by the body (Hermaya et al., 2021). This is also supported by Tapal and Tiku (2019) that enzymatic hydrolysis is generally considered to be the most efficient method in the hydrolysate preparation process. This is because the enzymes can increase the nutritional value of various foods by breaking down fats, carbohydrates, and complex proteins into smaller and simpler compounds.

Table 1. Mean results of soluble protein test on different concentrations of earthworms and papain enzyme concentration

Treatment		Soluble Protein (%)
Earthworm Concentration (% (w/v)	**Papain Enzyme Concentration (% (w/v))**	
20	0	4.74±0.28 [a]
20	8	5.04±0.19 [ab]
20	10	5.25±0.07 [b]
20	12	5.62±0.15 [c]
30	0	6.63±0.21 [d]
30	8	7.16±0.21 [e]
30	10	7.70± 0.19 [f]
30	12	8.14±0.25 [g]
40	0	8.90±0.19 [h]
40	8	9.55±0.28 [i]
40	10	10.30±0.19 [j]
40	12	11.47±0.15 [k]

Notes: The value presented is the mean of 3 replicates, ± represents the standard deviation from three measurements, and different letter indicate significant differences.

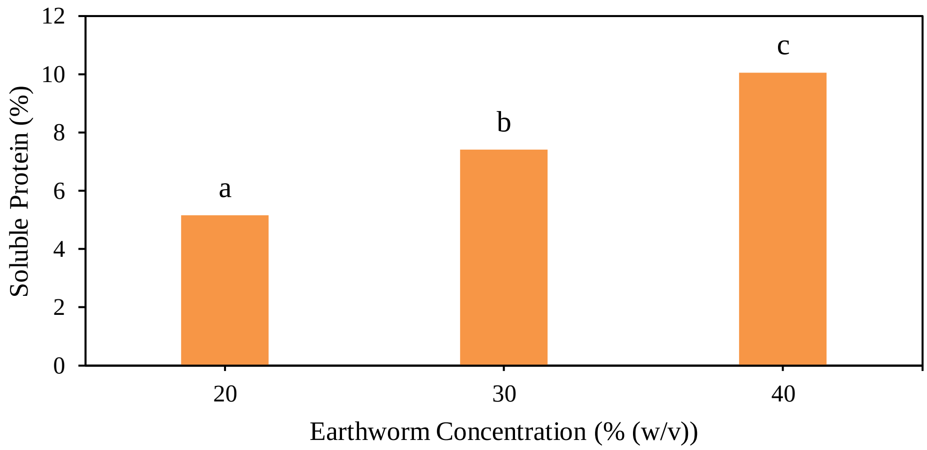

Figure 1. The average soluble protein of earthworm hydrolysate at different earthworm concentration. different notations indicate significantly differences from 3 replicates.

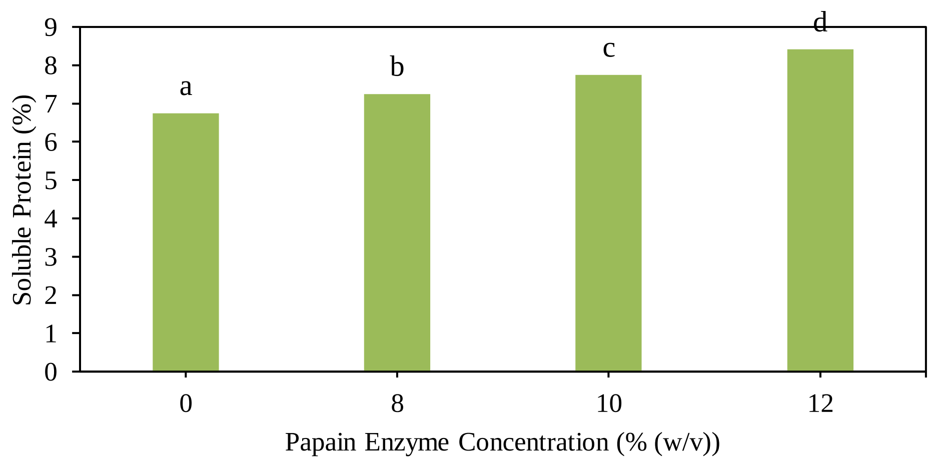

Figure 2. The average soluble protein of earthworm hydrolysate at difference papain enzyme concentration. Different notations indicate significantly differences from 3 replicates.

