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Antioxidant characteristics of hydrolysate from low-value sea cucumber: *In vitro* and *in vivo* activities of *Caenorhabditis elegans*

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ABSTRACT

The antioxidant activity *in vitro* and *in vivo* of *Actinopyga miliaris* hydrolysate (AMH) was investigated. The proportion of oligopeptides with 150–1000 Da in AMH was 65.48%. The IC_{50} values of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), hydroxyl radical scavenging ability, and ferric ion reducing power of AMH were 0.37, 3.43, and 24.15 mg/mL, respectively. Compared with the control group, the body length of *Caenorhabditis elegans* fed with 8 mg/mL AMH extended from 632.08 μm to 1009.57 μm, and the swallowing frequency and head-swing frequency increased significantly. After being fed with AMH, the lifespan of *C. elegans* can be prolonged even under stress conditions, primarily due to superoxide dismutase activity, catalase activity, reduced glutathione content, and total antioxidant capacity in *C. elegans* increased, whereas reactive oxygen species level was reduced. The results showed that AMH had *in vitro* and *in vivo* antioxidant activity, which can alleviate oxidative damage and prolong life of *C. elegans*.

1. Introduction

Body cells produce reactive oxygen species (ROS) and other free radicals when subjected to various stress factors, including environmental pollution, mental stress, and strenuous exercise (Barati, Nikzad, & Karimian, 2020). When the accumulated free radicals exceed the removal capacity of the antioxidant defense system, oxidative stress is prone to occur, leading to apoptosis, tissue damage, and disease in the body (Lv et al., 2022). The occurrences of cancer, atherosclerosis, and other chronic diseases are reportedly associated with increased ROS level (Lv et al., 2022). Exogenous antioxidants can help reduce or inhibit the production of free radicals, thereby reducing the risk of diseases caused by oxidative stress (Liang et al., 2020). However, synthetic antioxidants are restricted or prohibited due to their potential carcinogenic effects (Pan et al., 2020). Therefore, the preparation of natural active substances like antioxidant peptides from food has become an active area of research.

Antioxidant peptides prepared from the by-products of aquatic processing and their high-quality proteins by enzymatic hydrolysis have been extensively reported. *Apostichopus japonicus* has the highest nutritional value among sea cucumber species, and the IC_{50} values of hydroxyl radical (•OH) scavenging abilities of hydrolysates from *A. japonicus* prepared by alcalase hydrolysis for 6 h is 1.63 mg/mL (Lee et al., 2021). Conversely, *A. lecanora* is a low-value sea cucumber encountered as a by-catch, and the IC_{50} of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical scavenging ability of hydrolysates from *A. lecanora* prepared by alcalase hydrolysis for 6 h is 0.35 mg/mL (Bordbar, Ebrahimpour, Zarei, Abdul Hamid, & Saari, 2018). These results suggest that low-value sea cucumber can be a low-cost raw material for preparing peptides with good antioxidant activity. However, the preparation of antioxidant peptide from low-value sea cucumber such as *Actinopyga miliaris* has not been reported.

Chemical and biological methods can be used to evaluate the antioxidant activity of peptides (Zhu, Lao, Pan, & Wu, 2022). The free-radical scavenging ability of $ABTS^{\bullet+}$ and •OH and reducing-capacity analysis are common chemical methods for evaluating antioxidant activity (Meng et al., 2021). However, relying solely on one kind of method may cause the antioxidant-activity results to be one sided, so choosing other methods for auxiliary analysis is necessary. *Caenorhabditis elegans* is a good *in vivo* model organism for studying antioxidant activity due to its simple structure, rapid reproduction rate, short lifespan, and high homology with the human genome (Mudd, San Martin-Gonzalez, Ferruzzi, & Liceaga, 2022). Therefore, the use of *C. elegans* assisted chemical methods to evaluate the antioxidant activity

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of protein peptides is gradually being recognized by researchers.

A. miliaris, which is primarily distributed in the Red Sea and Mozambique, has the advantages of low cost and application as a raw material in domestic dishes for Chinese consumers (Dong, Pan, & Wang, 2013). In the present study, sea cucumber hydrolysate was prepared by enzymatic hydrolysis by using *A. miliaris* as a raw materials. We determined the proximate composition of sea cucumber hydrolysate and investigated the *in vitro* antioxidant activities, including the $ABTS^{\bullet+}$ and •OH scavenging ability and ferric ion reducing antioxidant power (FRAP). The *in vivo* antioxidant activities of sea cucumber hydrolysate were also studied using the model of *C. elegans*. This work aimed to develop oligopeptides with antioxidant activity using low-value sea cucumber, providing a raw material to produce cost-effective antiaging functional foods.

2. Material and methods

2.1. Material and chemicals

A. miliaris was acquired from a local aquatic wholesale market. Pancreatin, collagenase, alcalase, and papain were purchased from Nanning Pangbo Biological Engineering Co., Ltd. (Nanning, China). *C. elegans* of N2 wild-type and *Escherichia coli* OP50 strains were obtained from Fujian Shangyuan Biotechnology Co., Ltd. (Fujian, China). Kits of total antioxidant (*T*-AOC), catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH) were obtained from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China). All other chemicals were analytical grade.

