

A comparative study on anti-ageing efficacy of feruloyl oligosaccharides with different polymerisation degrees in *Caenorhabditis elegans*

S. Yu[#], D.J. Wang[#], Y.M. Lyu, H. Chen, M.Y. Long, S.H. Lyu and X.H. Yu^{*} 

School of Marine and Bioengineering, Yancheng Institute of Technology, Yancheng 224051, China

ORIGINAL RESEARCH PAPER

Received: January 10, 2026 • Accepted: May 23, 2026

Published online: June 15, 2026

© 2026 The Author(s)



ABSTRACT

This study explored the anti-ageing effects of feruloyl oligosaccharides (FOs) with varying degrees of polymerisation (DP) through *in vivo* and *in vitro* experiments. Low-DP FOs (LFOs, DP 2–5, predominantly 2) were prepared by acidolysis, whereas high-DP FOs (HFOs, mainly DP 6) were produced by fermentation. *In vitro*, LFOs showed greater ABTS radical scavenging activity and reducing power than HFOs. Treatment with 20 mg mL⁻¹ LFOs increased the lifespan and body length of *Caenorhabditis elegans* by 22.4 and 16.01%, respectively, compared to 16.30 and 3.8% with HFOs. Both FOs enhanced oxidative and heat stress resistance. Compared with the HFOs, the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) antioxidant enzymes of LFOs increased by 9.50, 13.70, and 12.28%, respectively. However, the malondialdehyde (MDA) content decreased by 9.49%. These results suggest that LFOs appear more effective than HFOs at reducing oxidative damage and prolonging lifespan.

KEYWORDS

feruloyl oligosaccharides, degree of polymerisation, antioxidant activity, anti-ageing effect

* Corresponding author. Tel.: +86 0515-13961981055. E-mail: yxh1127@ycit.edu.cn

[#] These authors are co-first authors.

1. INTRODUCTION

Ageing is a complex process of progressive functional decline and is most frequently explained by the widely accepted free radical theory. Excessive accumulation of ROS induces oxidative stress, damaging cellular components (López-Otín et al., 2023). The body's primary antioxidant defence system against such damage relies on key enzymes, including SOD, CAT, and GSH-Px (Lessel et al., 2017). Supplementation with exogenic antioxidants is a key strategy to counter the system's inevitable weakening from ageing and environmental stress. Due to safety concerns regarding synthetic antioxidants, identifying safe and effective bioactive compounds from natural food sources is a key research focus (Wang et al., 2025). The anti-ageing effects of the natural extract, tea tree mushroom extracellular polysaccharides, was mediated by scavenging free radicals *in vitro* and upregulating SOD, CAT, and GSH-Px *in vivo* (Zhao et al., 2017). *Rhizoma Polygonati Odorati* counteracted ageing and memory deficits by elevating brain SOD/GSH-Px activities and reducing MDA levels (Bian et al., 2022). These natural compounds are safe and show great promise against ageing.

FOs, functional carbohydrates primarily located in cereal bran cell walls, are structurally characterised by ferulic acid ester-linked to arabinoxylan-based sugar chains (Gong et al., 2022). This unique structure endows them with potent antioxidant activity *via* the ferulic acid component and, alongside water solubility, offers prebiotic potential through the sugar chains (Yin et al., 2024). Current research on FOs prioritises extraction optimisation and *in vitro* antioxidant assessment (Zhang et al., 2024a).

FOs bioactivity is predominantly determined by structural features such as DP, since DP critically influences their absorption and utilisation *in vivo* (Zeng et al., 2021). Owing to the predominant use of simplified *in vitro* models, the *in vivo* absorption and enzyme-regulatory effects of FOs with varying DP remain unclear, necessitating systematic studies on their anti-ageing mechanisms.

C. elegans is an ideal model for anti-ageing research due to its short life cycle, clear genetics, and conserved ageing pathways relevant to humans (Zhang et al., 2024b). Manno-protein-rich yeast cell wall enzymatic hydrolysate extracts extended the lifespan and improved locomotion in *C. elegans* by reducing ROS and MDA levels while elevating SOD activity (Zeng et al., 2023). Walnut protein hydrolysate extended the lifespan of *C. elegans*, even under oxidative stress, mediated by enhanced antioxidant activity and reduced cellular damage (Gao et al., 2024). The biological efficacy of natural products can be more comprehensively and accurately evaluated using the *C. elegans* model in combination with traditional *in vitro* analyses.

This study aimed to elucidate the structure, antioxidant capacity, and anti-ageing benefits of FOs with different DP. This was achieved through the preparation and structural characterisation of two distinct FO fractions. For clarity and comparative purposes in this study, the fraction with smaller fragments (DP 2–5) generated via acidolysis is operationally referred to as “low-DP FOs” (LFOs), while the relatively larger fraction (predominantly DP 6) produced via fermentation is termed “high-DP FOs” (HFOs). This research helps explain the link between DP and function, providing a solid basis for developing new functional foods.