Table 2. Mean results of total phenolic content on different concentrations of earthworms and papain enzyme concentration

Treatment		Total Phenolic Content (mg GAE/mL hydrolysate)
Earthworm Concentration (% (w/v)	**Papain Enzyme Concentration (% (w/v))**	
20	0	9.89±0.23 [a]
20	8	11.01±0.39 [b]
20	10	11.83±0.34 [c]
20	12	13.26±0.45 [d]
30	0	14.01±0.47 [d]
30	8	14.98±0.34 [e]
30	10	16.10±0.34 [f]
30	12	17.00±0.34 [g]
40	0	18.28±0.69 [h]
40	8	19.25±0.34 [i]
40	10	21.42±0.47 [j]
40	12	23.07±0.72 [k]

Notes: The value presented is the mean of 3 replicates, ± represents the standard deviation from three measurements, and different letter indicate significant differences.

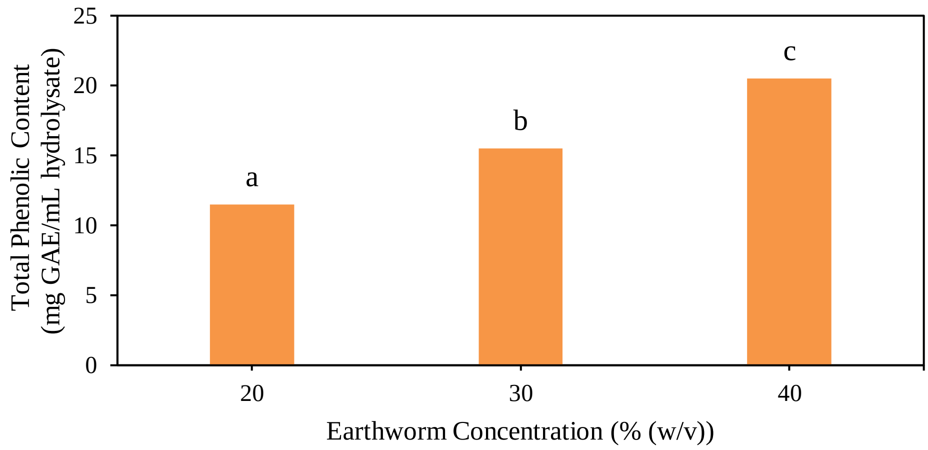

Figure 3. The average total phenolic content of earthworm hydrolysate at different earthworm concentration. Different notations indicate significantly differences from 3 replicates.

Total phenolic content

The interaction between the two treatments had a significant effect ($p<0.05$) on the total phenol produced by earthworm protein hydrolysate (Table 2). The average total phenol content ranged from 9.89-23.07 mg GAE/mL hydrolysate. The highest total phenol value of 23.07 mg GAE/mL hydrolysate was obtained from with the treatment combination of adding earthworm concentration of 40% (w/v) and papain enzyme concentration of 12% (w/v). The lowest total phenol content of 9.89 mg GAE/mL hydrolysate was from the treatment with earthworm concentration of 20% (w/v) and papain enzyme concentration of 0% (w/v). The results show that increasing the amount of earthworm concentration and papain enzyme concentration increases the total phenol content produced in earthworm hydrolysate. According to Samatra et al. (2017), phenol has antioxidant effects and can be used as a natural antioxidant to cure diseases related to inflammation and oxidative stress. The phenol in earthworm hydrolysate can come from plants consumed by earthworms that enter their digestive system (Deswal et al., 2020).