2.2. Preparation of *A. miliaris* homogenate (AM) and *A. miliaris* hydrolysate (AMH)

Dried sea cucumber body wall (1 kg) was cleaned and immersed in citric acid aqueous solution (10 L) for 16 h. After heating the immersed sea cucumber body at 90 °C for 90 min, it was broken into small pieces with size of 1–3 mm using a food processor. These small pieces of *A. miliaris* were homogenized in distilled water to prepare AM. On the other hand, AMH was prepared according to the report (Weng et al., 2014) with slight modifications. At pH 8.0 and 55 °C, the small pieces of *A. miliaris* were hydrolyzed for 2 h using pancreatin, collagenase and alcalase at an enzyme/substrate ratio of 0.5/100, 0.3/100 and 0.2/100 (w/w, dry matter), respectively. Enzymatic hydrolysis was continued by adding 1% papain (w/w, dry matter) for 2 h at pH 7.0 and 55 °C. The enzyme was deactivated by heating for 10 min at 100 °C. The hydrolysate was cooled to ambient temperature and then passed through a 50 nm ceramic membrane and reverse osmosis membrane in turn to obtain AMH. The collected AM and AMH were freeze dried for the following experimental analysis.

2.3. Proximate composition

The protein and ash contents of AMH were measured by AOAC 992.15 and AOAC 920.153 (AOAC 2005). Polysaccharide content was determined by phenol–sulfuric acid method (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956) according to the standard curve drawn with glucose standard solution. The saponin content of AMH was measured at 560 nm according to Liu et al (2023).

2.4. Molecular-weight distribution (MWD)

The MWD of AMH was determined according to the method reported by Zheng, Hao, Weng, and Ren (2019). HPLC was used for analysis and carried out on a TSK gel G2000 SWXL column (7.8 × 300 mm, TOSOH, Tokyo, Japan) with UV detection at 214 nm. The mobile phase comprised acetonitrile (A) and 0.18% trifluoroacetic acid in water (B) (A:B = 45:55, v/v) and was eluted at a flow rate of 0.5 mL/min. Cytochrome *C* (12,327 Da), trasylol (6533 Da), oxidized glutathione (613 Da), triglycine (Gly-Gly-Gly, 189 Da), and glycine (Gly, 75 Da) were used as molecular-weight standards to establish calibration curve.

2.5. Antioxidant activity assay in vitro

2.5.1. $ABTS^{\bullet+}$ scavenging ability

ABTS assay was conducted according to Zheng, Hao, Weng, and Ren (2019). The $ABTS^{\bullet+}$ working solution included 2 mM ABTS solution (10 mL) and 2.45 mM potassium persulfate (10 mL) and was placed in darkness at ambient temperature for 12 h. Then, the $ABTS^{\bullet+}$ reagent was diluted in 95% ethanol to prepare a detection wavelength of 0.80–1.00 at 734 nm. Different concentrations of AMH (0.3 mL) and AM (0.3 mL) were blended with $ABTS^{\bullet+}$ working solution (1.2 mL) respectively, and stored in a cabinet for 6 min, and the absorbance was measured at 734 nm. The $ABTS^{\bullet+}$ scavenging ability of AM and AMH was calculated according to Eq. (1).

$$\text{Scavenging ability}(\%) = \left(1 - \frac{A_s - A_0}{A}\right) \times 100 \quad (1)$$

where A_S is the absorbance of the sample, A_0 is the absorbance of distilled water instead of $ABTS^{\bullet+}$ working solution, and A is the absorbance of the initial concentration of $ABTS^{\bullet+}$ reagent.

2.5.2. •OH scavenging ability

The •OH scavenging ability of AM and AMH was determined according to Weng et al. (2014) with slight modifications. AMH and AM were prepared at different concentrations in test tubes. Then, 1 mL of AMH solution, 0.3 mL of 8 mM $FeSO_4$ solution, 0.25 mL of 30% 0.02 mol/L H_2O_2, and 1 mL of 0.3 mmol/L salicylic acid were sequentially poured into the test tubes. Uniform mixing was conducted at 37 ℃ for 0.5 h, after which the mixture was cooled rapidly and subjected to centrifugation (5000 × g, 10 min). The detection wavelength of the supernatant was 510 nm. The •OH scavenging rate of the sample was performed based on Eq. (2), where A_S is the absorbance of the sample, A_0 is the absorbance of distilled water instead of salicylic acid, and A is the absorbance of distilled water instead of the sample.

$$\text{Scavenging ability}(\%) = \left(1 - \frac{A_s - A_0}{A}\right) \times 100 \quad (2)$$

2.5.3. FRAP assay

FRAP assay was conducted according to Zheng, Hao, Weng, and Ren (2019). AMH and AM were dissolved in distilled water to prepare the required concentration of aqueous sample, respectively. The detection wavelength of the mixture was 700 nm. IC_{50} was the effective concentration when the absorbance of the AMH solution was 0.5.