2. MATERIALS AND METHODS

2.1. Experimental materials

The wheat bran was purchased from a local market in Heze, Shandong Province, China. Macroporous adsorbent resin Amberlite XAD-2 was obtained from Zhengzhou Aino Chemical Technology Co., Ltd. (Zhengzhou, China). C18 column packing material YMC ODS-A-HG (50 μm) was supplied by Nanyang Zhigu Biological Co., Ltd. (Nanyang, China). HPLC-grade monosaccharide standards were purchased from Yuan Ye Biotechnology Co., Ltd (Shanghai, China). All other analytical reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. *Bacillus amyloliquefaciens* IT-45 was isolated, identified, and maintained in our laboratory. Uracil-deficient *Escherichia coli* (*E. coli* OP50) was provided by the Research Laboratory of Food Additives and Nutritional Regulation, Tianjin University of Science and Technology. The wild-type N2 *C. elegans* strain was obtained from the Caenorhabditis Genetics Center (CGC). Kits for the determination of total protein (TP), CAT, SOD, GSH-Px, and MDA were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2. Experimental methods

2.2.1. Pretreatment of wheat bran. Wheat bran was dried, ground, and sieved (60-mesh). The sieved powder (100.0 g) was suspended in 1200.0 mL of deionised water, dextrinised (95.0 $^{\circ}\text{C}$, 30.0 min) and cooled to room temperature. The mixture was first adjusted to pH 5.0 and treated with thermostable α -amylase (0.3% w/w, 90.0 $^{\circ}\text{C}$, 30.0 min). Following enzyme inactivation (100.0 $^{\circ}\text{C}$, 10 min) and cooling, the mixture was adjusted to pH 7.0 and treated with 1.00% (w/v) papain (800 U mg^{-1}) (50.0 $^{\circ}\text{C}$, 120 min), which was subsequently inactivated by heating at 100.0 $^{\circ}\text{C}$ for 10 min. The mixture was filtered, the residue was rinsed three times and dried at 55.0 $^{\circ}\text{C}$.

2.2.2. LFOs obtained by acid hydrolysis. After acid hydrolysis (1.2% *p*-toluenesulfonic acid, 120 $^{\circ}\text{C}$, 30 min), the supernatant was concentrated and subjected to two-step ethanol precipitation (1 $\times$ and 4 $\times$ ethanol, 4 $^{\circ}\text{C}$ overnight). The precipitate was then dissolved, deproteinised with Sevag reagent (chloroform:*n*-butanol, 4:1, v/v), and lyophilised to obtain crude LFOs.

2.2.3. HFOs obtained by fermentation. LB liquid medium and wheat bran fermentation medium (150.0 g wheat bran, 3.0 g peptone, 3000.0 mL water, pH 8.0) were autoclaved. 300 mL LB liquid medium was inoculated with 10.0 μL of *Bacillus amyloliquefaciens* seed suspension and incubated (37.0 $^{\circ}\text{C}$, 200 r.p.m., 12 h). The seed culture (10.0%, v/v) was then inoculated into wheat bran fermentation medium, and incubated (28.0 $^{\circ}\text{C}$, 200 r.p.m., 48 h). The supernatant was treated as for LFOs to obtain crude HFOs.

2.2.4. Purification of FOs. Purification was performed using an Amberlite XAD-2 column (2.6 $\times$ 40 cm). The crude FOs (4.0 mg) were dissolved in 10 mL of deionised water and loaded onto the column at a flow rate of 5 mL min^{-1} . To remove salts and free sugars, the column was first washed with 4 bed volumes of deionised water. Subsequently, the target compounds were eluted with 50 and 80% ethanol at a flow rate of 5 mL min^{-1} , using 2 bed volumes for each gradient. The 50% ethanol fraction was collected and lyophilised. Further separation was conducted on a C18 column (2.1 $\times$ 3 cm). The column was washed prior to sample loading

with 30 mL of deionised water, followed by 30 mL of 20% methanol and 30 mL of 40% methanol. The column was then activated and equilibrated with 10 mL of 100% methanol and 10 mL of deionised water. A 5 mg sample was dissolved in 1 mL of deionised water, loaded onto the column, and allowed to flow through by gravity into a collection beaker. The column was subsequently washed with 15 mL of deionised water to elute salts and oligosaccharide without FA. The retained compounds were then eluted sequentially with 20 and 40% methanol. Fractions were collected at 0.5 mL per tube, with 6 tubes collected for each methanol concentration, yielding a total volume of 3 mL per elution step. The 20 and 40% methanol elution fractions were collected, combined, concentrated, and lyophilised to obtain the final purified FOs.

