DOI: 10.26599/FSHW.2026.9250963

Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

China Food Publishing Co.

Investigating Antioxidant and Anti-aging Properties of *Katsuwonus pelamis* Peptides in *Drosophila* Subjected to High-Fat Diets

Xixi Cai [a, b, #], Taher Abdelnaby [a,c,#], Jie Liang [d], Lichan Li [a], Fengchao Zhou [d], Mohamed Ali Farag [e,f], Xu Chen [a, g,*], Shaoyun Wang [a,*]

[a] *College of Biological Science and Engineering, Fuzhou University, Fuzhou 350108, China*

[b] *Fujian Province - Indonesia Marine Food Joint Research and Development Center (Fujian Polytechnic Normal Univeristy), Fuzhou 350300, China*

[c] *Food science and technology department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt*

[d] *College of Environmental and Biological Engineering, Putian University, Putian 351100, China*

[e] *Department of Pharmacognosy, Cairo University, Cairo, Egypt*

[f] *Health Care Faculty, Saxony Egypt University for Applied Sciences, Badr City, Egypt*

[g] *Qingdao Chenland Health Industry Group Co., Ltd. Qingdao 266061, China*

ABSTRACT: This study investigates the antioxidant and anti-aging properties of *Katsuwonus pelamis* peptides (KPPs) using a high-fat diet (HFD) *Drosophila* melanogaster model. *In vitro* analysis revealed KPPs exhibit potent radical scavenging activity (e.g., DPPH IC_{50} = 1.80 mg/mL). In HFD-fed flies, KPPs supplementation significantly extended median lifespan by 23.4% ($P < 0.01$) and improved motor function (e.g., climbing index increased by 30.0%). In the model, KPPs significantly prolonged lifespan, improved motor function, lowered peroxide, malondialdehyde (MDA) levels, and triglyceride content. Concurrently, KPPs enhanced antioxidant enzyme activity, increasing T-SOD by 20.9% and GSH-Px by 80.1%. Furthermore, KPPs attenuated aging by upregulating key antioxidant genes (*Sod1*, *Cat*) and inhibiting the insulin/IGF-like signaling (*IIS*) pathway. KPPs also demonstrated significant intestinal protection: reducing lipid droplet accumulation and ROS levels, decreasing apoptosis in epithelial cells by 43.2%, while suppressing intestinal stem cell proliferation. These effects collectively preserved gut integrity, maintained homeostasis, and contributed to delayed aging. This study provides compelling evidence for KPPs as a novel intervention to counteract HFD-induced damage and mitigate aging processes.

Keywords: *Katsuwonus pelamis*, peptides, antioxidant, anti-aging, *Drosophila*

1. Introduction

High-fat diets have become prevalent in modern society due to several interrelated factors, including economic, cultural, and health-related influences. Economically, high-fat, processed foods are often cheaper and more accessible than healthier options, particularly appealing to individuals with lower socioeconomic status [1]. The food industry's profitability from producing energy-dense, long-shelf-life products further

# These authors contributed equally to this work.
***Corresponding author**
chenxu@fzu.edu.cn (X. Chen)
shywang@fzu.edu.cn (S.Y. Wang)

Received 24 March 2025
Received in revised from 14 April 2025
Accepted 22 October 2025

drives this trend [2]. Culturally, the widespread adoption of the Western diet, characterized by high fat and sugar content, influences dietary habits globally [3, 4]. Additionally, the convenience of quick, easy meal options aligns with fast-paced lifestyles that are typically high in fats and sugars [5]. High-fat diet (HFD) disrupts energy metabolism in the organism. Excessive fat intake promotes the generation of cytotoxic peroxidation products and is accompanied by excessive production of reactive oxygen species (ROS), ultimately triggering oxidative stress. Oxidative stress is a key mechanism of aging. It leads to oxidative modification of biological macromolecules (such as lipids, proteins, and nucleic acids), disrupts related physiological functions, causes oxidative damage to tissues and organs, and dysregulates various metabolic activities [1, 5]. Moreover, the oxidative damage induced by HFD is responsible for lipid peroxidation, insulin resistance, and heat shock protein modifications, among other responses. It plays a significant role in modeling the human lifespan process and the onset of aging-related diseases. Studies have established a link between oxidative stress and obesity in *Drosophila* melanogaster. By introducing a HFD in the Drosophila model, researchers can simulate the oxidative stress and cellular damage caused by HFD in humans, thereby enabling the evaluation of the antioxidant and anti-aging effects of potential drugs or bioactive substances [6, 7]. Thus, a high-fat diet may not only lead to a series of health problems but also accelerate the aging process of the human body [8].

Denham Harman first postulated the free radical theory of aging, which posits that the accumulation of oxidative damage from free radicals, particularly from mitochondrial sources, is a primary driver of aging and age-related diseases [9]. This theory has evolved to incorporate the impact of mitochondrial DNA (mtDNA) mutations and oxidative stress, emphasizing the interplay between free radicals and cellular processes. Mitochondrial DNA is particularly vulnerable to oxidative damage, leading to mutations and deletions that promote further dysfunction and increased free radical production in a feedback loop [9]. With increasing age, the accumulation of reactive oxygen species (ROS) rises while antioxidant defenses decline, resulting in oxidative stress associated with various disorders [10]. According to this widely recognized doctrine, aging is induced by the damaging attack of excessive free radicals on the components of cells [11]. While dietary supplementation with antioxidants can help prevent diseases associated with oxidative stress by effectively scavenging free radicals and reducing oxidative damage, it does not significantly extend lifespan. Still, it may reduce the incidence of age-related diseases [12].

Marine biological resources, particularly fish-derived peptides, are gaining attention for their rich nutritional content and unique biological activities, making them promising candidates for antioxidant defense and anti-aging research. *Katsuwonus pelamis*, a marine economic fish widely distributed in tropical and subtropical seas, contains fish oil and protein with high nutritional value [13]. With an annual global catch exceeding 3 million tons, it ranks among the top three most heavily fished species worldwide. These factors collectively endow skipjack tuna with immense development potential and market prospects. Moreover, studies have shown that *Katsuwonus pelamis* peptides (KPPs) are rich in biological activities, such as anti-inflammatory [14], antioxidant [15], uric acid-lowering [16], and blood pressure-lowering effects [17].

Furthermore, KPPs are abundant in amino acids associated with antioxidant activity, as shown in Table S1. However, there is a limited understanding of how KPPs specifically contribute to mitigating oxidative stress and delaying aging processes, particularly in the context of a high-fat diet.

KPPs demonstrate potential in mitigating oxidative stress primarily through their antioxidant properties. Derived from skipjack tuna, these peptides exhibit strong radical scavenging activities, effectively countering oxidative stress caused by ROS. Specific KPPs, such as AEM, QDHKA, and others, have shown significant scavenging abilities against various radicals and the capacity to inhibit lipid peroxidation, indicating high efficacy [15]. Mechanistically, KPPs may enhance the expression of antioxidative enzymes like superoxide dismutase and catalase, which are vital for neutralizing ROS [18]. Similar peptides from other sources, like the Manila clam, have shown the ability to modulate cellular defense by reducing intracellular ROS levels and increasing glutathione levels, suggesting a potential mechanism for KPPs [19]. The strong antioxidant properties of KPPs make them suitable for pharmaceutical and functional products to prevent or treat oxidative stress-related conditions [15, 20]. However, additional research is needed to investigate their mechanisms and efficacy in *vivo* to alleviate oxidative stress fully. Therefore, studying the antioxidant activity of KPPs is of significant scientific interest and practical application. To investigate these potential effects of KPPs, we utilize *Drosophila* as a model organism due to its short life cycle and genetic similarities to mammals. *Drosophila* is characterized by ease of handling, a clear genetic background, and a high degree of conservation with mammals in molecular and cellular mechanisms [21]. It has been widely used in studying aging and related diseases and evaluating anti-aging functional factors. The high-fat diet *Drosophila* model can simulate the oxidative stress and pro-aging processes caused by a high-fat diet in humans, providing an ideal experimental platform for studying antioxidant and anti-aging mechanisms [22].

This study specifically aims to elucidate the antioxidant mechanisms of KPPs and assess their impact on aging-related markers in a *Drosophila* model that fed a high-fat diet. The findings could support the high-value utilization and industrial development of *Katsuwonus pelamis* while providing theoretical foundations for new nutritional interventions addressing health issues linked to high-fat diets and aging.

2. Materials and methods

2.1. Materials and reagents

Fresh *Katsuwonus pelamis* was sourced from Zhangpu Fengziya Food Co., Ltd. in Zhangzhou, China, while 1,1-diphenyl-2-picrylhydrazyl (DPPH) was bought from Tokyo Chemical Industry Co., Ltd. in Tokyo, Japan. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and Linoleic acid were purchased from Macklin Biochemical Technology Co., Ltd. in Shanghai, China. Ferrozine was obtained from Sigma-Aldrich, Inc. in Saint Louis, USA. Other chemical reagents were supplied by Xilong Science Co., Ltd. in Shantou, China. Assay kits for superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), and 4',6-diamidino-2-phenylindole (DAPI) were purchased from Nanjing Jiancheng Bioengineering Research Institute in Nanjing, China. Pyridinium iodide (PI), dihydroethidium (DHE), mouse-GFP antibody, rabbit-PH3 antibody, mouse Alexa Fluor 488 antibody, and rabbit Alexa Fluor 555 antibody were acquired

from Shanghai Beyotime Biotechnology Co., Ltd. in Shanghai, China. The Fluorescence real-time quantitative PCR (qPCR) premix (SYBR) was sourced from Tiangen Biotechnology Co. in Beijing, China. The total RNA isolation kit and HiScript III RT SuperMix for qPCR (+gDNA wiper) were obtained from Vazyme Biotech Co., Ltd. in Nanjing, China.

2.2. Preparation of KPPs

Katsuwonus pelamis was gutted, had its gills removed, cleaned, cut into pieces, and minced using a meat grinder. *Katsuwonus pelamis* peptides (KPPs) with antioxidant activity were obtained from *Katsuwonus pelamis* using an optimized enzymatic hydrolysis procedure with alkaline protease. The hydrolysis conditions were as follows: enzyme-to-substrate ratio (E/S) of 4.4%, solid-to-liquid ratio of 1:37, pH 8.0, at 50°C for 4 hours.