2.6. Antioxidant activity assay in C. elegans

2.6.1. C. elegans culture and synchronization

C. elegans culture and synchronization were performed according to Farias-Pereira, Oshiro, Kim and Park (2018) with sight modifications. *C. elegans* was cultured in nematode growth medium (NGM) spread with *E. coli* OP50 at 25 °C. *C. elegans* cultured for 40 h was collected and repeatedly washed with sterilized ultrapure water. Gravid adult nematodes were lysed with bleaching solution (0.3 mL of 10 M NaOH, 0.54 mL of 10 M bleach water, and 2.16 mL of ultrapure water). Then, the collected eggs were washed with M9 buffer (250 mL of distilled water, 0.75 g of KH_2PO_4, 1.5 g of Na_2HPO_4, 1.25 g of NaCl, and 0.25 mL of 1 M $MgSO_4$). Finally, the eggs in an S-complete solution were hatched on a shaker at 25 °C for 12 h, and L1-stage nematodes were obtained.

AMH was dissolved in ultrapure water and passed through a 0.22 μm membrane filter to prepare 0.5, 2.0, and 8.0 mg/mL solutions. In the same way, the AM solution (8.0 mg/mL) was prepared. Ultrapure water

and 0.50 mg/mL GSH were used instead of AMH solution as the control group and positive control, respectively. L1-stage nematodes were collected and subsequently transferred onto the 12-well plate containing nematodes, sample solution, *E. coli* OP50, 5-fluoro-2′-deoxyuridine (FUDR) solution, and S-complete solution. The 12-well plate containing nematode solution was cultured and shook (150 rpm) at 25 °C for 60 h. Worms that reached the L4-stage were collected for subsequent experiments.

2.6.2. Lifespan assay

Lifespan assay was conducted based on the method reported by Shi et al. (2021) with minor modifications. Synchronous L4-stage nematodes were transferred onto NGM plates with *E. coli* OP50, sample solution, and FUDR. The first transfer day was recorded as 0th day. Nematodes were transferred onto fresh NGM plates every 2 days. The survival of nematodes was observed and recorded every day under an optical microscope until the last nematode died. *C. elegans* showing no signs of moving and swallowing after touching were considered as nematode death. About 20 nematodes were randomly selected for each assay, and all experiments were repeated three times.

2.6.3. Body length

Body length was measured as described by Zhang, Jiao, and Jing (2021a). In each group, synchronized nematodes were randomly chosen and transferred onto glass slides and then photographed with a microscope. The body length of nematodes was determined along the animal axis by using Motic Images Plus 3.0 software.

2.6.4. Pharyngeal pumping assay

Pharyngeal pumping assay was performed according to Shi et al. (2021) with sight modifications. In each group, synchronized nematodes were randomly picked onto NGM plates, and the times of pharyngeal pumping for 1 min was observed with an optical microscope. Pharyngeal pumping frequency was recorded based on the principle of one contraction of the posterior bulb/grinder during swallowing.

2.6.5. Motoricity assay

The locomotivity class and head swing of nematodes were examined according to Chen et al. (2020) and Lin et al. (2020), respectively. Synchronized nematodes from each group were randomly transferred onto flesh NGM plates and allowed to acclimate for 1 min on days 2, 6, and 10 of the lifespan assay. Then, the times of head swings of nematodes was recorded for 30 s. Locomotivity class was determined according to Chen et al. (2020). Nematodes that can move symmetrically and spontaneously were recorded as “I” nematode; after being prodded, nematodes with uncoordinated movement were recorded as “II” nematode; nematodes that cannot move but were obviously alive were recorded as “III” nematode.

2.6.6. Stress-resistance assay

Thermal stress was slightly modified according to Zhang et al. (2021). The synchronized nematodes in each group were incubated at 35 °C to measure thermotolerance. Nematode vitality was recorded every 2 h until all *C. elegans* died.

Oxidative stress was slightly modified according to Lin et al. (2020). In each group, the synchronized nematodes, 1 μL of 30% H_2O_2, and 1 mL of S-complete solution were added onto a 12-well plate. The number of surviving nematodes was counted every 1 h until all nematodes died.

2.6.7. ROS level

ROS level was determined according to Shen et al. (2017) with some modifications. A nematode solution containing 1 μL of 30% H_2O_2 was placed at 25 °C for 24 h. The cultured nematode solution was washed with M9 buffer and centrifuged (2000 rpm, 2 min), and the supernatant was discarded, and these steps were repeated three times. After adding 100 μL of M9 buffer and 1 μL of 5 mM 2′,7′-dichlorodihydrofluorescein diacetate solution to the centrifuge tube with nematodes, they were cultured in darkness for 1.5 h at 25 °C (100 rpm, 1.5 h). The nematode solution was washed with 0.01 mol/L PBS buffer and centrifuged (2000 rpm, 2 min), and then the supernatant was discarded; these steps were repeated three times. Finally, 100 μL of 0.01 mol/L PBS buffer was added to a centrifuge tube with nematodes. After nematodes were photographed by fluorescence microscope, the fluorescence intensity *in vivo* was quantitatively analyzed using ImageJ. Relative fluorescence intensity was calculated by comparing the sample group and the control group.