Figure 3 shows differences in the mean results of the total phenol test of earthworm hydrolysate due to differences in earthworm concentration. The concentration of 20% (w/v) earthworms has significantly different results with 30% (w/v) and 40% (w/v) of earthworm concentration. The highest total phenol content of earthworm hydrolysate (20.51 mg GAE/mL hydrolysate) was shown in the treatment with 40% (w/v) earthworm concentration. While, the lowest total phenol content of 11.50 mg GAE/mL hydrolysate was from the treatment with 20% (w/v) earthworm concentration. The results indicate that adding a higher concentration of earthworms lead to an increase in the total phenol content produced in earthworm hydrolysate. It is stated that phenolic compounds are the main elements responsible for antioxidant properties in earthworms. Phenolic compounds have various biological effects and roles, such as acting as reducing agents, capturing free radicals, and chelating pro-oxidant metal ions (Asma et al., 2023; Suparmi et al., 2020). According to Dewi et al. (2017), phenolic compounds are a group of secondary metabolites that can capture free radicals causing oxidative damage.

Figure 4 indicates that without addition of papain enzyme concentration (0% (w/v)) had significantly different results from that of with adding 8%, 10%, and 12% (w/v) papain enzyme concentration. The highest total phenol content was shown in the treatment with 12% (w/v) papain enzyme concentration, giving the value of 17.78 mg GAE/mL hydrolysate. In comparison, the lowest total phenol content was found in the treatment without papain enzyme addition, at value of 14.06 mg GAE/mL hydrolysate. The results show that the higher the concentration of papain enzyme added in the hydrolysate-making process, the higher the total phenol content produced by earthworm hydrolysate. This is because phenol compounds are closely related to peptide bonds and amino acids, so increasing the enzyme concentration may increase the availability of enzyme molecules to break down proteins into smaller peptide units (Deswal et al., 2020). A previous study by Samatra et al. (2017), showed that earthworm phenolic acid compounds can neutralize ROS (Reactive Oxygen Species) reactions caused by infection from *Salmonella typhi*.

Antioxidant activity

Table 3 shows that the highest antioxidant activity of earthworm hydrolysate of 3301.41 ppm was from the treatment of adding 40% (w/v) earthworm concentration and 12% (w/v) papain enzyme concentration. While, the lowest antioxidant activity of 7957.57 ppm was obtained from the treatment with 20% (w/v) earthworm concentration and 0% (w/v) papain enzyme concentration. The results indicate that adding more concentration of earthworms and papain enzyme used in making earthworm protein hydrolysate, may contribute to increase the antioxidant activity. The antioxidant activity value increased due to increased free amino acids and the number of peptides in the hydrolysate (Baehaki et al., 2015). However, the category of antioxidant activity produced in this study is classified as very weak at 3301.41 ppm. This can be due to the lack of time in hydrolysis, causing the enzyme to work less optimal (Hermaya et al., 2021). Enzymatic hydrolysis can be influenced by various factors (i.e., the length of hydrolysis and the concentration used). Nonoptimal conditions of those factors can cause the enzyme to lose its ability to bind to the substrate (Agustin et al., 2023).

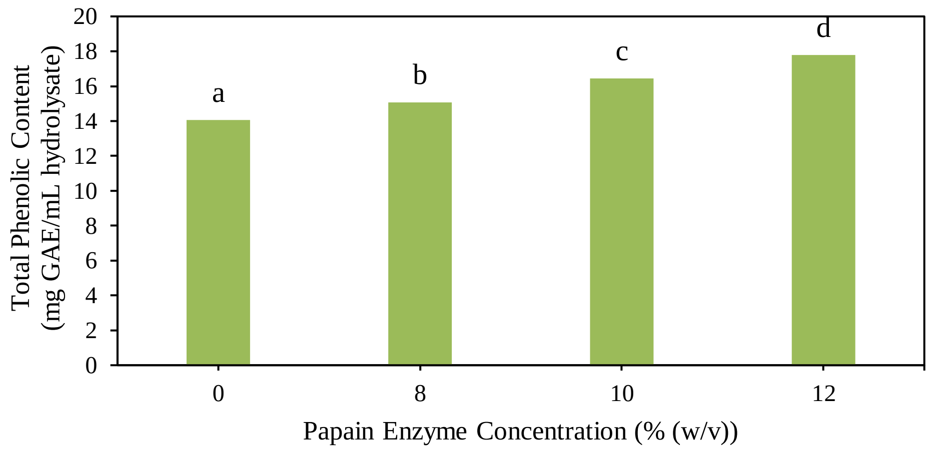

Figure 4. The average total phenolic content of earthworm hydrolysate at different papain enzyme concentration. Different notations indicate significantly differences from 3 replicates.