2.3. Antioxidant Activity of KPPs

KPPs (50–200 mg) and hydrochloric acid (10 mL, 6 M) were placed in a hydrolysis tube, followed by hydrolysis in a digestion oven at 110 °C for 22–24 hours. The resulting hydrolysate was analyzed using an automated amino acid analyzer [23].

2.4. Antioxidant Activity of KPPs

2.4.1. DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was assessed following the method outlined by Tu et al. [24], with some modifications. The sample solution was combined with an equal volume of 0.1 mM DPPH solution, and the absorbance was recorded at 512 nm after the mixture stood for 0.5 hours at room temperature in the dark. The scavenging activity was determined using the following formula:

$$\text{DPPH}\ (\%) = (1 - (A_i - A_j) / A_0) \times 100$$

In this formula, A_i represents the absorbance of the sample group, A_j denotes the absorbance of the sample reference group, and A_0 is the absorbance of the control group.

2.4.2. ABTS Radical Scavenging Activity

The ABTS radical scavenging activity was evaluated following the method described by Wang et al. [25]. To prepare the ABTS working solution, 7 mM ABTS was mixed with 2.45 mM persulfuric acid in a 1:1 ratio and allowed to stand for 16 hours at room temperature in the dark. The solution was diluted with a 5 mM phosphate buffer (pH 7.4) to absorb 0.70 ± 0.02 at 734 nm. The working solution was then combined with varying concentrations of KPPs in a 1:1 ratio and incubated at room temperature for 10 minutes, after which the absorbance was measured at 734 nm. The scavenging activity was determined using this formula:

$$\text{ABTS}\ (\%) = ((A_c - A_s) / A_c) \times 100$$

In this equation, A_c refers to the absorbance of the blank group, and A_s denotes the absorbance of the sample group.

2.4.3. Hydroxyl Radical Scavenging Activity

The Hydroxyl radical scavenging activity was evaluated using the method described by Zhang et al. [26]. One mL sample was combined with 0.3 mL of 8 mM FeSO4, 1 mL of 3 mM salicylic acid, and 0.25 mL of 20 mM H2O2 and then incubated at 37 ℃ for 0.5 hours before being cooled under running water. After adding 0.45 mL of distilled water, the mixture was centrifuged at 3000 rpm for 10 minutes, and the absorbance was measured at 510 nm. The hydroxyl radical scavenging activity was determined using this formula:

Hydroxyl radical scavenging activity (%) = $(A_c - A_s) / A_c \times 100$

In this equation, A_c represents the absorbance of the blank group, while A_s indicates the absorbance of the sample group.

2.4.4. Metal Chelating Activity

The metal chelating activity was assessed based on the method outlined by Dinis et al. [27] with minor modifications. A mixture of 1 mL of various concentrations of KPPs, 4.7 mL of distilled water, and 0.1 mL of 2 mM ferrous chloride mixed with 0.2 mL of 5 mM phenanthroline. This reaction mixture was allowed to stand at room temperature for 20 minutes before measuring the absorbance at 562 nm. A blank group was created using 1 mL of distilled water, while the sample reference group consisted of 1 mL of the sample combined with 5 mL of distilled water. The metal chelating activity was determined using this formula:

Metal chelating activity (%) = $(1 - (A_i - A_j) / A_0) \times 100$

In this formula, Ai represents the absorbance of the sample group, Aj denotes the absorbance of the sample reference group, and A0 is the absorbance of the control group.

2.4.5. Lipid Peroxidation Inhibitory Activity

The lipid peroxidation inhibitory activity of KPPs was assessed using the linoleic acid system with minor modifications [28]. In a test tube, 1 mL of the sample was combined with 2 mL of 95% ethanol, 2 mL of phosphate buffer (50 mM, pH 7.0), and 26 μL of linoleic acid. The mixture was shaken well and kept at 40 ℃ in the dark. The degree of peroxidation was measured every 24 hours. The blank group was treated with 1 mL of distilled water instead of the sample. The ferric thiocyanate (FTC) method was used to measure the amount of lipid peroxidation [29]. For this, 0.1 mL of reaction mixture was mixed with 0.1 mL of 30% thiocyanate solution, 4.7 mL of 75% ethanol, and 0.1 mL of 20 Mm FeCl2 and allowed to react for 3 minutes. The absorbance was then measured at 500 nm.

2.4.6. Reducing power

The assay was performed following the method described by GüLCIN et al. [30]. One mL sample was combined with 2.5 mL of phosphate buffer (pH 6.6, 0.2 M), 2.5 mL of 10% K3[Fe (CN)6], and 2.5 mL of 10% TCA. The mixture was incubated for 20 minutes at 50 ℃ and then centrifuged at 3000 rpm for 10 minutes. Subsequently, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 1% $FeCl_3$ solution. After standing at room temperature for 10 minutes, the absorbance was measured at 700 nm. The blank group consisted of 1 mL of distilled water in place of the sample. The absorbance values are positively correlated with the reducing power.

2.5. Drosophila Rearing and Diets

Fruit flies (*Drosophila*. melanogaster strains Canton-S and ESG-Gal4/UAS-GFP) obtained from the Institute of Biological Sciences, Fuzhou University (Fuzhou, China) were cultured on a standard medium prepared from yeast, cornmeal, sucrose, and agar. All *Drosophila* were maintained in a controlled environment with a 12-hour light/dark cycle, a constant temperature of 25°C, and 60% relative humidity. The high-fat medium was created by incorporating 10% (v/v) lard into the basal medium. To assess the effects of KPPs, they were added to the high-fat medium at final concentrations of 1, 3, and 5 mg/mL (w/w), designated as KPPs(L), KPPs(M), and KPPs(H), respectively.

2.6. Lifespan and Behavior Experiments of Drosophila

2.6.1. Lifespan Analysis

Newly fledged *Drosophila* were randomly grouped and placed in blank diet (ND), high-fat diet (HFD), and KPPs(L), KPPs(M), and KPPs(H) groups. The number of surviving *Drosophila* in each group was counted daily, and the medium was changed every three days while dead *Drosophila* were removed. Average, maximum, and median lifespans were calculated after the experiment.

2.6.2. Climbing Ability Test

Newly fledged *Drosophila* were transferred to an empty tube marked 7 cm from the bottom for measurement. The tube was gently shaken to ensure all *Drosophila* fell to the bottom, and the number that crawled more than 7 cm in 30 seconds was recorded. The percentage of flies that reached this distance represented the climbing ability of each group.

2.6.3. Food Intake Assay

Food intake rates were measured using the approach provided by Xin et al. [31] with minor changes. After 3 days of culture, *Drosophila* were subjected to 1 hour of starvation, then moved to fresh vials containing food with 0.5% edible brilliant blue dye for 5 hours. The flies were homogenized in saline, and the supernatant was collected following centrifugation for 2 minutes at 10,000 rpm. The absorbance was determined at 625 nm, and the calculations were based on a standard curve of absorbance vs medium mass.

2. 6.4. Measurement of Body Weights

On days 7 and 28, the body weights of *Drosophila* in each group were recorded. Flies were anesthetized with CO_2 before being weighed on a balance, and mean body weights were calculated for each group.

2.7. Triglycerides Quantification

After 7 and 28 days of rearing, *Drosophila* were ground on ice with anhydrous ethanol in a 1:9 (w/v) ratio. The mixture was homogenized and centrifuged at a low temperature for 10 minutes at 2500 rpm. The supernatant was collected to determine the triglyceride content following the instructions provided with the TG kit.

2.8. Determination of Antioxidant Index In Vivo

On days 7 and 28, anhydrous ethanol was added to the total weight of *Drosophila* in a 1:9 (w/v) ratio and homogenized on ice. The homogenate was centrifuged for 10 minutes at 2500 rpm to extract the supernatant for assays of total protein content, SOD, GSH-Px activities, and MDA levels, following the instructions provided with the assay kits.

2.9. Drosophila Gut Experiments

Female *Drosophila* were used for gut experiments due to easier dissection and greater structural stability than male *Drosophila* [32].

2.9.1. Intestinal Morphology

Female *Drosophila* from the 28-day-old ND, HFD, and KPPs(H) groups were collected. Ten to twelve intestines were extracted in pre-cooled PBS and placed on slides with PBS for morphology observation and measurement using ImageJ software.

2. 9.2. Intestinal Integrity

Female *Drosophila* from various treatment groups were incubated in a medium with 2.5 % (w/v) blue dye for 12 hours. The number of "Smurf" *Drosophila*, showing blue coloration, was counted.

2. 9.3. Analysis of Apoptosis and ROS Content in Intestinal Epithelial Cells

After 28 days, female *Drosophila* intestines were collected and immersed in pre-chilled PBS. Intestines were stained with propidium iodide (PI, 100 μg/mL) or dihydroethidium (DHE, 10 μM) for 30 minutes at ambient temperature, then immersion in 4% paraformaldehyde for 30 minutes and counterstaining with DAPI for 10 minutes. After washing with PBS, intestines were examined using a Nikon Ti2 fluorescent microscope.

2. 9.4. Determination of Intestinal Progenitor Cell Morphology and Quantity

The ESG-Gal4/UAS-GFP strain of *Drosophila* was used to label precursor cells because it has its own specific green fluorescent protein (GFP) [33]. Female *Drosophila* from ND, HFD, and KPPs(H) groups were dissected in cold PBS and fixed in 4% formaldehyde for 30 minutes. The samples were rinsed three times for 5 minutes with PBST and blocked for 30 minutes with PBST containing 5% goat serum. Primary antibodies (Mouse-GFP: 1:200; Rabbit-PH3: 1:200; 488: 1:200 and Rabbit Alexa Fluor 555 1:1000) were incubated overnight at 4°C for 2 hours. After rinsing with PBST, samples were stained with DAPI and examined with a Nikon A1 laser scanning confocal microscope.

2. 9.5. Observation of Intestinal Lipid Droplet Staining

Intestinal lipids were stained using oil red O, following the method of Eugenio Gutierrez et al. [34] with minor adjustments. *Drosophila* intestines were dissected in cold PBS, fixed with 4% paraformaldehyde for 10 minutes, then treated in 0.1% Oil Red O stain for 30 minutes in the dark, and washed with distilled water. Lipid droplets were photographed using a Nikon Ti2 fluorescence microscope.