2.6.8. SOD, CAT, T-AOC activities, and GSH content

The treatment method of nematode solution was slightly modified according to Teng et al. (2022). The nematode solution was centrifuged at 2000 rpm and washed to remove excess *E. coli* OP50, and these steps were repeated three times. About 200 μL of 0.01 mol/L PBS buffer was added to the nematode solution and placed in an ultrasonic cell disruptor for 3.5 min under ice-bath conditions. The supernatant was obtained by centrifugation (2000 rpm, 2 min). According to kit instructions, the protein concentration, SOD, CAT, *T*-AOC activity, and GSH content in the supernatant were determined.

2.7. Statistical analysis

Each experiment was repeated three times, and data are expressed as the mean ± standard deviation. SPSS 26.0 software was used for significance analysis, and Origin 2022 and GraphdPad Prism 8.0 were used for plotting.

3. Results and discussion

3.1. Proximate composition and MWD of AMH

The protein content in the AMH was the highest, followed by polysaccharide, ash, and saponin (Fig. 1A). Fig. 1(B) presents the MWD of AMH. The major peaks of 470 and 1630 Da were observed in the MWD of the AMH. GPC analysis results further indicated that oligopeptides with a molecular weight of 150–1000 Da accounted for up to 65.48% (data not shown). The molecular weight of peptides can reportedly affect their antioxidant activity, and peptides with a M_w below 3000 Da can exhibit higher antioxidant activity because short-chain peptides can expose more electron donors to interrupt free radical chain reaction (Rao, Bajaj, & Mann, 2020; Li et al., 2022). According to the MWD result (Fig. 1B), we speculated that the prepared AMH may have good antioxidant activity.

3.2. In vitro antioxidant activities of AMH

Scavenging ability of •OH can be used to assess hydrogen atom donating abilities of the sample (Mazumder, Nabila, Aktar, & Farahnaky, 2020). Assay results of FRAP indicate the ability to donate electrons that convert ferric ion to the ferrous form (Hunsakul et al., 2022). Activity of $ABTS^{\bullet+}$ is used to determine the ability to provide electrons and hydrogen atoms (Hunsakul et al., 2022). Table 1 presents the IC_{50} values of ABTS, OH radical scavenging, and FRAP of AMH. A lower IC_{50} indicated that the sample had stronger free-radical scavenging capability. The IC_{50} of $ABTS^{\bullet+}$ scavenging ability of AMH was 0.37 mg/mL, which was much lower than those of sea cucumber (*Holothuria scabra*) hydrolysates (IC_{50} = 1.33–1.63 mg/mL; Doungapai et al., 2022). This finding suggested that the AMH prepared in this study had a good ability to donate electrons or hydrogen atoms, which may affect the MW of the hydrolysate (Zhang, Huang, Chen, Wang, & Wang, 2021b). The IC_{50} of OH radical scavenging ability of AMH was 3.43 mg/mL, indicating that AMH can reduce the damage caused by •OH in the body. Conversely, AMH showed an IC_{50} of 24.15 mg/mL for FRAP, also suggesting that AMH had a certain degree of ability to denote electrons.

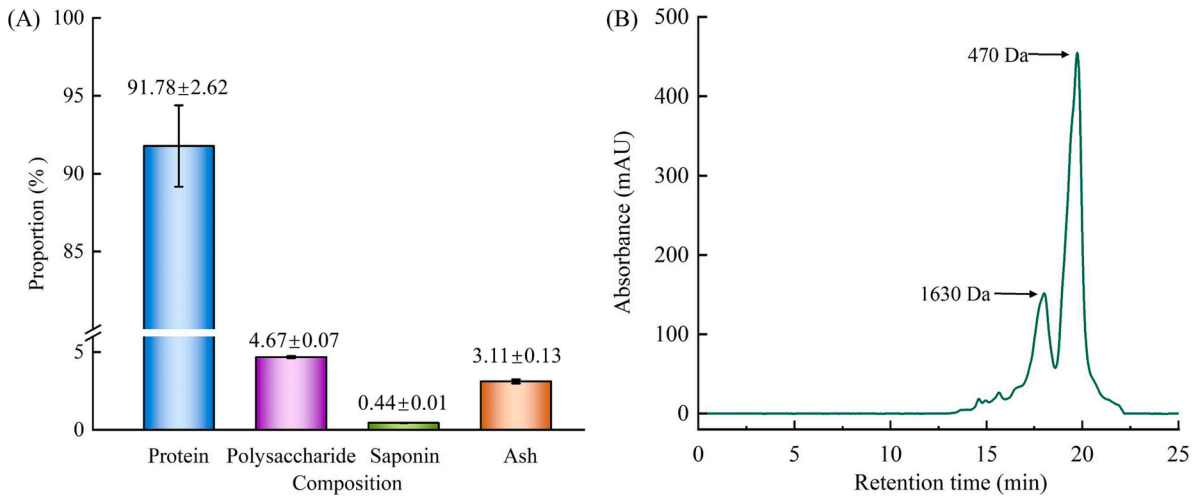

Fig. 1. Proximate composition (A) and molecular-weight distribution (B) of AMH.