Table 3. Mean results of antioxidant activity test on different concentrations of earthworms and papain enzyme concentration

Treatment		IC$_{50}$ (ppm)
Earthworm Concentration (% (w/v)	Papain Enzyme Concentration (% (w/v))	
20	0	7957.57±114.25 ^{j}
20	8	7165.71±142.87 ^{i}
20	10	6642.75±29.59 ^{h}
20	12	6412.12±57.65 ^{g}
30	0	6148.46±124.41 ^{f}
30	8	5201.97±206.74 ^{e}
30	10	4410.24±169.33 ^{d}
30	12	4274.71±205.40 ^{cd}
40	0	4079.90±79.05 ^{c}
40	8	3754.16±82.23 ^{b}
40	10	3582.22±90.80 ^{b}
40	12	3301.41±79.70 ^{a}

Notes: The value presented is the mean of 3 replicates, ± represents the standard deviation from three measurements, and different letter indicate significant differences.

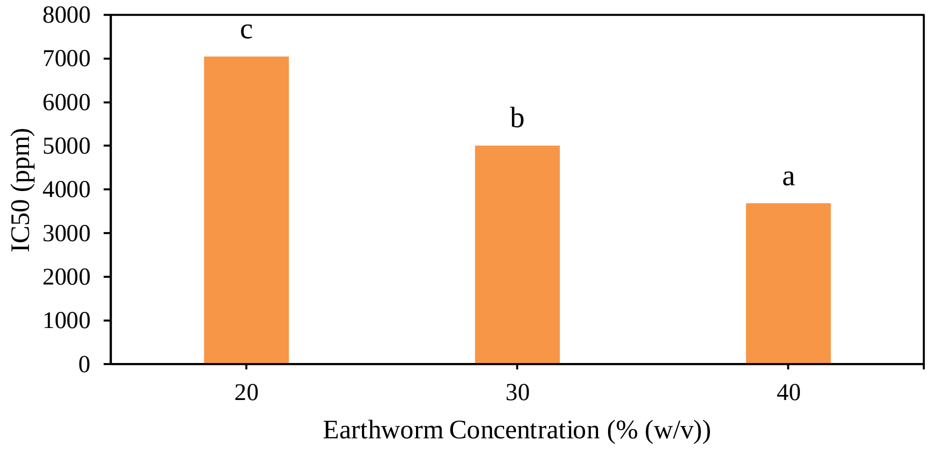

Figure 5. The average antioxidant activity content of earthworm hydrolysate at different earthworm concentration. Different notations indicate significantly differences from 3 replicates.

Figure 5 shows differences in the mean results of the antioxidant activity on earthworm hydrolysate due to earthworm concentration. The results confirmed that concentration of earthworms affected the antioxidant activity. The highest antioxidant activity of 3679.42 ppm was obtained from the treatment with a 40% (w/v) earthworm concentration. While, the lowest antioxidant activity of 7044.54 ppm was from treatment with a 20% (w/v) earthworm concentration. These findings show that the higher the concentration of earthworms used in the hydrolysate preparation, the higher the antioxidant activity produced. This result was in line with Nurjanah et al. (2021), who also showed that the lowest antioxidant activity of 24.09% was produced from the lowest concentration (500 ppm) sample solution, while the highest antioxidant activity of 93.12% was produced from the highest concentration (2000 ppm) of sample solution. Thus, the higher the concentration of the sample added to the hydrolysate preparation, the higher the antioxidant activity produced. This is because the substrate concentration used for the enzymatic hydrolysis process affects the peptides produced in the hydrolysate product, which functions as an antioxidant agent (Mutamimah et al., 2018). Earthworm antioxidants can reduce oxidative damage to the human body without causing side effects (Dewi et al., 2017).