2.10. Quantitative Real-Time PCR Analysis

Drosophila from the 28-day-old ND, HFD, and KPPs (H) groups were collected to assess gene expressions related to antioxidant activity and the IIS signaling pathway. Total RNA was isolated using the FastPure Cell/Tissue Total RNA Isolation Kit V2, and cDNA was created with HiScript® II Q RT SuperMix for qPCR (+gDNA wiper). A reaction volume of 20 μL was prepared using Taq Pro Universal SYBR qPCR Master Mix, with primers specified in Table S2. The Bio-Rad CFX Connect™ Real-Time System (Bio-Rad Laboratories, Inc., USA) performed quantitative polymerase chain reaction (qPCR). Data was standardized using the 2−ΔΔCt technique, with RP49 as the reference gene.

2.11. Statistical Analysis

Data are reported as mean ± SD. The statistical analyses were carried out with GraphPad Prism 8 software. A t-test was used to determine significance, and findings were reported as $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

3. Results and Discussion

3.1. Antioxidant Properties of KPPs

3.1.1. DPPH Radical Scavenging Activity

The assay evaluated the antioxidant activity of KPPs by measuring their ability to neutralize DPPH radicals, which are stable free radicals. The results demonstrated a concentration-dependent scavenging effect, with the scavenging rate increasing progressively from 0.1 to 6.0 mg/mL (Fig.1A). The maximum scavenging efficiency (83.33%) was achieved at 6.0 mg/mL, after which the activity exhibited a plateau phase. The half-maximal inhibitory concentration (IC_{50}) for DPPH radicals was calculated as 1.80 mg/mL, indicating that KPPs effectively inhibit DPPH radical formation while demonstrating robust antioxidant activity. These findings suggest that KPPs could serve as promising natural antioxidants, potentially mitigating lipid peroxidation through their free radical scavenging capabilities.

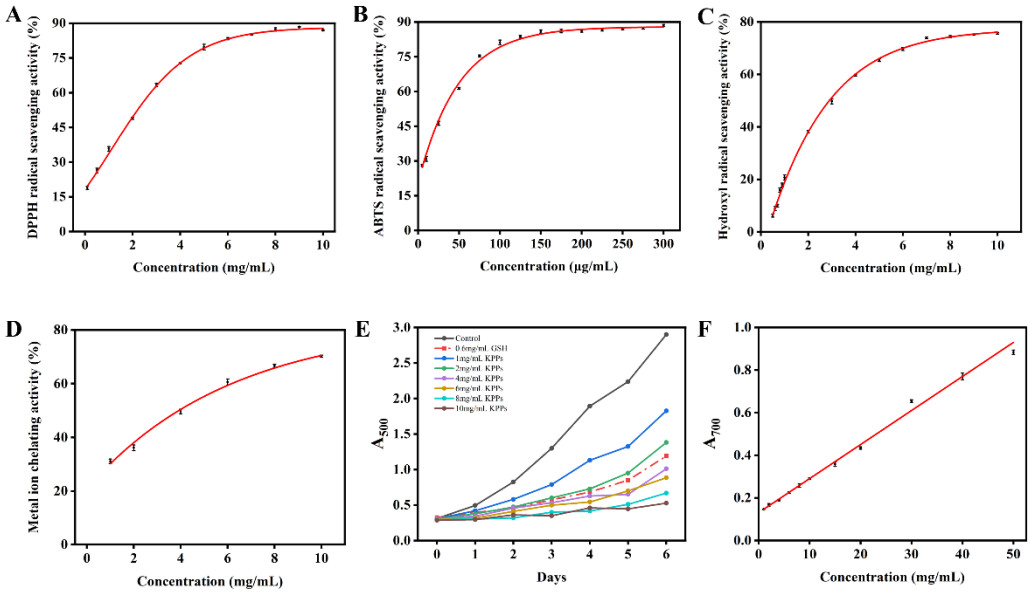


Fig. 1. *In vitro* antioxidant activities of KPPs. (A) DPPH radical scavenging activity, (B) ABTS radical scavenging activity, (C) hydroxyl radical scavenging activity, (D) metal chelating activity, (E) inhibition capacity of lipid peroxidation, (F) reducing power.

3.1.2. ABTS Radical Scavenging Activity

The ABTS assay was employed to evaluate the capacity of KPPs to scavenge ABTS radicals, chosen for its rapidity, simplicity, high sensitivity, and reproducibility. KPPs exhibited a concentration-dependent scavenging effect, with significant activity observed in the range of 10 – 100 μg/mL (Fig. 1B). The maximum scavenging capacity (90%) was achieved at 200 μg/mL, corresponding to a half-maximal inhibitory concentration (IC_{50}) of 32.93 μg/mL. These results confirm the potent ABTS radical scavenging ability of KPPs, thereby reinforcing their role as effective antioxidants.

3.1.3. Hydroxyl Radical Scavenging Activity

Hydroxyl radicals are highly reactive and destructive free radicals that can interact with lipids, proteins, and other biomolecules, leading to oxidative damage in cells and tissues and contributing to aging [35]. The ability of KPPs to scavenge hydroxyl radicals was also evaluated (Fig.1C). KPPs exhibited significant scavenging activity, with the scavenging rate positively correlated with KPP concentration from 0.1 to 4.0 mg/mL. Significant hydroxyl radical scavenging was observed, with an IC_{50} of 2.90 mg/mL, demonstrating the ability of KPPs to effectively neutralize highly reactive hydroxyl radicals, suggesting their potential as antioxidants in mitigating oxidative damage.

3.1.4. Metal Ion Chelating Ability

Antioxidants can chelate metal ions, inhibiting their pro-catalytic effects and reducing free radical generation, thereby exerting antioxidant effects [36]. KPPs were assessed for their ability to chelate metal ions, a crucial mechanism in preventing oxidative stress. The results showed that KPPs exhibited an increased capacity for Fe^{2+} chelation with rising concentrations (Fig.1D). This is attributed to their richness in glutamic and aspartic acids, which provide binding sites for ferrous ions; thus, their chelating ability may be linked to their amino acid composition. KPPs exhibited strong metal ion chelation, with an IC_{50} of 3.92 mg/mL, suggesting their potential to mitigate oxidative stress through metal ion binding.

3.1.5. Inhibition Capacity of Lipid Peroxidation

The capacity of KPPs to inhibit lipid peroxidation was evaluated using a linoleic acid system (Fig.1E). As a polyunsaturated fatty acid, linoleic acid is prone to autoxidation and peroxide generation [37], indicating a weaker antioxidant capacity. The findings revealed a positive association between KPP concentration and lipid peroxidation inhibition. By day 7, the inhibition rate at 4 mg/mL was about four times that of the control group, highlighting KPPs' potential to protect against lipid peroxidation. These findings demonstrate that KPPs significantly inhibit lipid peroxidation, underscoring their protective effects.

3.1.6. Reducing Power

The reducing power assay demonstrated that KPPs function as effective reducing agents, reflecting their ability to donate electrons and participate in scavenging free radicals and reducing oxidized intermediates. The absorbance at 700 nm positively correlated with concentration (Fig.1F), showing a strong quantitative relationship. At a concentration of 10 mg/mL, the reducing power of KPPs was approximately 0.32, indicating

strong reducing capabilities that surpassed those of the carnosine peptide from pearl gentian grouper prepared by Xu et al. [38]. This highlights their significant electron-donating ability, contributing to their overall antioxidant capacity. Collectively, these results suggest that KPPs possess promising antioxidant activities, which could be beneficial in preventing oxidative stress-related diseases.

3.2. The Effect of KPPs on the Lifespan of Drosophila on a High-Fat Diet

Excessive dietary fat is linked to reduced lifespan and various physiological and metabolic disorders [39], which trigger oxidative damage, inflammation, apoptosis, and other adverse biological effects. These factors accelerate cellular aging and the decline of organ function, potentially leading to death. Lifespan serves as one of the most visible indicators of the aging process. To assess the potential anti-aging effects of KPPs, we investigated their impact on the lifespan of *Drosophila* subjected to a high-fat diet (HFD) by supplementing the high-fat medium with an appropriate amount of KPPs. As shown in Fig. 2 and Table 1, *Drosophila* in the HFD exhibited significantly decreased mean lifespan, maximum lifespan, and half-death time compared to the normal diet (ND) group. KPPs improved the lifespan of both female and male *Drosophila* on the HFD. The survival curve for female *Drosophila* indicates a decrease in lifespan, although the rate of decline may differ from that of males. In male *Drosophila*, the mean lifespan extended by 7.0%, 15.2%, and 19.1% for KPPs (L), KPPs (M), and KPPs (H), respectively. The maximum lifespan increased by 7.6%, 14.5%, and 23.4%. Female *Drosophila* similarly benefited from KPPs, with mean lifespan rising from 24.1 days in the HFD group to 28.0 days, demonstrating a clear lifespan-extending effect. These results indicate a clear dose-dependent relationship between KPPs and lifespan extension. This suggests that female flies may have a different metabolic response to high-fat diets, potentially allowing them to tolerate higher fat levels before experiencing significant health impacts. The observed differences in lifespan between female and male *Drosophila* may be attributed to varying metabolic responses to high-fat diets, enabling them to handle higher fat levels more effectively. Additionally, biological factors such as hormonal differences or genetic predispositions may significantly influence how each sex responds to dietary fat. Their differing adaptability and sensitivity to KPPs align with the results of Chen et al. [40] regarding the antioxidant effects of KPPs on high-fat *Drosophila*.