Table 1
IC_{50} values (mg/mL) in the antioxidant activities of AMH and AM.

	AMH	AM
$ABTS^{\cdot+}$ scavenging	0.37 ± 0.02	21.64 ± 0.21
$^{\cdot}OH$ scavenging	3.43 ± 0.05	22.87 ± 0.08
FRAP assay	24.15 ± 0.28	56.32 ± 0.44

Data are expressed as mean ± standard deviation.

It was worth noting that the IC_{50} values of ABTS, OH radical scavenging, and FRAP of AMH were much lower than those of AM, suggesting that enzymatic hydrolysis can improve the antioxidant activity of *A. miliaris*.

3.3. Effect of AMH on the antioxidant activity of *C. elegans* in vivo

3.3.1. Lifespan assay

The effect of AMH on the lifespan of *C. elegans* was investigated (Fig. 2A and Table 2). The survival curve of *C. elegans* revealed that the AMH shifted to the right in contrast to the control group (Fig. 2A). The average lifespan of *C. elegans* of the control group and 0.5 mg/mL GSH (positive control) were 8.18 d and 10.45 d, respectively (Table 2). The mean lifespan of *C. elegans* fed with 0.5 mg/mL AMH was higher than that of the control group but it is same as that of the positive control and AM group (8.0 mg/mL). The mean lifespan of *C. elegans* was also significantly prolonged with increased AMH concentration ($P < 0.05$), suggesting that AMH may help retard *C. elegans* aging. Guo et al. (2020) discovered that the mean lifespan of *C. elegans* treated with 2 mg/mL of

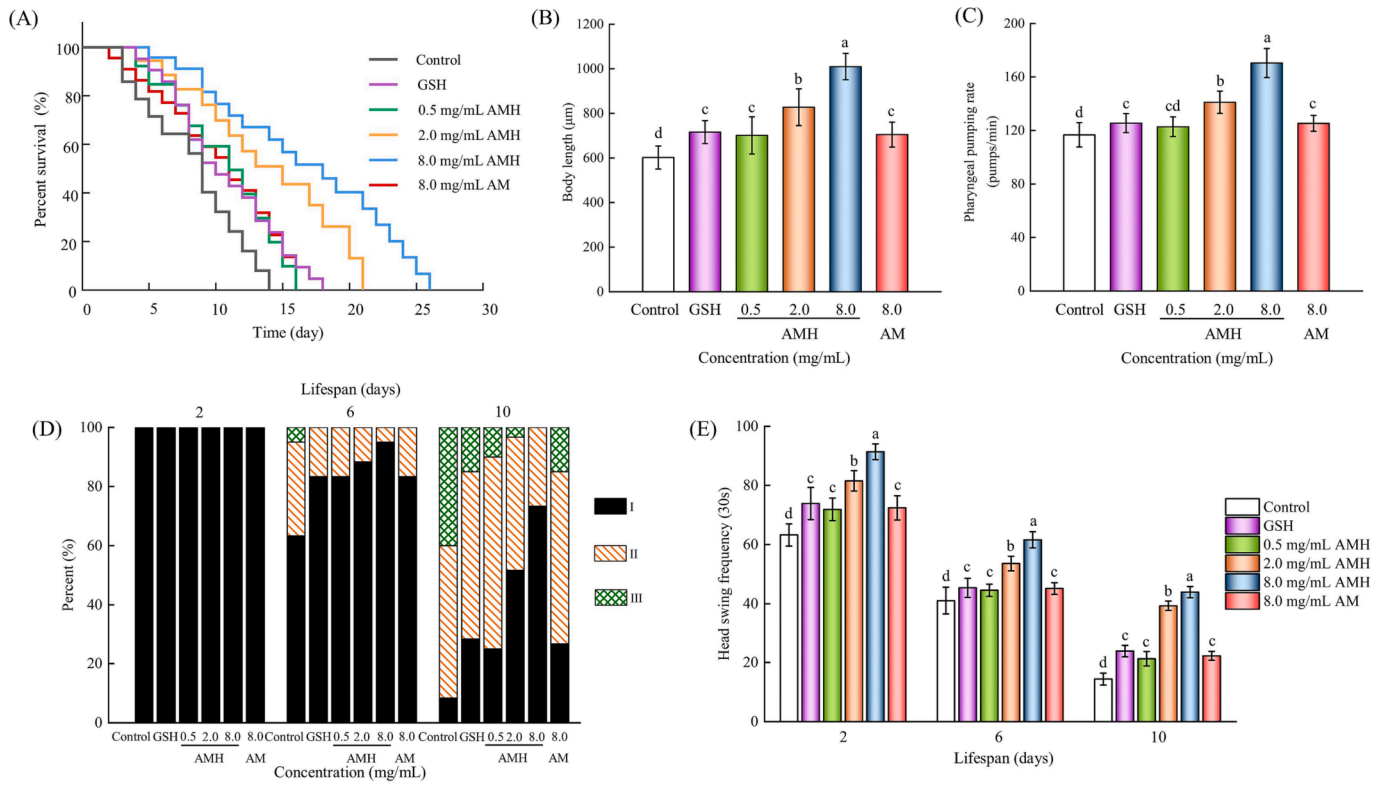

Fig. 2. Effect of AMH on the lifespan (A), body length (B), pharyngeal pumping rate (C), locomotivity (D), and head-swing frequency (E) of *C. elegans*.