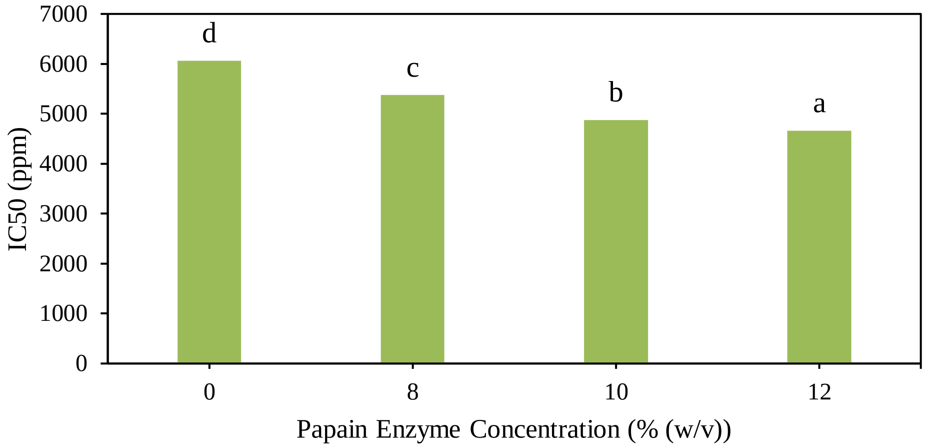

Figure 6. The average antioxidant activity content of earthworm hydrolysate at different papain enzyme concentration. Different notations indicate significantly differences from 3 replicates.

Figure 6 shows that 0% (w/v) papain enzyme concentration has significantly different results to that of with 8%, 10%, and 12% (w/v) of papain enzyme concentration. The highest antioxidant activity of 4662.75 ppm was shown at 12% (w/v) papain enzyme concentration, while the lowest antioxidant activity of 6061.97 ppm was shown at 0% (w/v) papain enzyme concentration. This finding also confirmed that the higher the papain enzyme added, the higher the antioxidant activity. This result aligned with Hermaya et al. (2021), who showed that the highest antioxidant activity in the fish hydrolysate (549.16 ppm) was obtained at the highest concentration of papain enzyme (6%). This is because the increase in papain enzyme concentration is proportional to the increase in the number of peptides and free amino acids produced in the hydrolysate product. Thus, the percentage of inhibition of free radical activity may increase along with the increase in the concentration of the hydrolyzing enzyme (Prastika et al., 2019). The hydrolysis process using papain enzyme can increase the amount of hydrophobic amino acids (i.e., methionine, isoleucine, valine, and phenylalanine) contributing to antioxidant activity (Witono et al., 2020). Aromatic amino acids (e.g. tryptophan and phenylalanine) can donate electrons to free radicals and form stable molecules. While amino acids (i.e., histidine, tyrosine, leucine, cysteine, and methionine) can neutralize free radicals by donating protons (Rabiei et al., 2019). A compound can be categorized as having antioxidant activity if the compound can donate hydrogen atoms, indicated by a change from purple to a pale-yellow color (Baehaki et al., 2015)

Best treatment selection

Table 4 shows that the treatment with the addition of 40% (w/v) earthworm concentration had better results than the other treatments. In particular, the treatment at 12% (w/v) papain enzyme concentration obtained the lowest total score value, thus getting the 1st rank and was selected as the best treatment. The 2nd and 3rd rankings were obtained in the treatment with the addition of concentration of earthworms at 10% and 8% papain enzyme concentration, respectively. The lowest rank was obtained in the treatment of 20% (w/v) earthworm concentration with papain enzyme concentration of 8% and 0%. The best treatment selected can produce the highest value in soluble protein content, total phenol content, and antioxidant activity of earthworm *Eudrilus eugeniae* hydrolysate.

Composition of amino acid

The amino acid composition of *Eudrilus eugeniae* protein hydrolysate was analyzed using the best treatment selected and without papain enzyme addition (Table 5). The results confirm that the protein hydrolysate of earthworm *Eudrilus eugeniae* contained 15 amino acids. According to Alahmad et al. (2022), amino acid composition plays an essential role as it can affect the functional properties and nutritional value of protein hydrolysates. According to Halim et al. (2018), amino acids in protein hydrolysates are critical water-binding capacity, emulsifying properties, solubility, and fat binding capacity. In addition, the amino acid composition in earthworm hydrolysate is important in the antioxidant activity (Wasunan et al., 2022).