Table 1 Statistical analysis of lifespan of high-fat damaged Drosophila

Group	Male			Female		
	Average lifespan (days)	half the time of death (days)	Maximum lifespan (days)	Average lifespan (days)	Half the time of death (days)	Maximum lifespan (days)
ND	52.97±4.27	52.29±3.40	78.14±3.44	53.84±2.67	53.57±2.70	75.86±4.45
HFD	25.66±3.6$^{###}$	24.86±2.27$^{###}$	41.43±6.50$^{###}$	24.09±3.35$^{###}$	22.29±2.36$^{###}$	40.14±4.88$^{###}$
KPPs (L)	27.46±3.23	26.14±3.34	44.57±5.91	25.57±3.45	24.00±3.21	40.29±5.62
KPPs (M)	29.56±2.97*	29.86±2.27*	47.43±7.18	27.91±4.50	25.71±3.82	42.29±7.57
KPPs (H)	30.56±2.22*	29.57±2.70**	51.14±8.38*	28.01±4.07	26.14±3.76*	48.43±7.07*

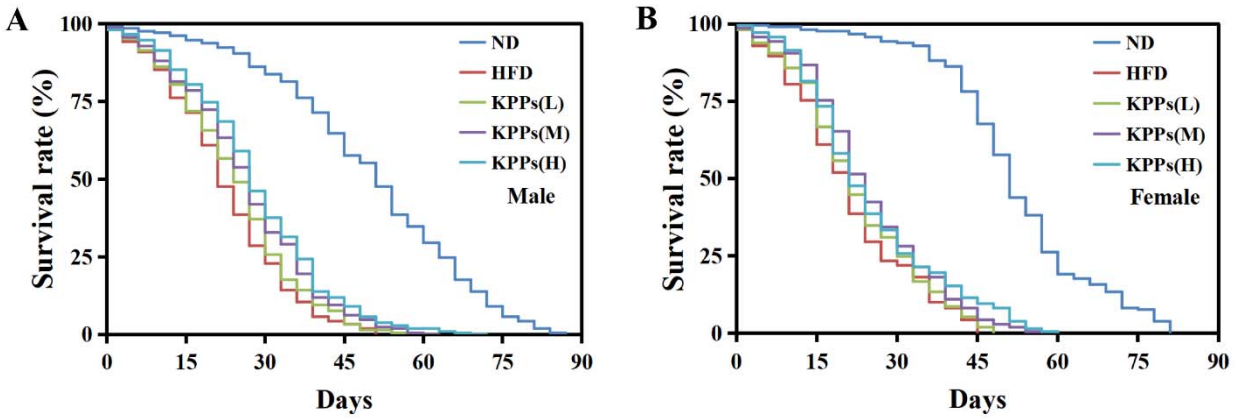


Fig. 2. Survival curve of high-fat damaged (A) male and (B) female Drosophila.

3.3. The Effect of KPPs on Behavioral Indicators of Drosophila on a High-Fat Diet

Fig. 3 illustrates the changes in behavioral indices of high-fat damaged *Drosophila* across different diets, focusing on three key parameters: climbing ability, food intake, and body weight. The results shed light on how high-fat diets impact *Drosophila*'s overall behavior and health.

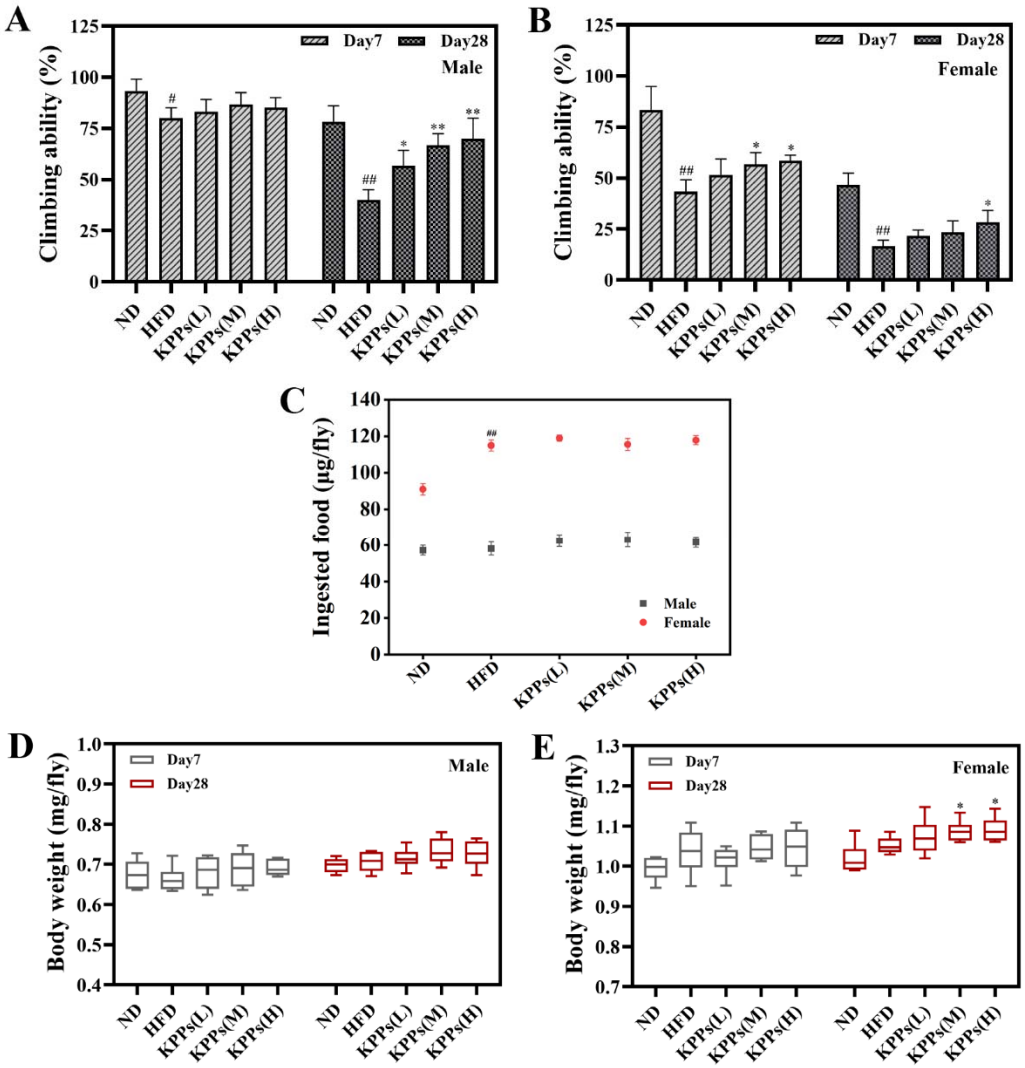


Fig. 3. Changes in behavioral indices of high-fat damaged *Drosophila* in different diets. (A-B) Climbing ability, (C) food intake, (D-E) body weight.

3.3.1. Climbing Ability

The climbing ability of *Drosophila* serves as a functional indicator of motor performance. Weakened skeletal muscle function, degraded metabolism, and declining motor capabilities often accompany the aging process in *Drosophila*. The effect of KPPs on the climbing ability of *Drosophila* under high-fat conditions are shown in Fig. 3A-B. Both male and female *Drosophila* displayed reduced locomotor ability, with females showing more severe impairment and moving more slowly. This decline in climbing ability accelerated with aging. KPPs significantly improved climbing ability on day 7, with male *Drosophila* showing increases of 3.3%, 6.7%, and 5.0% for KPPs (L), KPPs (M), and KPPs (H), respectively. In females, KPPs at 3 mg/mL and 5 mg/mL led to increases of 56.7% and 58.3% in climbing ability, respectively. Notably, the KPPs (M) and KPPs (H) groups saw increases of 13.3% and 15.0%, respectively, compared to the HFD group. After 28 days, KPPs significantly mitigated the decline in climbing ability, with the highest enhancements of 11.7% for females and 30.0% for males, demonstrating their potential to enhance motor function and alleviate age-related deterioration. Overall, the research results imply that KPPs could greatly improve the locomotor ability of *Drosophila*, particularly in males, while alleviating the deterioration caused by aging and oxidative damage.

3.3.2. Food Intake

Dietary restriction is one of the most effective strategies for slowing aging and increasing lifespan [41]. To determine whether the lifespan extension was attributable to the effects of KPPs, we measured the food consumption of *Drosophila* fed a medium containing edible brilliant blue. Fig. 3C-D shows that there were no significant variations in food intake among male *Drosophila* across all groups, indicating that KPPs and lard did not affect consumption. In contrast, female *Drosophila* exhibited increased food intake on the high-fat diet, suggesting a higher susceptibility to dietary fat and its effects. Overall, these findings indicate that the observed lifespan extension was not due to caloric restriction.

3.3.3. Body Weight

Body weight changes are closely correlated with nutritional status and serve as a critical indicator of organismal health. The body weight dynamics across all experimental groups are summarized in Fig. 3D-E. At both 7 and 28 days of age, no significant differences in body weights were observed among male flies in different treatment groups. Neither high-fat diet supplementation nor the addition of KPPs significantly affected male fly body weights. In contrast, female flies exhibited distinct responses: high-fat diet-fed females showed increased body weights compared to the normal diet group, and moderate-to-high-dose KPPs treatment groups demonstrated statistically significant weight gains compared to the high-fat diet group by day 28 of feeding. With age progression, male fly body weights showed only minimal increases and remained largely stable, whereas female fly weights increased markedly, indicating a stronger susceptibility to high-fat diet-induced weight gain. Collectively, these results demonstrate that no diet-restriction-induced weight loss was observed in any experimental group.

3.4. Effect of KPPs on the Triglyceride Content of Drosophila on a High-Fat Diet

The data presented in Fig. 4 offers valuable insights into the effects of high-fat diets on triglyceride levels in *Drosophila*, analyzed separately for male and female flies. This information is crucial for understanding how dietary choices influence survival and overall health. Chronic high-fat diets are known to elevate triglyceride levels, which represent a primary form of stored fat. Triglyceride content was measured in each group on day 28. Our results indicated that both male and female *Drosophila* on a high-fat diet exhibited significantly increased triglyceride levels, with females showing a more pronounced increase compared to the normal diet (ND) group. The survival curves for *Drosophila* (Panels A & B for Males and Females) demonstrate a significant reduction in lifespan for those subjected to high-fat diets, particularly in females, suggesting potential sex-specific responses to dietary fat. The data reveal a steep decline in survival rates for both sexes, indicating that high-fat consumption adversely affects the longevity of *Drosophila*. This finding is particularly relevant, as high-fat diets are associated with increased mortality rates. KPPs led to reductions of 9.0% in triglycerides for males and 3.7% for females, highlighting a differential response in lipid metabolism between male and female *Drosophila*.