Table 2
Effect of AMH on the lifespan of *C. elegans*.

Group	Mean lifespan (days)	Mean fold increase (%)
Control	8.18 ± 0.65^{d}	0
GSH	10.45 ± 0.14^{c}	27.88
0.5 mg/mL AMH	10.19 ± 0.16^{c}	24.64
2.0 mg/mL AMH	12.90 ± 0.14^{b}	57.79
8.0 mg/mL AMH	15.18 ± 0.20^{a}	85.73
8.0 mg/mL AM	10.22 ± 0.09^{c}	25.00

Data are expressed as the mean ± standard deviation.
Values with different lowercase letters in the same column indicate significant differences at $P < 0.05$.

A. japonicus protein hydrolysate was 18.51% longer than the positive control. In this study, the mean lifespan of *C. elegans* was extended by 57.79% after being treated with 2 mg/mL AMH.

3.3.2. Body length

The effect of AMH on the body length of *C. elegans* is illustrated in Fig. 2(B). The body length of *C. elegans* in the control group and the positive control were 601.95 and 701.09 μm, respectively. The body length of *C. elegans* fed with 0.5 mg/mL AMH was 690.23 μm, near that of the positive control and 8.0 mg/mL AM. The body length of *C. elegans* increased significantly with increased AMH concentration ($P < 0.05$). A previous study has reported that the intake of rice bran peptide KF-8 with antioxidant activity in nematodes can promote the increase in body length (Cai et al., 2022). This phenomenon could be due to that peptides with antioxidant activity inhibited the physiological damage caused by free radicals, thereby prolonging the body length of *C. elegans*.

3.3.3. Pharyngeal pumping assay

Pharyngeal pumping rate reflects the intaking capability, aging, and mobility of *C. elegans* (Yuan et al., 2019). Hence, the effect of AMH on the pharyngeal pumping rate of *C. elegans* was investigated, and results are shown in Fig. 2(C). The pharyngeal pumping rate of the control group and the positive control group were 117 and 126 times/min, respectively. No obvious significant effect was found on the swallowing frequency between nematodes treated with 0.5 mg/mL AMH, 0.5 mg/mL GSH and 8.0 mg/mL AM, whereas the swallowing frequency of 2 mg/mL AMH-treated nematodes was remarkably higher than that of the positive control ($P < 0.05$), indicating that AMH can improve the food intake of *C. elegans*. Chen et al. (2020) discovered that the pharyngeal pumping rate in nematodes was enhanced after feeding antioxidant peptides extracted from the protein hydrolysate of defatted round scad. A previous study reported that the volatile metabolites produced by bacteria can attract *C. elegans* and affect its feeding preference (Niu et al., 2010). AMH prepared in this study had a weak fishy smell, but the effect on the feeding preference of *C. elegans* remains to be further studied.

3.3.4. Motoricity assay

Movement behavior indicators including locomotivity and head-swing frequency can be used to evaluate the aging changes of nematodes (Lin et al., 2019). Accordingly, the effect of AMH on the spontaneous movement of nematodes during growth was investigated (Fig. 2D and 2E).

On the second day of *C. elegans* growth, each group was in the autonomous locomotivity of class I, and the head-swing frequency of *C. elegans* treated with GSH and the AMH were markedly higher than that of the control group, indicating that AMH helped improve the movement ability of nematodes. Cai et al. (2020) also reported that the head-swing frequency of nematodes can be increased by feeding 0.1 M rice bran active peptide KF-8. On the 6th day, the nematodes in each group showed class II movement, and 5% of the nematodes in the control group began to show class III movement. The head-swing frequency of *C. elegans* in each group was lower than that in the second day. The head-swing frequency of nematodes feeding with AMH was markedly higher than that of the control ($P < 0.05$). *C. elegans* motility remarkably increases reportedly after being fed with antioxidant peptides prepared from defatted round scad (Chen et al., 2020). On the 10th day, the proportion of nematodes with class III movement in the control group had reached 40%, whereas nematodes fed with the 8 mg/mL AMH group did not show class III movement. Moreover, the head-swing frequency of nematodes fed with 8 mg/mL AMH was 44 times/30 s, which was significantly higher than that in the control (14 times/30 s). The above results showed that AMH can improve physiological activity in nematodes and delayed the decline in exercise capacity caused by nematode aging.