Table 4. Determination of the best treatment

Treatment		Score				
Earthworm Concentration (% (w/v)	**Papain Enzyme Concentration (% (w/v))**	**L1**	**L2**	**L∞**	**Result Total**	**Rank**
20	0	0.81	0.17	0.20	1.17	12
20	8	0.82	0.15	0.19	1.16	11
20	10	0.83	0.14	0.18	1.15	10
20	12	0.84	0.13	0.17	1.14	9
30	0	0.86	0.11	0.15	1.13	8
30	8	0.88	0.09	0.12	1.10	7
30	10	0.90	0.08	0.11	1.09	6
30	12	0.91	0.07	0.10	1.08	5
40	0	0.93	0.05	0.07	1.06	4
40	8	0.95	0.04	0.06	1.04	3
40	10	1.00	0.00	0.03	1.03	2
40	12	1.00	0.00	0.00	1.00	1

Table 5. Amino acid composition of protein hydrolysate from earthworm *Eudrilus eugeniae*

Amino Acid	**Earthworm protein hydrolysate without using papain enzyme (mg/L)**	**Earthworm protein hydrolysate using papain enzyme (mg/L)**
L-Serine	44.92	537.33
L-Glutamic Acid	210.97	1423.94
L-Phenylalanine*	142.82	142.82
L-Isoleucine*	51.3	182.56
L-Valin*	38.63	316.96
L-Alanine	241.95	756.08
L-Arginine*	115.87	550.33
Glycine	185.79	840
L-Lysine*	100.82	576.97
L-Aspartic acid	57.17	785.71
L-Leucine*	50.19	325.01
L-Tyrosine	182.4	182.4
L-Proline	38.51	651.4
L-Threonine*	48.93	790.61
L-Histidine*	88.53	88.53

Notes: *Essential amino acid group

Table 5 shows that protein hydrolysate using enzymes can produce higher amino acid content than without adding enzymes. This is because the hydrolysis process using papain enzyme can increase the amount of hydrophobic amino acids (i.e., valine, phenylalanine, methionine, and isoleucine), which can contribute to the antioxidant activity of earthworm hydrolysate (Witono et al., 2016). According to Mutamimah et al. (2018), during hydrolysis, insoluble proteins are converted by papain enzyme into soluble proteins which will then be hydrolyzed into smaller components (e.g., peptides and amino acids). Enzymes could also improve absorption, metabolism, and digestibility (Nguyen et al., 2022). According to Bhaskar et al. (2019) and Knežević-Jugović et al. (2023), enzymatic hydrolysis is carried out not only to improve the nutritional and functional properties of protein sources, but also to produce hydrolysates with better antioxidant capacity. These antioxidant properties relate to the hydrolysate's ability to donate electrons or metal ions and remove free radicals.

According to Zielińska et al. (2017), the amount of amino acids can indicate protein hydrolysate’s ability to capture or inhibit free radicals. This is because the amino acid composition influences the DPPH free radical inhibitory activity of protein hydrolysate from earthworms. Islam et al. (2022) stated that isoleucine, valine, and threonine could contribute to the inhibition of DPPH free radicals. In addition, aromatic amino acids tyrosine and phenylalanine found in hydrolysates can stabilize DPPH free radicals through a proton donor mechanism to electron-deficient free radicals. The proton donor process can terminate the free radical reaction (Firmansyah and Abduh, 2019). Therefore, based on the amino acid composition and ability to inhibit DPPH free radicals, the protein hydrolysates from the earthworm *Eudrilus eugeniae* have potential as bioactive hydrolysates with antioxidant activity.

Conclusions

The concentration of earthworms and papain enzyme concentration, as well as the interaction between the two treatments, had a significant effect ($p<0.05$) on the soluble protein content, total phenolic content, and antioxidant activity from *Eudrilus eugeniae* earthworm hydrolysate. The highest soluble protein content, total phenolic content, and antioxidant activity was 11.47%, 23.07 mg GAE/mL hydrolysate, and 3301.42 ppm. These were obtained from the treatment with the addition of earthworm concentration (40% (w/v)) and papain enzyme concentration (12% (w/v)).

Declarations

Conflict of interests The authors declare no competing interests.

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