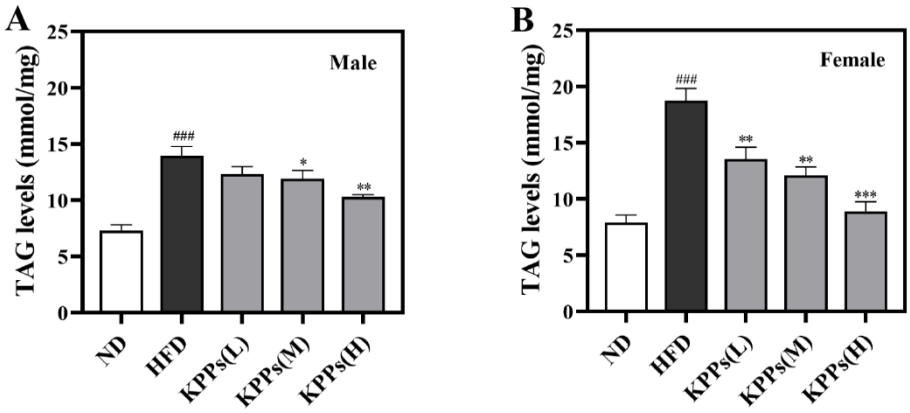


Fig. 4. Variations in TAG level of high-fat damaged (A) male and (B) female Drosophila in different diets.

3.5. Effect of KPPs on Antioxidant Indicators of Drosophila on a High-Fat Diet

To assess the *vivo* antioxidant effects of KPPs, we measured the activities of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) levels in *Drosophila*, particularly under high-fat damage conditions. Antioxidants are necessary for neutralizing free radicals, which may induce cellular damage and lead to disease and aging. *Drosophila* on the high-fat diet exhibited significantly lower antioxidant enzyme activities (T-SOD and GSH-Px) compared to the normal diet (ND) group. The observed decreases in antioxidant indexes suggest that high-fat diets may impair the ability of *Drosophila* to manage oxidative stress effectively, especially with aging, as shown in Fig. 5. Notably, T-SOD activity in female *Drosophila* significantly increased after 28 days of supplementation with 5 mg/mL KPPs. The supplementation with KPPs, particularly at 5 mg/mL, significantly enhanced T-SOD activities in the KPPs (M) and KPPs (H) groups, with increases of 17.4% and 20.9% in males compared to the high-fat diet (HFD) group. The enhancement of GSH-Px activity by KPPs was particularly significant on day 28, with the highest

increases of up to 53.5% in females and 102.6% in males, compared to the HFD group. Due to the accumulation of lipid peroxides linked to aging and high-fat diets, MDA levels were higher in older *Drosophila* compared to younger ones. On day 7, supplementation with 5 mg/mL KPPs effectively reduced MDA levels in both female and male *Drosophila* compared to the HFD group. On day 28, the MDA levels in the HFD group for female and male *Drosophila* (3.0 and 2.8 nmol/mg protein, respectively) were significantly greater than those in the ND group (1.8 and 1.5 nmol/mg protein). The effect of KPPs on reducing MDA accumulation, indicative of lipid peroxidation, was pronounced, demonstrating a clear dose-dependent relationship and highlighting KPPs' efficacy in mitigating oxidative stress.

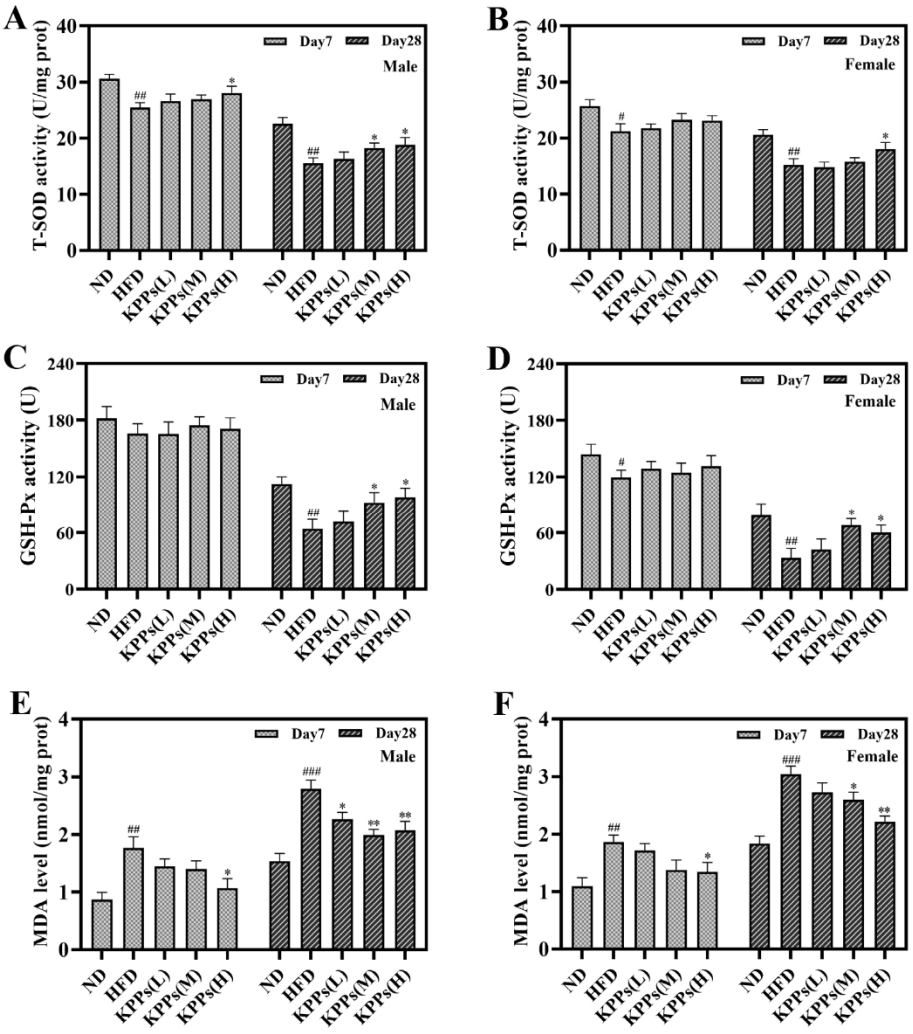


Fig. 5. Change of antioxidant indexes in high-fat damaged Drosophila in different diets. (A-B) T-SOD activity, (C-D) GSH-Px activity, and (E-F) MDA level.

3.6. Protective Effects of KPPs Against High-Fat-Induced Intestinal Damage in Drosophila

3.6.1. Analysis of Intestinal Barrier Integrity

The analysis of intestinal barrier integrity, illustrated in Fig. 6, provides crucial insights into how different diets affect gut health in *Drosophila*, especially under high-fat damage conditions. Understanding intestinal barrier integrity is essential for assessing overall gut health and functionality. The results indicate that various diets significantly affect the intestinal barrier integrity in *Drosophila*. While *Drosophila* typically

possesses strong intestinal function and integrity, external factors, such as oxidative damage, can disrupt intestinal homeostasis, leading to impaired barrier function. Furthermore, the decline in physiological regulation associated with aging can exacerbate these effects, compromising intestinal integrity and increasing permeability [42]. Maintaining a robust intestinal barrier is vital for preventing the translocation of bacteria and toxins, which can lead to chronic inflammation and contribute to metabolic disorders. High-fat diets are shown to compromise gut integrity, leading to potential dysbiosis and enhanced permeability, causing harmful substances to leak into the bloodstream, which is a situation linked to various health issues. This may be attributed to inflammation and oxidative stress compared to those on low-fat or control diets, suggesting that excessive fat intake disrupts gut homeostasis. The proportion of "Smurf" *Drosophila* increased with age, and those on a high-fat diet displayed a significantly higher leakage rate than the ND group. KPPs demonstrated protective effects on intestinal integrity, evidenced by a reduction in the number of "Smurf" *Drosophila*, indicating improved barrier function. Specifically, the leakage rate decreased by 32.3% in females receiving 5 mg/mL KPPs (Fig. 6), suggesting that KPPs can mitigate oxidative damage and support intestinal health. These findings imply that diets lower in fat may help preserve intestinal barrier integrity, informing dietary recommendations aimed at improving gut health and preventing high-fat-diet-induced damage. The results underscore the importance of maintaining gut integrity to prevent adverse health outcomes associated with high-fat diets.

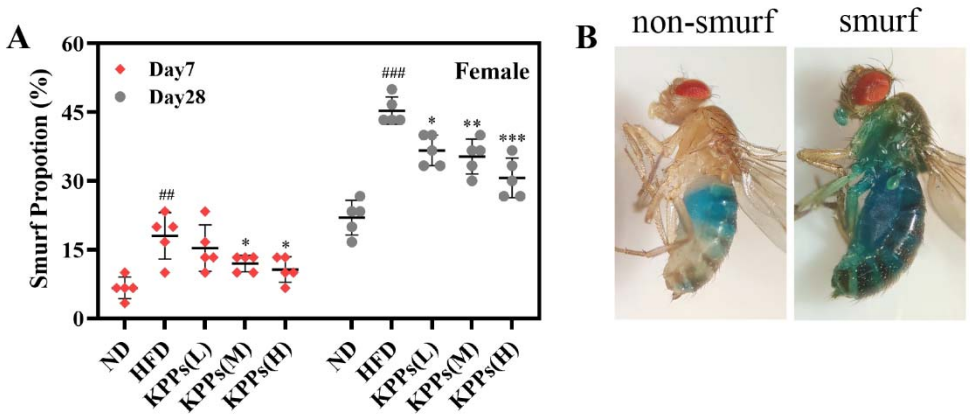


Fig. 6. Effects of different diets on gut integrity in high-fat damaged female Drosophila. (A) Number of "Smurfs", (B) morphology of "Smurfs."

3.6.2. Analysis of Intestinal Morphology

The analysis of intestinal morphology, presented in Fig. 7, provides valuable insights into the structural changes occurring in the intestines of *Drosophila* under different dietary conditions, mainly focusing on high-fat diets. Intestinal morphological integrity is crucial for preserving a healthy gut barrier. Morphological disruptions can cause enhanced permeability, enabling harmful substances to enter the bloodstream, triggering chronic inflammation, and contributing to various diseases. In the ND group, the intestinal structure was intact, with no abnormal changes in dimensions, wall breakage, thickening, or accumulation of melanoma or hyperplasia. However, under oxidative damage and aging effects associated with a long-term high-fat diet, the intestines of *Drosophila* displayed significant atrophy, with reduced length and accumulation of cellulite.