3.3.5. Stress-resistance assay

C. elegans produces highly reactive free radicals under stress conditions, including ROS, which can lead to biomolecular damage, physiological dysfunction, or apoptosis (Li, Ma, Cui, Li, & Wang, 2018). Thus, the lifespan and aging of nematodes are closely related to environmental stresses, such as thermal stress and oxidative stress (Li, Wang, Lam, & Shui, 2021). In the current study, the effect of AMH on thermal stress-resistance ability and oxidative stress-resistance capability of nematodes were investigated, and results are shown in Fig. 3.

The survival rates of *C. elegans* of the different groups cultured at 35 °C for 2 h were 100%, but the survival rate of *C. elegans* decreased when the culture time was extended. The mean lifespan of nematodes of the control group was only 8.13 h, whereas the positive control can reach 9.07 h (Table S1). With increased AMH concentration, the survival curve of nematodes gradually shifted to the right (Fig. 3A). This finding indicated that AMH can improve the survival rate of nematodes under thermal stress.

A similar trend was noticed in the effect of feeding AMH on the antioxidant stress ability of nematodes (Fig. 3B). The average lifespan of nematodes in the control group under oxidative stress condition was 2.19 h, whereas the survival rate of *C. elegans* in the positive control group, 8 mg/mL AM-treated and 8 mg/mL AMH-treated nematodes increased by 19.96%, 19.08% and 70.40%, respectively (Table S1). This phenomenon may be due to the antioxidant activity of AMH that enhanced antioxidant defense in nematodes (Lu et al., 2021).

3.3.6. ROS level

Fluorescence intensity can reflect the level of intracellular ROS in nematode (Hu et al., 2021). Thus, the effect of AMH on ROS level in nematodes was investigated by fluorescence microscopy (Fig. 3C). The ROS level in the positive control was 13.66% lower than that in the control group, which was near the result of feeding 0.5 mg/mL AMH and 8.0 mg/mL AM. Accompanied with the improved AMH concentration, the intracellular ROS level of *C. elegans* decreased gradually, indicating that AMH can inhibit ROS production in *C. elegans*. Cricket peptides prepared by simulated gastrointestinal digestion can reportedly reduce the ROS level of nematodes under oxidative stress conditions primarily due to the antioxidant activity of peptides (Mudd et al., 2022). The effect of 2 mg/mL AMH on the intracellular ROS level of *C. elegans* was significant ($P < 0.05$). Given that the accumulation of oxidative damage affected the aging of *C. elegans*, the intracellular ROS level of nematodes was negatively correlated with lifespan (Fig. 3C, Table 2). This finding was consistent with previous reports that AMH can prolong the lifespan of C. elegans by scavenging intracellular ROS (Chen et al., 2020).

3.3.7. SOD, CAT, T-AOC activities, and GSH content

SOD protects cells from oxidative stress by scavenging free radicals in nematodes, whereas CAT protects cells by decomposing excessive H_2O_2 *in vivo* (Xiong, Deng, Zheng, Li, & Liu, 2021). As an antioxidant, reduced glutathione has the effect of protecting cells from oxidative stress (Pormohammad, Hansen, & Turner, 2022). T-AOC is one of the indicators that directly measure the total antioxidant capacity of the body, indicating the body's ability to resist oxidative damage (Jiang

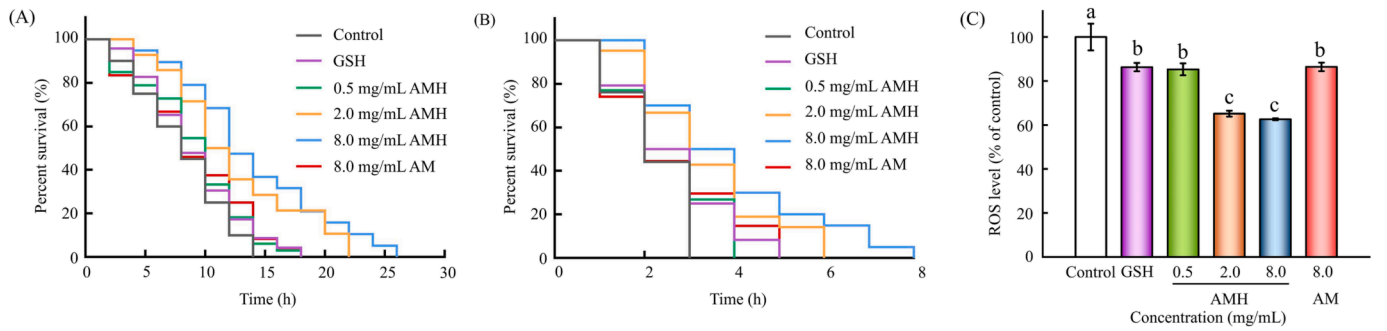

Fig. 3. Effect of AMH on the thermal stress (A), oxidative stress (B), and ROS level (C) of *C. elegans*.

et al., 2020). Therefore, the effect of AMH on SOD activity, CAT activity, GSH content, and *T*-AOC in nematodes was explored (Fig. 4).