2.2.5. Monosaccharide composition of FOs and FA characterisation. FOs (50 mg) were hydrolysed with 2 mol L⁻¹ trifluoroacetic acid (5 mL) at 110 °C for 6 h. After removing excess acid, the hydrolysate was redissolved in water (2.5 mL). An aliquot (200 µL) was derivatised with 0.5 mol L⁻¹ NaOH and 0.5 mol L⁻¹ 1-phenyl-3-methyl-5-pyrazolone (PMP) in methanol (1:1:1, v/v/v), followed by incubation at 70 °C for 1 h. The solution was then neutralised with 0.5 mol L⁻¹ NaOH and 0.5 mol L⁻¹ HCl, extracted with chloroform, and centrifuged (12,000 r.p.m.). The aqueous layer was filtered through a 0.22 µm membrane and analysed by HPLC under the following conditions: chromatographic column: Agilent SB-C18 (4.6 × 250 mm, 5 µm), mobile phase: 0.1 mol L⁻¹ ammonium acetate:acetonitrile (87:13, v/v), flow rate: 1.0 mL min⁻¹, sample volume: 20 µL, column temperature: 30 °C, detection wavelength: 250 nm.

The FOs solution (1 mg mL⁻¹) was hydrolysed with an equal volume of 1 mol L⁻¹ NaOH at 90 °C under light for 2 h. After cooling and neutralisation with HCl, the sample was filtered (0.22 µm) and analysed using an Agilent 1260 HPLC system with a C18 column (5 µm, 4.6 × 250 mm). HPLC conditions: column temperature: 30 °C, mobile phase: methanol:1% acetic acid (1:1, v/v), 0.8 mL min⁻¹, detection: 320 nm, injection volume: 10 µL.

2.2.6. Fourier transform infrared spectroscopy. FOs (1.0 mg) were blended with KBr and pressed into a pellet for FT-IR analysis, and spectra were acquired from 4,000 to 400 cm⁻¹ at a resolution of 4 cm⁻¹ over 32 scans.

2.2.7. UV absorption spectra. FOs (5.0 mg) were dissolved in 0.1 mol L⁻¹ MOPS buffer (pH 6.0, 1.0 mL) and analysed by UV-Vis spectrophotometry between 200 and 400 nm.

2.2.8. High-resolution mass spectrometry. For MS analysis, FOs were dissolved in 50% aqueous methanol, filtered (0.22 µm), and analysed in positive ion mode with a spray voltage of 3.5 kV. The mobile phase was 50% aqueous methanol at 200 µL min⁻¹, with an injection volume of 2 µL. The ion source and desolvation temperatures were 110 °C and 400 °C, respectively, using an N₂ flow of 5 Arb and scanning *m/z* 200–2,000.

2.2.9. In vitro antioxidant assays.

2.2.9.1. ABTS free radical scavenging activity. The ABTS radical scavenging activity was measured with reference to the reported method and slightly modified (Wang et al., 2024). The ABTS working solution was prepared by reacting 2.5 mmol L⁻¹ potassium persulfate with 7.0 mmol L⁻¹ ABTS (1:1, v/v) in the dark for 12 h and diluting with 95% ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Fifty microliters of sample was mixed with 200 µL of the

working solution, incubated for 5 min in the dark, and the absorbance at 734 nm was recorded in triplicate. ABTS radical scavenging activity was calculated as:

$$\text{ABTS (\%)} = \left(1 - \frac{X_1 - X_2}{X_3}\right) \times 100\%$$

X_1 : sample + ABTS, X_2 : sample + ethanol, X_3 : ABTS + ethanol.

2.2.9.2. DPPH free radical scavenging activity. The DPPH radical scavenging capacity was determined referring to the reported method by Wang et al. (2024) with minor modifications. The assay was performed in triplicate by mixing 30 μL sample with 150 μL of 0.1 mM DPPH (in ethanol) in a 96-well plate, incubating in the dark for 30 min at room temperature, and measuring absorbance at 517 nm, with vitamin C as the positive control. DPPH radical scavenging activity was calculated as:

$$\text{DPPH (\%)} = \left[1 - \frac{X_1 - X_2}{X_3}\right] \times 100\%$$

X_1 : sample + DPPH, X_2 : sample + ethanol, X_3 : DPPH + ethanol

2.2.9.3. Reducing capacity. The reducing capacity was assayed according to Wang et al. (2024), using vitamin C (VC) as a positive control, with minor modifications. The reaction mixture containing phosphate buffer, potassium ferricyanide, and sample was incubated at 50 °C for 20 min. After the addition of trichloroacetic acid and centrifugation, the supernatant was reacted with ferric chloride, and the absorbance was recorded at 700 nm.