Such morphological changes can significantly affect nutrient absorption, as a damaged intestinal lining may reduce the efficiency of absorbing essential nutrients, impacting overall health and metabolic status. High doses of KPPs effectively alleviated these issues. The results revealed that KPP supplementation preserved intestinal structure, alleviating atrophy and cellular damage associated with high-fat diets, thereby maintaining intestinal morphology and integrity.

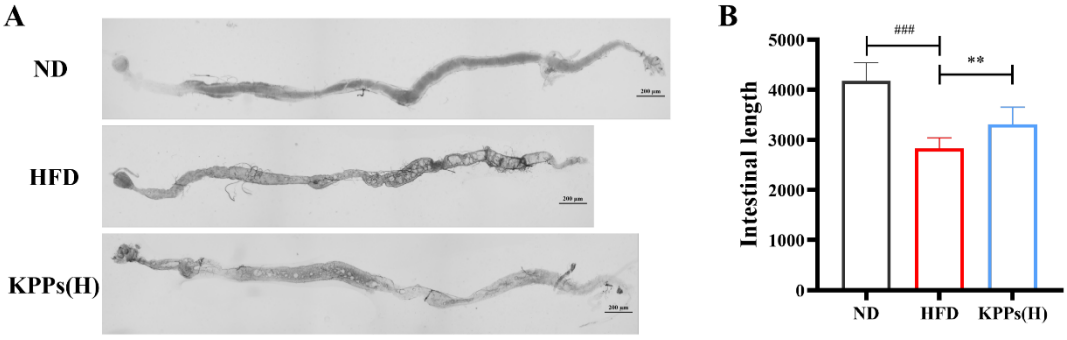
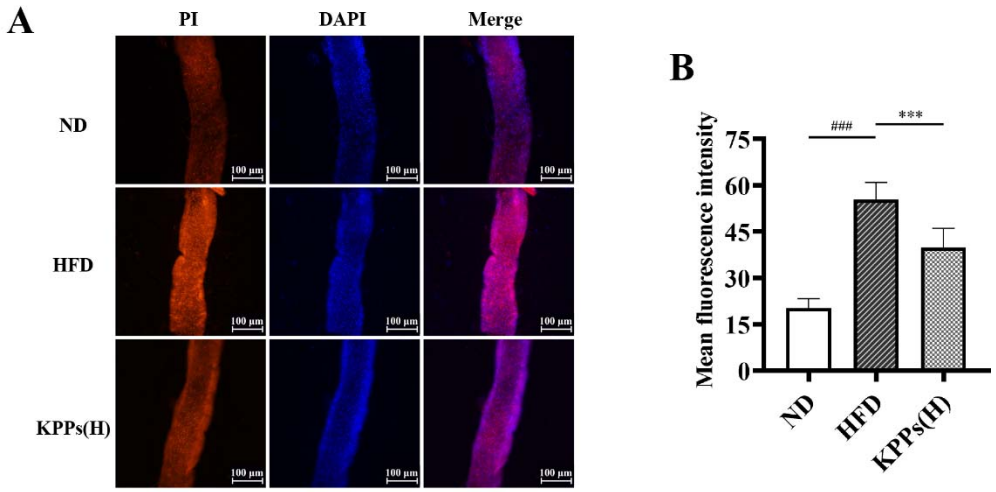


Fig. 7. Morphology (A) and length (B) of the intestine in high-fat damaged female *Drosophila* of different diets.

3.6.3. Analysis of Apoptosis in Intestinal Epithelial Cells

The analysis of apoptosis in intestinal epithelial cells, depicted in Fig. 8A-B, provides critical insights into cellular processes affected by dietary conditions, particularly high-fat diets. As shown, there were few apoptotic cells with red fluorescence in the intestines of *Drosophila* in the ND group; however, the incidence of apoptotic cells in the intestinal epithelium increased significantly in *Drosophila* on a high-fat diet. This increase in apoptosis can compromise gut barrier integrity, trigger inflammatory responses, and impair nutrient absorption, potentially leading to malnutrition and metabolic issues. KPPs at 5 mg/mL reduced apoptotic cell counts by 43.2%, indicating a protective effect against oxidative damage compared to the HFD group. These data suggest that KPPs could inhibit or reduce apoptosis of intestinal epithelial cells induced by high-fat oxidative damage, thereby preserving gut integrity and function. However, KPPs do not completely restore the tissue to a normal state. These data emphasize the necessity of keeping a balanced diet to support intestinal integrity and prevent excessive cell death, which can lead to broader health issues.



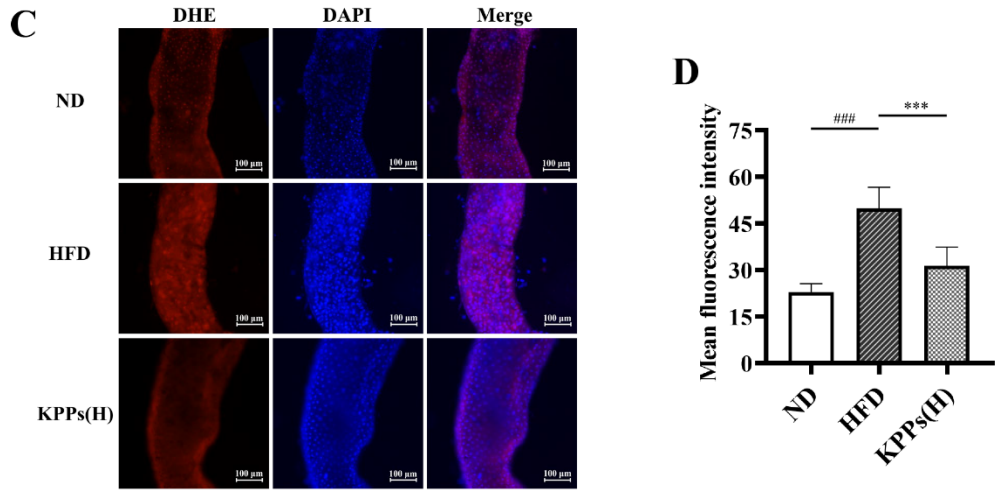


Fig. 8. Effects of different diets on apoptosis of intestinal epithelial cells in high-fat damaged female *Drosophila*. Apoptosis of intestinal epithelial cells (A) observed by PI staining and (B) the mean PI fluorescence intensity; ROS level in intestinal epithelial cells (C) observed by DHE staining and (D) the mean ROS fluorescence intensity.

3.6.4. Analysis of ROS Levels in Intestinal Epithelial Cells

The analysis of reactive oxygen species (ROS) levels in intestinal epithelial cells, illustrated in Fig. 8C-D, is essential for understanding the oxidative stress response in these cells, especially under high-fat diet conditions. Data indicates a significant increase in ROS levels within the intestinal epithelial cells of *Drosophila* exposed to high-fat diets. Low levels of ROS were detected in the intestines of *Drosophila* on a normal diet; however, a prolonged high-fat diet resulted in a substantial increase in ROS production, averaging approximately 2.17 times that of the normal group. The increase in ROS is often associated with cellular damage and oxidative stress, compromising cell viability and gut health, triggering inflammation, and impairing nutrient absorption. Natural nutrients, such as peptides, can protect intestinal health by lowering ROS levels. In contrast, 5 mg/mL KPPs significantly reduced ROS levels in the intestines of *Drosophila* on a high-fat diet, effectively scavenging excessive ROS and maintaining intestinal homeostasis.

3.6.5. Analysis of Intestinal Stem Cell Proliferation

The analysis of intestinal stem cell (ISC) proliferation, depicted in Fig. 9, provides insights into the regenerative capacity of the intestinal epithelium in *Drosophila* under different dietary conditions. ISCs play a critical function in regulating homeostasis within the intestinal environment, renewing and replenishing damaged or apoptotic epithelial cells through proliferation and differentiation [43]. Increased proliferation in response to dietary changes can aid regeneration; however, an imbalance favoring excessive proliferation may lead to dysplastic changes and increase the risk of tumorigenesis. It has been shown that improving and enhancing ISC function in aging senescent organisms promotes epithelial cell homeostasis and prolongs lifespan [44]. Under oxidative stress caused by a long-term high-fat diet, the intestinal epithelium showed a considerable increase in GFP-positive and PH3-positive cells compared to the ND group. KPPs normalized the number of PH3-positive and GFP-positive cells, suggesting a restoration of intestinal homeostasis. Immunostaining results indicated that KPPs inhibited oxidative damage in intestinal stem cells, aiding in the

maintenance of intestinal homeostasis. These findings highlight the need to follow a well-balanced diet to promote intestinal health and prevent adverse effects associated with excessive stem cell proliferation.

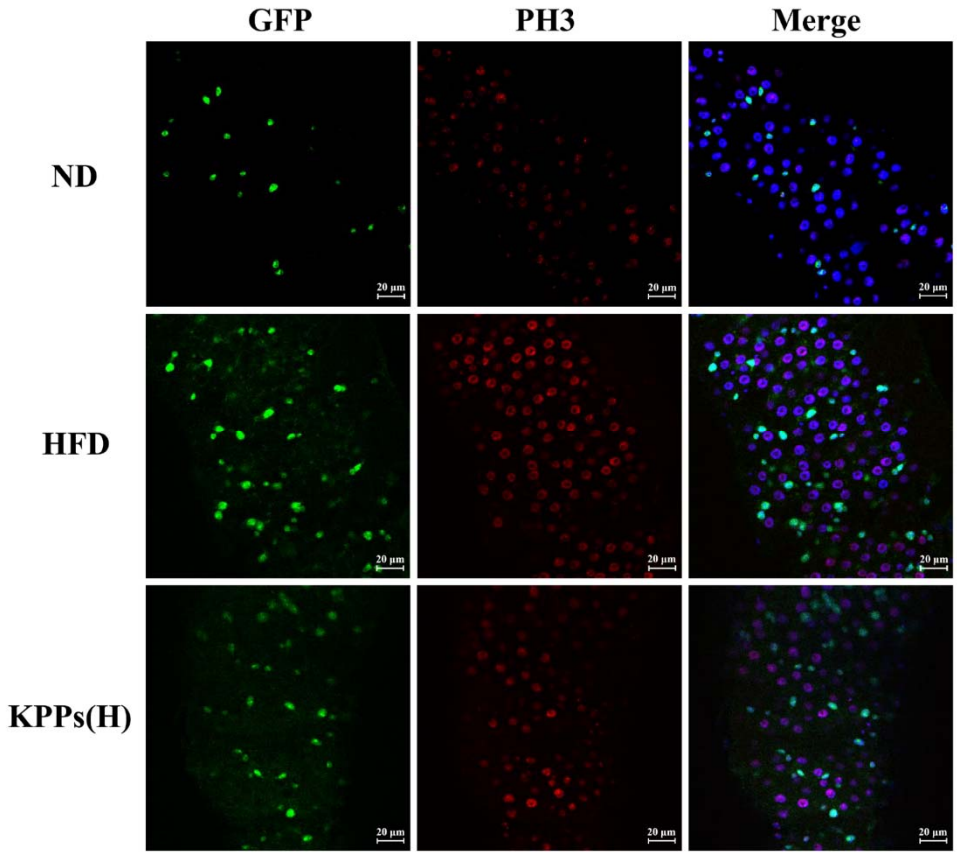


Fig. 9. Effects of different diets on intestinal stem cells over proliferation in high-fat damaged female Drosophila.