The SOD activity of *C. elegans* in the positive control increased by 12.04% compared with that of the control group, which was close to the results of feeding 0.5 mg/mL AMH and 8.0 mg/mL AM (Fig. 4A). SOD activity in nematodes increased with increased peptide concentration. A similar trend was also appeared in the effect of AMH on CAT activity in nematodes (Fig. 4B). GSH contents in the control group and the positive control were 94.93 μmol/g pro and 137.12 μmol/g pro, respectively (Fig. 4C). The GSH content in *C. elegans* fed with 0.5 mg/mL AMH was similar to that in the positive control and 8.0 mg/mL AM. With increased AMH concentration, the GSH content in nematodes gradually increased. As shown in Fig. 4(C), AMH can promote GSH production in nematodes. According to Fig. 4(D), the T-AOC of nematodes fed with 0.5 mg/mL AMH was close to that of the positive control and 8.0 mg/mL AM, indicating that AMH can improve the ability of nematodes to resist oxidative damage. As shown in Fig. 4, feeding AMH can increase antioxidant enzyme activity and GSH content in *C. elegans*, thereby enhancing T-AOC, prolonging the lifespan of *C. elegans*, and affecting its related physiological changes.

4. Conclusion

This study revealed the *in vitro* antioxidant activity of AMH and their effects on the physiological function of *C. elegans* and antioxidant activity *in vivo*. AMH showed higher DPPH• and •OH scavenging ability, which may be due to the high proportion of peptides with 1000 Da. AMH can increase the activity of antioxidant enzymes, GSH content, and T-AOC in *C. elegans*, thereby extending the lifespan and tolerance to environmental stresses. The results of this study suggested that low-value sea cucumber can be used to prepare peptides with antioxidant activity, which can serve as a theoretical guidance for developing anti-aging functional foods.

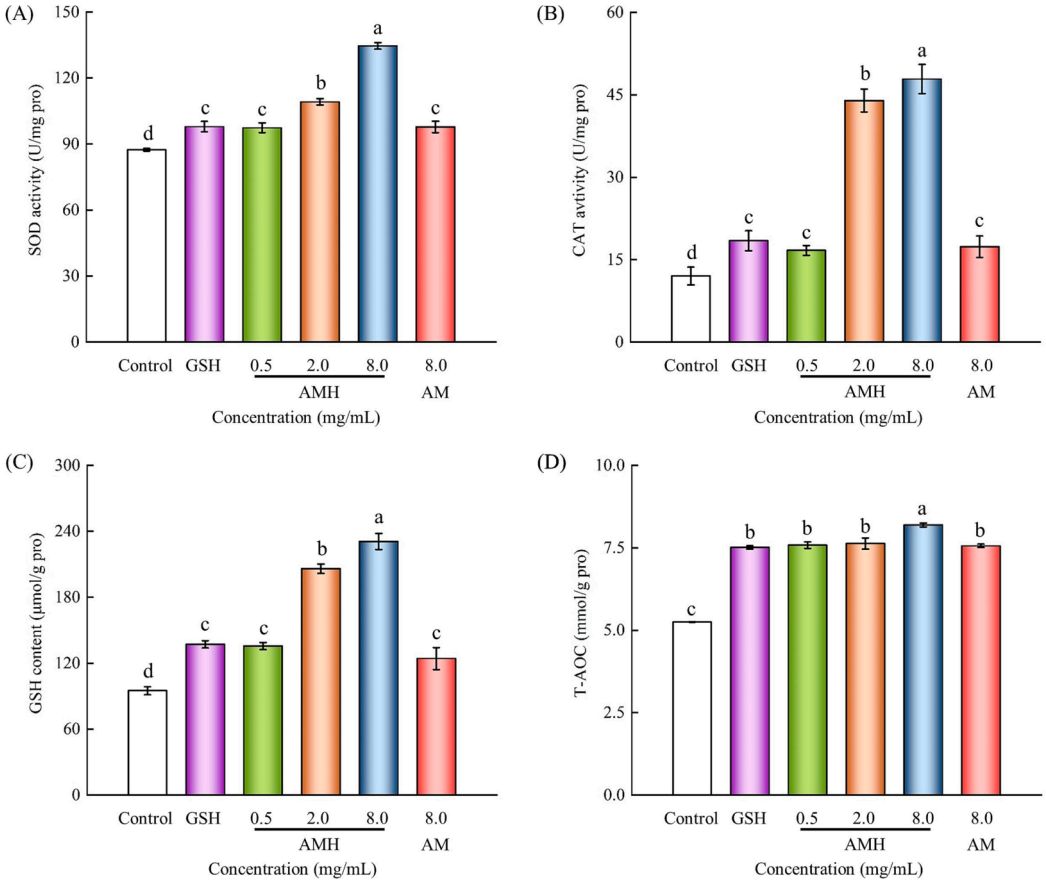

Fig. 4. Effect of AMH on SOD activity (A), CAT activity (B), GSH content (C), and *T*-AOC (D) of *C. elegans*.

CRediT authorship contribution statement

Kexin Chen: Methodology, Data curation. **Linfan Shi:** Supervision. **Zhongyang Ren:** Supervision. **Wuyin Weng:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100836.

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