$$\text{Reduction capacity} = X_1 - X_0$$

X_1 : Sample group, X_0 : Blank control group

2.2.10. Physiological activity and antioxidant activity of *C. elegans*.

2.2.10.1. *C. elegans* culture conditions and experimental groups. The intervention concentrations were selected based on our preliminary toxicity assays. On solid NGM plates, *C. elegans* maintained normal vitality and showed no signs of severe osmotic stress even when exposed to FOs at concentrations up to 50.0 mg mL^{-1} . Therefore, 20.0 mg mL^{-1} was established as a safe and effective maximum dose for this study, ruling out dominant osmotic toxicity, which was further supported by the positive growth phenotypes. All *C. elegans* strains were maintained at 20 °C on NGM plates seeded with *E. coli* OP50. To eliminate the potential confounding effect of FOs acting as an additional carbon source for bacterial proliferation, the *E. coli* OP50 seeded on the experimental NGM plates was pre-sterilised (inactivated) prior to transferring the *C. elegans*. Specifically, the NGM plates seeded with OP50 were grown overnight and subsequently exposed to ultraviolet (UV) light for 30 min in a biosafety cabinet to halt bacterial replication. To prevent the potential carry-over of bacterial sources during the transfer, an unseeded plate transition protocol was applied. Specifically, L4-stage *C. elegans* were initially transferred onto sterile, unseeded NGM agar plates and left to crawl for 10–15 min. This brief transition physically reduces the number of bacteria adhering to the cuticle *via* friction with the

agar surface and supports the clearance of bacteria from the digestive tract. The nematodes were subsequently transferred to the target experimental plates using a flame-sterilised platinum pick. Synchronised L1 larvae were obtained by hypochlorite treatment and grown to the L4 stage, after which they were transferred to fresh plates and divided into nine experimental groups for treatment.

1. Control, FUDR + OP50,
2. L1, FUDR + OP50 + 2.5 mg mL⁻¹ of LFOs,
3. L2, FUDR + OP50 + 5.0 mg mL⁻¹ of LFOs,
4. L3, FUDR + OP50 + 10.0 mg mL⁻¹ of LFOs,
5. L4, FUDR + OP50 + 20.0 mg mL⁻¹ of LFOs,
6. H1, FUDR + OP50 + 2.5 mg mL⁻¹ of HFOs,
7. H2, FUDR + OP50 + 5.0 mg mL⁻¹ of HFOs,
8. H3, FUDR + OP50 + 10.0 mg mL⁻¹ of HFOs,
9. H4, FUDR + OP50 + 20.0 mg mL⁻¹ of HFOs.

2.2.10.2. Lifespan assay. The lifespan assay was performed according to Wang et al. (2023) with minor modifications. Synchronised L4 larvae were maintained on experimental plates, which were replaced every 24 h. Survival was scored daily, and *C. elegans* were considered dead if they showed no response to gentle touch with a platinum wire.

2.2.10.3. Determination of body size. The *C. elegans* body length assay was performed according to Lu et al. (2024) with minor modifications. Synchronised L4 larvae were transferred to experimental groups (≥ 30 worms per plate) and their body lengths were measured on days 2, 5, and 8 using ImageView software, with three replicate measurements per worm averaged for the final value.

2.2.10.4. Thermotolerance assay and oxidative stress resistance. The thermotolerance assay and oxidative stress resistance assay were performed following Lu et al. (2024) with minor modifications. Synchronised L4 larvae were transferred to experimental plates ($n \geq 60$ per group) and cultivated at 20 °C for 5 days. For the thermotolerance assay, the original plates were subsequently transferred to a 37 °C incubator. For the oxidative stress assay, the adult worms were carefully transferred onto freshly prepared, unseeded NGM agar plates containing 10 mM H₂O₂. Under both stress conditions, the viability of the nematodes was recorded hourly until complete mortality was observed.

2.2.10.5. Determination of antioxidant enzyme activity and MDA content. The determination of *C. elegans* biochemical indices was performed according to the method described by Chen et al. (2023) with minor modifications. Synchronised L4 larvae were incubated for 5 days at 20 °C in the experimental groups, collected, washed with M9 buffer, and centrifuged. The worms were ultrasonically disrupted (400 W, 2 s, 5 min, 4 °C) and centrifuged (12,000 r.p.m., 5 min) to obtain the protein extract. Following the kit instructions, TP, ROS, CAT, SOD, GSH-Px, and MDA were measured in triplicate.

2.2.11. Data analysis. All experiments were performed in triplicate. Data were analysed using IBM SPSS Statistics 27 and graphed with Origin 2024. Statistical comparisons were made by

one-way ANOVA or *t*-test, with significance set at $P < 0.05$ and $P < 0.01$. Survival data were analysed with the Kaplan–Meier method (Kwon et al., 2025).

3. RESULTS AND DISCUSSION

3.1. FA qualitative and UV spectral analysis

HPLC analysis confirmed the presence of ferulic acid (FA) in FOs, with a retention time of 13.74 min matching that of the FA standard (Fig. 1A–C). UV spectra showed characteristic absorption peaks near 286 and 325 nm in both the standard and FOs (Fig. 1D–F), consistent with esterified FA as previously reported (Yin et al., 2024). The peak around 325 nm corresponds to the typical backward shift associated with FA esterification, collectively indicating that the prepared FOs contain esterified FA.