3.6.6. Analysis of Intestinal Lipid Droplet Staining

The analysis of intestinal lipid droplet staining, shown in Fig. 10, provides important insights into lipid metabolism and storage within the intestinal cells of *Drosophila*. This analysis highlights lipid accumulation in response to dietary changes, improving understanding of how dietary fat influences lipid storage in the intestine. A high-fat diet increases the intake of saturated fatty acids and cholesterol, leading to excessive fat deposition as lipid droplets in the intestines and other organs of *Drosophila*, negatively impacting metabolism. *Drosophila* on a normal diet exhibited no lipid droplets in their intestines, while chronic high-fat diets resulted in significant lipid accumulation. Increased lipid droplet accumulation may suggest enhanced lipid uptake or impaired lipid metabolism, which can have implications for overall energy balance and metabolic health. KPPs at 5 mg/mL markedly reduced lipid droplet formation, highlighting the peptides' role in lipid metabolism. Insights gained from lipid droplet analysis could identify potential targets for therapeutic interventions aimed at managing obesity and related metabolic diseases. By understanding how dietary fats affect lipid storage, strategies can be developed to mitigate adverse health effects.

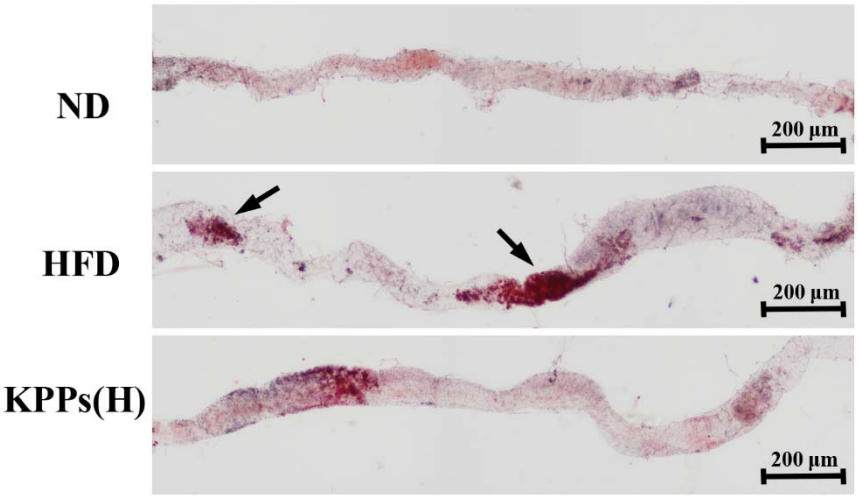


Fig. 10. Variations in intestinal lipid droplets in high-fat-damaged female *Drosophila* on different diets.

3.7. Effects of KPPs on Antioxidant-Related Genes and IIS Signaling Pathways in High-Fat Drosophila Melanogaster In Vivo:

The analysis presented in Fig. 11A focuses on the effects of KPPs on antioxidant-related genes and the Insulin/IGF-like signaling (*IIS*) pathways in *Drosophila* melanogaster subjected to a high-fat diet. Understanding how KPPs enhance antioxidant defense mechanisms is crucial for mitigating oxidative stress induced by high-fat diets. To examine the antioxidant mechanism of KPPs, we did qPCR to assess the expression levels of antioxidant-related genes, such as *SOD1*, *GSTs*, and *CNC*, in *Drosophila* melanogaster. *SOD1* is a key antioxidant enzyme that effectively scavenges free radicals and protects cells from oxidative stress. Glutathione S-transferases (*GSTs*) play a vital function in synthesizing glutathione, which is vital for maintaining redox balance and detoxification, thereby supporting cellular stability and health [45]. The *CNC* protein, a direct homolog of *Nrf2* in *Drosophila*, regulates the expression of antioxidant genes [46]. Our qPCR analysis revealed that expression levels of *SOD1*, *GSTs*, and *CNC* were significantly lower in *Drosophila* on a high-fat diet compared to the ND group, likely due to free radical accumulation and lipid peroxidation. KPP supplementation upregulated these genes, particularly *GSTs* in females and *SOD1* in males, thereby enhancing the antioxidant defense system. These results suggest that KPPs enhance the expression of antioxidant-related genes, activating antioxidant enzymes and improving the overall defense system, which increases the capacity to resist oxidative damage and delay aging in *Drosophila*. The insulin/IGF-1 signaling pathway (*IIS*) plays a crucial role in aging, growth, and metabolism. Studies indicate that *IIS/Foxo* signaling affects lipid metabolism, and inhibiting the *IIS* pathway can extend lifespan and reduce age-related ISC proliferation and developmental abnormalities [47]. Lipid metabolism is critical in the aging process, and downregulation of the insulin signaling pathway promotes lipid turnover and prolongs lifespan [48]. In our findings, the high-fat diet significantly reduced the expression of PI3K and dFoxO while increasing the expression of *Akt1* in both female and male *Drosophila* compared to the ND group. KPPs at 5 mg/mL appear to inhibit the IIS pathway, as evidenced by increased expression of *PI3K* and *dFoxO* in both sexes and a decreasing trend in *Akt1* expression. However, no significant difference was noticed in males compared to the HFD group. This

regulation could lead to improved insulin sensitivity and overall metabolic health in *Drosophila*. These insights suggest that the anti-aging mechanism of KPPs may be related to their inhibition of the insulin signaling pathway (Fig.11B). Furthermore, since the insulin signaling pathway also reduces age-related *ISC* proliferation and developmental abnormalities, KPPs may promote lipid turnover and maintain intestinal homeostasis by modulating the *PI3K-Akt* signaling pathway. Overall, these findings could help in the development of nutritional supplements and dietary strategies aimed at enhancing antioxidant defenses and improving metabolic health.

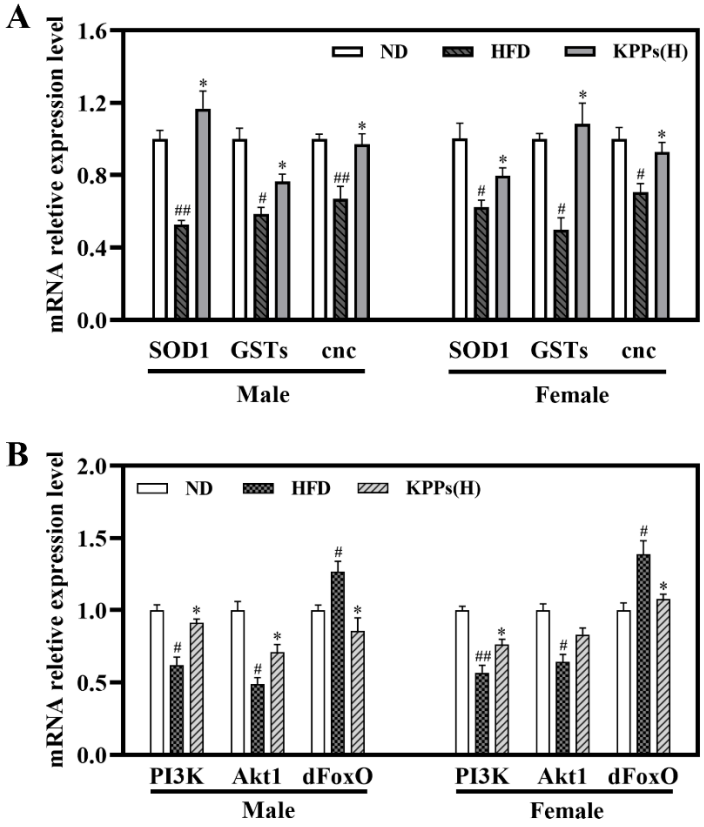


Fig. 11. The expression of antioxidant genes (A) and IIS signaling pathway-related genes (B) in *Drosophila* in different diets.

4. Conclusion

This research underscores the therapeutic potential of *Katsuwonus pelamis* peptides in combating oxidative stress and promoting longevity in *melanogaster* on a high-fat diet. The significant antioxidant activity of KPPs contributes to lifespan extension and improvement in behavioral indices, suggesting their role in enhancing overall health. Additionally, the protective effects on intestinal integrity further emphasize the importance of dietary interventions in managing the health risks associated with high-fat diets. KPPs inhibited the IIS signaling pathway by upregulating the expression of antioxidant-related genes *SOD1*, *CNC*, and *GSTs* and regulating gene expressions such as *Akt*, *PI3K*, and *dFoxO* in a high-fat diet. KPPs improve intestinal homeostasis and delay aging by protecting the intestinal barrier, reducing lipid accumulation, and decreasing ROS levels and apoptosis. Future research is needed to investigate the mechanisms behind these effects and determine the application of KPPs in broader nutritional and therapeutic contexts.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Acknowledgements

This work was supported by National Key R&D Plan, China (No. 2023YFD2100205), Young Elite Scientists Sponsorship Program by CAST (No. 2023QNRC001), Opening Project of Fujian Province - Indonesia Marine Food Joint Research and Development Center (Y1-KF2209), the Research Project of Fuzhou University (XRC-25016), and Qingdao Chenlan Health Industry Group Co., Ltd. Technology Development Project (2025041109).