3.2. Infrared spectrum, mass spectrum and monosaccharide composition

Figure 2A shows the infrared spectra of LFOs and HFOs. Specifically, LFOs showed prominent peaks at 3398.15, 2935.50, 1660.21, and 1033.73 cm^{-1} , whereas HFOs displayed major peaks at 3420.84, 2930.43, 1647.64, and 1040.08 cm^{-1} . Peaks at 3,600–3,200 and 3,000–2,800 cm^{-1} were assigned to O–H and C–H stretching vibrations, respectively (Chu et al., 2024). Additionally, peaks in the 1,900–1,650 cm^{-1} range arose from C=O stretching vibrations, indicating the presence of carbonyl groups in both samples (He et al., 2022). Peaks in the 1,600–1,450 and 880–680 cm^{-1} regions were attributed to aromatic C=C and C–H vibrations. Strong absorptions at 1,200–900 cm^{-1} suggested C–O stretching and glycosidic linkages (Wei et al., 2022), with

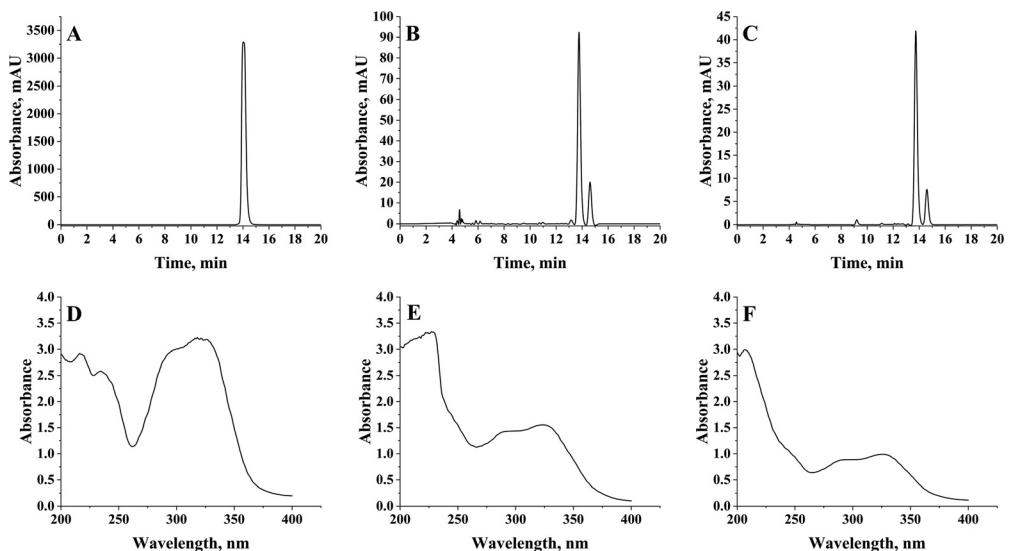


Fig. 1. HPLC chromatograms and UV spectra of ferulic acid (FA) and feruloylated oligosaccharides (FOs). HPLC chromatograms of (A) FA, (B) LFOs, and (C) HFOs. UV spectra of (D) FA, (E) LFOs, and (F) HFOs

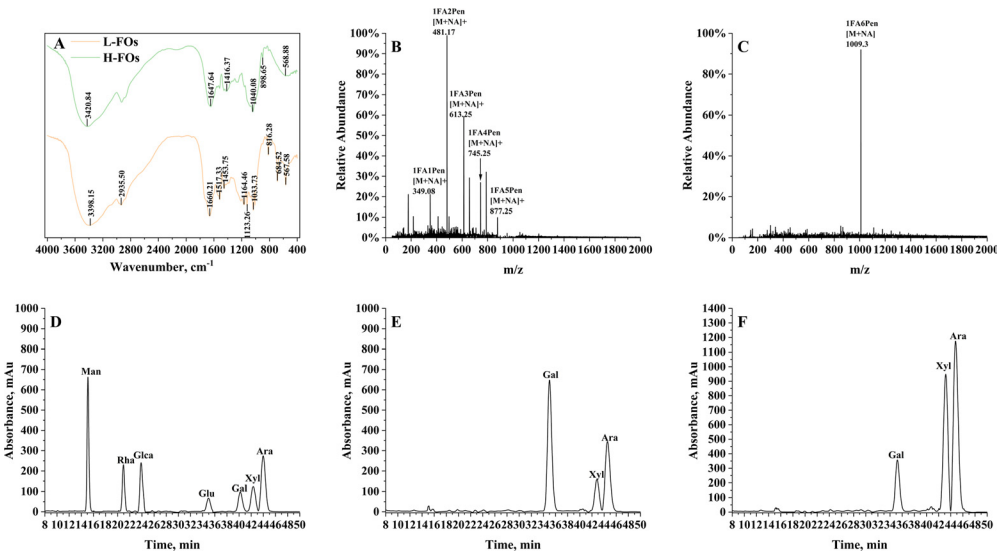


Fig. 2. Structural characterisation and monosaccharide composition analysis of feruloylated oligosaccharides (FOs). (A) FT-IR spectra of LFOs and HFOs. MS spectra of (B) LFOs and (C) HFOs. Monosaccharide composition analysis of (D) monosaccharide standards, (E) LFOs, and (F) HFOs
Man: mannose; Rha: rhamnose; GlcA: glucuronic acid; Glu: glucose; Gal: galactose; Xyl: xylose; Ara: arabinose

minor shifts suggesting possible differences in chain linkage or monosaccharide composition. Overall, both FOs exhibited consistent functional group profiles with only localised variations.

A consistent 132 *m/z* interval between adjacent peaks in the mass spectrum was attributed to dehydrated arabinose or xylose units. As shown in Fig. 2B and C, the peak at *m/z* 481 was assigned to the sodium adduct of feruloyl-arabinose-xylose. Subsequent peaks at *m/z* 613, 745, 877, and 1,009 were accordingly identified as feruloylated arabinoxyylan oligomers with increasing numbers of xylose units (di- to penta-saccharides). Environmentally sensitive xylanase and feruloyl esterase activities collectively yielded a mixture of feruloylated oligosaccharides, as reflected in the profile (Yu et al., 2016). The DP distribution observed here aligns with reported FO components whose antioxidant activity correlates with side-chain length (Zhao et al., 2018). By optimising *Bacillus amyloliquefaciens* fermentation (pH 8, 2 days), we obtained FOs predominantly with DP 6, whereas acid hydrolysis yielded FOs with DP 2–5. Consequently, these two fractions were designated as HFOs and LFOs, respectively, representing a relative size comparison to evaluate the impact of DP on their subsequent biological activities.

HPLC analysis revealed distinct monosaccharide profiles for the bran-derived FOs (Fig. 2D–F). LFOs consisted of glucose (48.63%), arabinose (34.31%), and xylose (17.06%), whereas HFOs were primarily composed of arabinose (40.18%), xylose (31.00%), and glucose (8.42%).

While FT-IR and MS analyses confirmed the primary structural features and DP distribution of the extracted FOs, precise elucidation of inter-glycosidic linkages and anomeric configurations requires advanced nuclear magnetic resonance spectroscopy. According to established

structural models of Gramineae cell walls, ferulic acid in wheat bran is typically attached *via* an ester linkage predominantly to the O-5 position of α -L-arabinofuranosyl residues, which substitute the β -(1 $\rightarrow$ 4)-linked D-xylopyranosyl backbone. This structural arrangement distinguishes cereal-derived FOs from those derived from dicotyledons, such as sugar beet pulp, where ferulic acid is often esterified at the O-2 position of arabinose residues (Colquhoun et al., 1994). The structural complexity, including these specific α/β configurations and linkage patterns, likely plays a fundamental role in shaping the distinct antioxidant capacities and *in vivo* bioactivities observed between LFOs and HFOs.

3.3. *In vitro* antioxidant assays

The antioxidant capacity of FOs was evaluated by DPPH and ABTS radical scavenging assays as well as reducing power determination. LFOs exhibited stronger ABTS radical scavenging and reducing power than HFOs (Fig. 3A). At 0.50 mg mL⁻¹, LFOs showed >80% ABTS scavenging activity, compared to 66.52% for HFOs. In the DPPH assay, HFOs exceeded 90% at 0.50 mg mL⁻¹, while LFOs surpassed HFOs only at 4 mg mL⁻¹; both reached plateaus near 94.7% at 8 mg mL⁻¹. The overall reducing capacity was higher for LFOs. These differences suggest distinct antioxidant mechanisms related to the degree of polymerisation. The smaller, more soluble LFOs likely favour electron transfer (ET), whereas the larger HFOs may act preferentially *via* hydrogen atom transfer (HAT), facilitating DPPH scavenging at higher concentrations (Sissi et al., 2022). Thus, the DP critically influences not only monosaccharide composition and structure but also the expression and pathway of antioxidant activity.

3.4. Physiological activity and antioxidant activity of *C. elegans*

3.4.1. Lifespan and body length assay. FOs with different DPs significantly extended the mean lifespan of *C. elegans*, with the greatest effect observed at 20 mg mL⁻¹ (Fig. 4A and B, Table 1). At this concentration, LFOs prolonged lifespan by 22.4% compared to 16.3% for HFOs, indicating stronger efficacy and biocompatibility for the low-DP FOs.

FOs affected *C. elegans* body length in a concentration-dependent manner (Fig. 4C–E). LFOs significantly promoted growth compared to the control, whereas HFOs had a minimal effect.