References

1. Mitchell, A., M. Selby, and E.L. Sullivan, The influence of maternal high-fat diet consumption on neurobehavioral development, in Factors Affecting Neurodevelopment. 2021, Elsevier. p. 593-606.
2. Freudenberg, N., The capitalist diet: Energy-dense and profitable. Journal of Agriculture Food Systems and Community Development, 2019. **9**(1): p. 139-140.
3. Lavrelashvili, L., M. Tsanatskenishvili, and L. Gabunia, Western Diet, as a vast Problem of the 21st Century. Junior Researchers, 2023. **1**(1): p. 58-63.
4. Clemente-Suárez, V.J., et al., Global impacts of western diet and its effects on metabolism and health: A narrative review. Nutrients, 2023. **15**(12): p. 2749.
5. Palomar-Cros, A., et al., Associations of meal timing, number of eating occasions and night-time fasting duration with incidence of type 2 diabetes in the NutriNet-Santé cohort. International Journal of Epidemiology, 2023, 52(5): 1486-1497.
6. Araujo, S. M., Paula, M. T. D., Poetini, M. R., et al. Effectiveness of γ-oryzanol in reducing neuromotor deficits, dopamine depletion and oxidative stress in a Drosophila melanogaster model of Parkinson's disease induced by rotenone[J]. Neurotoxicology, 2015, 51: 96-105.
7. Gujjala, S., Bangeppagari, M., Devarakonda, V., et al. Pleiotropic effects of Salacia reticulata and Simvastatin on oxidative stress and insulin resistance in a rat model. Biomedicine & Pharmacotherapy, 2023, 164: 114960.
8. Takahashi, S., et al., A preliminary therapeutic study of the effects of molecular hydrogen on intestinal dysbiosis and small intestinal injury in high-fat diet-loaded senescence-accelerated mice. Nutrition, 2024. 122: p. 112372.
9. Harman, D. Aging: a theory based on free radical and radiation chemistry. Journal of Gerontology. 1956, **11**: 298-300.
10. Alberto Sanz, OP-20 - Mitochondrial ROS and ageing. Free Radical Biology and Medicine, 2017. 108(1): p. S9.
11. Peng, C., et al., Biology of ageing and role of dietary antioxidants. BioMed Research International, 2014. **2014**(1): p. 831841.
12. Wang, Q., et al., Antiaging function of Chinese pond turtle (Chinemys reevesii) peptide through activation of the Nrf2/Keap1 signaling pathway and its structure-activity relationship. Frontiers in Nutrition, 2022. **9**: p. 961922.
13. Meng, L., et al., Preparation, identification, activity prediction, and protective effects on IR-HepG2 cells of five novel DPP-IV inhibitory peptides from protein hydrolysate of skipjack tuna dark muscles. Food & Function, 2023. **14**(24): p. 10991-11004.
14. Wu, Q., et al., Studies on blocking the JAK2/STAT3 signaling pathway with elastin peptides from skipjack tuna (Katsuwonus pelamis) bulbus cordis to alleviate osteoarthritis. Food Bioscience, 2023. **56**: p. 103253.
15. Wang, J., et al., Twelve antioxidant peptides from protein hydrolysate of Skipjack tuna (Katsuwonus pelamis) roe prepared by flavourzyme: Purification, sequence identification, and activity evaluation. Frontiers in Nutrition, 2022. **8**: p. 813780.
16. Wei, W., et al., Katsuwonus pelamis peptide and its complexes protect zebrafish and mice from hyperuricemia through promoting kidney excretion of uric acid and inhibiting liver xanthine oxidase activity. Frontiers in Chemistry, 2022. **10**: p. 924371.
17. Zheng, S.-L., et al., Preparation, identification, molecular docking study and protective function on HUVECs of novel ACE inhibitory peptides from protein hydrolysate of skipjack tuna muscle. Marine Drugs, 2022. **20**(3): p. 176.
18. Shashoua, V., Peptide compounds for counteracting reactive oxygen species and free radicals. 2005, Google Patents.

19. Jin, H., et al., Unlocking the therapeutic potential of a manila clam-derived antioxidant peptide: Insights into mechanisms of action and cytoprotective effects against oxidative stress. Foods, 2024. **13**(8): p. 1160.
20. Zhang, L., et al., Identification and active evaluation of antioxidant peptides from protein hydrolysates of skipjack tuna (Katsuwonus pelamis) head. Antioxidants, 2019. **8**(8): p. 318.
21. Luo, J., et al., Ageing, age-related diseases and oxidative stress: What to do next? Ageing Research Reviews, 2020. **57**: p. 100982.
22. Heinrichsen, E.T., et al., Metabolic and transcriptional response to a high-fat diet in Drosophila melanogaster. Molecular Metabolism, 2014. **3**(1): p. 42-54.
23. Yang, Q., Cai, X., Huang, M., et al. Immunomodulatory effects of Pseudostellaria heterophylla peptide on spleen lymphocytes via a Ca^{2+}/CaN/NFATc1/IFN-γ pathway[J]. Food & Function, 2019, 10(6): 3466-3476.
24. Tu, J., et al., Identification of moracin N in mulberry leaf and evaluation of antioxidant activity. Food and Chemical Toxicology, 2019. **132**: p. 110730.
25. Wang, B., et al., Preparation and evaluation of antioxidant peptides from ethanol-soluble proteins hydrolysate of Sphyrna lewini muscle. Peptides, 2012. **36**(2): p. 240-250.
26. Zhang, S. and R. Zhang, Effects of microwave pretreatment of apple raw material on the nutrients and antioxidant activities of apple juice. Journal of food processing, 2014. **2014**(1): p. 824050.
27. Dinis, T.C., V.M. Madeira, and L.M. Almeida, Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of Biochemistry and Biophysics, 1994. **315**(1): p. 161-169.
28. Osawa, T. and M. Namiki, A novel type of antioxidant isolated from leaf wax of Eucalyptus leaves. Agricultural and Biological Chemistry, 1981. **45**(3): p. 735-739.
29. Akbar, S., et al., Phytochemical analysis and antioxidant studies of leaf, stem, root and fruit of Malva parviflora L. Boiscience Research, 2022, 18(4): 2626-2634
30. GÜLÇin, I., H.A. Alici, and M. Cesur, Determination of in vitro antioxidant and radical scavenging activities of propofol. Chemical and Pharmaceutical Bulletin, 2005. **53**(3): p. 281-285.
31. Xin, X.-x., et al., Supplementation with major royal-jelly proteins increases lifespan, feeding, and fecundity in Drosophila. Journal of Agricultural and Food Chemistry, 2016. **64**(29): p. 5803-5812.
32. Liang, Y., et al., Study on the sleep-improvement effects of Hemerocallis citrina Baroni in Drosophila melanogaster and targeted screening to identify its active components and mechanism. Foods, 2021. **10**(4): p. 883.
33. Vef, O., et al., A new strategy for efficient in vivo screening of mutagenized Drosophila embryos. Development Genes and Evolution, 2006. **216**: p. 105-108.
34. Gutierrez, E., et al., Specialized hepatocyte-like cells regulate Drosophila lipid metabolism. Nature, 2007. **445**(7125): p. 275-280.
35. Sakai, T., et al., The novel antioxidant TA293 reveals the role of cytoplasmic hydroxyl radicals in oxidative stress-induced senescence and inflammation. Biochemical and Biophysical Research Communications, 2017. **482**(4): p. 1183-1189.
36. Yu, D., et al., Protein degradation and peptide formation with antioxidant activity in pork protein extracts inoculated with Lactobacillus plantarum and Staphylococcus simulans. Meat Science, 2020. **160**: p. 107958.
37. Jeong, J.-B. and H.-J. Jeong, Schisandra chinensis inhibits oxidative DNA damage and lipid peroxidation via antioxidant activity. Korean Journal of Plant Resources, 2009. **22**(3): p. 195-202.
38. XU, J. and Z. LIN, Optimization of enzymatic preparation process of peptides from pearl gentian grouper by response surface methodology and the antioxidant activity of the enzymatic hydrolysis products. Science and Technology of Food Industry, 2020. **41**(19): p. 205-211.
39. Liao, S., M. Amcoff, and D.R. Nässel, Impact of high-fat diet on lifespan, metabolism, fecundity and behavioral senescence in Drosophila. Insect Biochemistry and Molecular Biology, 2021. **133**: p. 103495.
40. Chen, S., et al., Bioactive peptides derived from crimson snapper and in vivo anti-aging effects on fat diet-induced high fat Drosophila melanogaster. Food & Function, 2020. **11**(1): p. 524-533.

41. Wilson, K.A., et al., Evaluating the beneficial effects of dietary restrictions: A framework for precision nutrigeroscience. Cell Metabolism, 2021. **33**(11): p. 2142-2173.
42. Rera, M., R.I. Clark, and D.W. Walker, Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in Drosophila. Proceedings of the National Academy of Sciences, 2012. **109**(52): p. 21528-21533.
43. Fan, X., et al., Rapamycin preserves gut homeostasis during Drosophila aging. Oncotarget, 2015. **6**(34): p. 35274.
44. Buchon, N., et al., Drosophila intestinal response to bacterial infection: activation of host defense and stem cell proliferation. Cell Host & Microbe, 2009. **5**(2): p. 200-211.
45. Albrecht, S.C., et al., In vivo mapping of hydrogen peroxide and oxidized glutathione reveals chemical and regional specificity of redox homeostasis. Cell Metabolism, 2011. **14**(6): p. 819-829.
46. Pitoniak, A. and D. Bohmann, Mechanisms and functions of Nrf2 signaling in Drosophila. Free Radical Biology and Medicine, 2015. **88**: p. 302-313.
47. Rodriguez-Fernandez, I.A., H.M. Tauc, and H. Jasper, Hallmarks of aging Drosophila intestinal stem cells. Mechanisms of Ageing and Development, 2020. **190**: p. 111285.
48. Li, Y., et al., DO-SRS imaging of metabolic dynamics in aging Drosophila. Analyst, 2021. **146**(24): p. 7510-7519.