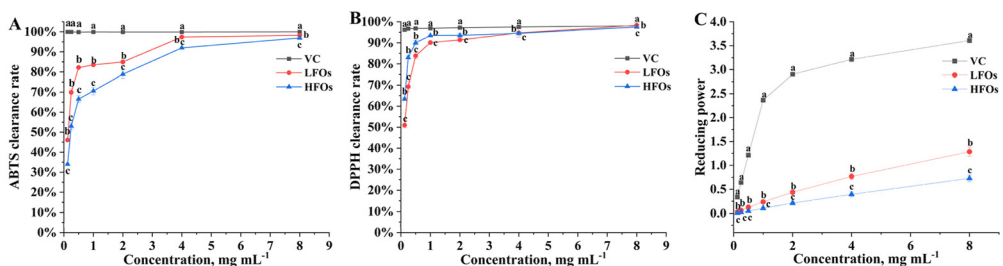


Fig. 3. Antioxidant activities of feruloylated oligosaccharides LFOs and HFOs: (A) ABTS radical scavenging activity, (B) DPPH radical scavenging activity, and (C) reducing capacity.

At the same concentration, identical lowercase letters indicate non-significant differences ($P > 0.05$), and different lowercase letters indicate significant differences ($P < 0.05$)

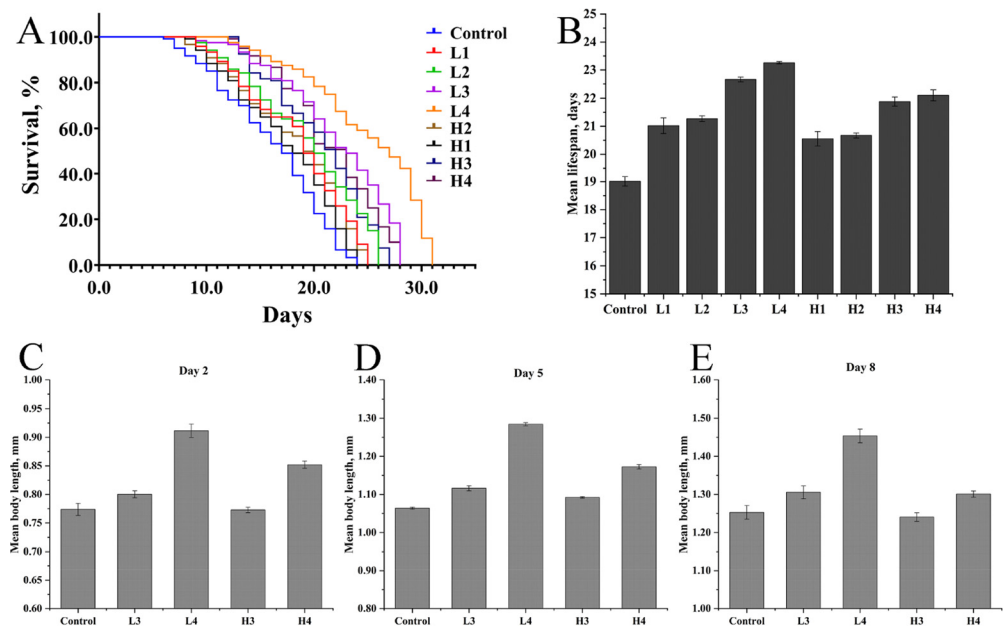


Fig. 4. Effects of feruloylated oligosaccharides (FOs) on the lifespan and body length of *C. elegans*. (A) Survival curves, (B) mean lifespan, and mean body lengths on (C) day 2, (D) day 5, and (E) day 8

Table 1. Effects of LFOs and HFOs on the lifespan of *C. elegans*

Tested material and concentration (mg mL ⁻¹)	Mean lifespan ±SEM (day)	Median lifespan ±SEM (day)	Change (%)	P
Control (0)	19.01 ± 0.04	23.38 ± 0.63		
LFOs (2.5)	21.02 ± 0.10	24.63 ± 0.48	10.6	<0.01
LFOs (5)	21.27 ± 0.15	25.50 ± 0.71	11.9	<0.01
LFOs (10)	22.66 ± 0.18	28.87 ± 0.31	19.2	<0.01
LFOs (20)	23.26 ± 0.08	29.61 ± 0.41	22.4	<0.01
HFOs (2.5)	20.55 ± 0.11	23.00 ± 0.42	8.1	<0.01
HFOs (5)	20.67 ± 0.19	23.88 ± 0.25	8.7	<0.01
HFOs (10)	21.88 ± 0.10	28.40 ± 0.50	15.1	<0.01
HFOs (20)	22.11 ± 0.13	29.13 ± 0.48	16.3	<0.01

Note: Data are expressed as mean ± SEM. All statistical comparisons were conducted against the untreated control group (0 mg mL⁻¹). Change: Percentage increase in lifespan relative to the mean lifespan of the control group. *P* < 0.01 indicates a highly significant difference compared with the control group.

While no difference was observed on day 2, LFO-treated worms grew faster than the HFO group by day 5, and this disparity further increased by day 8, with the LFO group attaining the greatest body length.

LFOs more effectively promoted growth and development than HFOs, and the positive correlation between increased body length and extended lifespan suggests that these traits are linked. LFOs outperformed HFOs primarily because of their lower DP. This structural feature improves bioavailability through reduced steric hindrance and higher solubility. Consequently, it promotes more efficient uptake and activation of antioxidant pathways in *C. elegans*, consistent with the documented higher bioactivity of lower-DP oligosaccharides (Wu et al., 2025).

3.4.2. Oxidative stress resistance and thermotolerance assay. FOs enhanced thermotolerance, prolonging survival beyond 7 h with LFOs showing viability until 9 h (Fig. 5A). Under oxidative stress, LFOs acted more rapidly and extended lifespan more effectively than HFOs (Fig. 5C).

FOs significantly enhanced both oxidative and thermal stress resistance, with LFOs outperforming HFOs. This is likely because their lower DP reduces steric hindrance and improves cellular uptake (Liu et al., 2020). The lower DP may allow greater exposure of ferulic acid, enhancing its role as a hydrogen donor for scavenging intracellular ROS and thus providing more efficient protection.

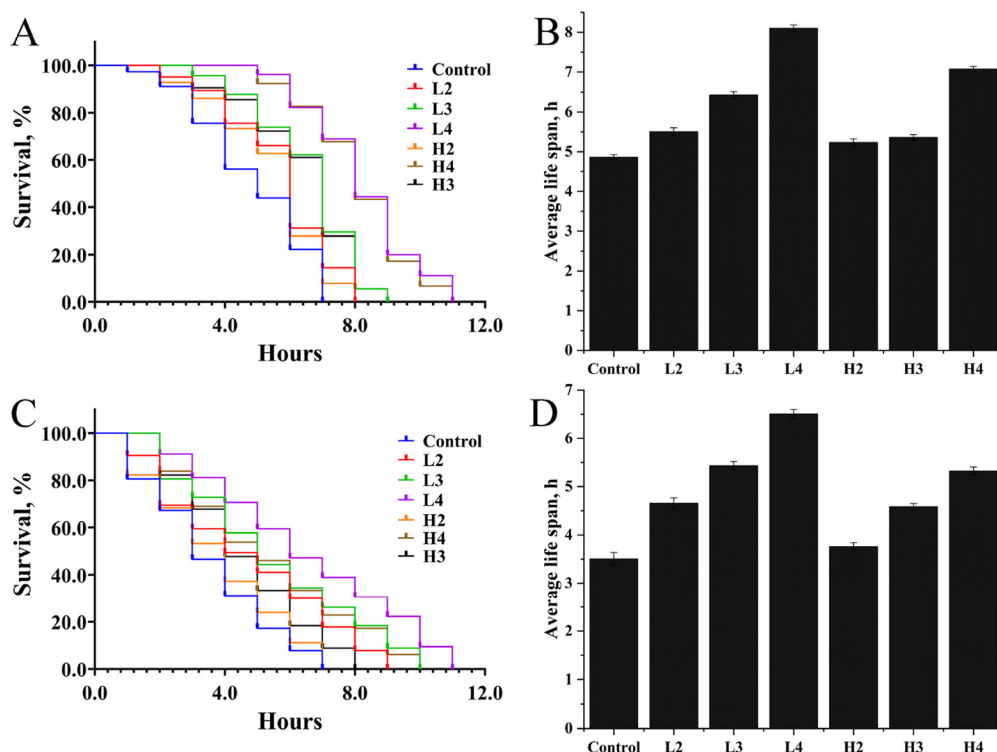


Fig. 5. Effects of feruloylated oligosaccharides (FOs) on thermotolerance and oxidative stress resistance in *C. elegans*. (A) Survival curves and (B) mean lifespan in the thermotolerance assay. (C) Survival curves and (D) mean lifespan in the oxidative stress assay

3.4.3. Effects of LFOs and HFOs on antioxidant enzyme activities and MDA content in *C. elegans*. To investigate the effects of FOs on antioxidant defense, the activities of SOD, CAT, and GSH-Px, as well as MDA levels, were measured.

Figure 6A–C show that treatment with FOs significantly increased the activities of antioxidant enzymes in *C. elegans*. Specifically, at a concentration of 20.00 mg mL⁻¹, LFOs and HFOs increased SOD activity by 3.09-fold ($P < 0.01$) and 2.74-fold ($P < 0.01$), CAT activity by 3.21-fold ($P < 0.01$) and 2.75-fold ($P < 0.01$), and GSH-Px activity by 33.21% ($P < 0.01$) and 17.16% ($P < 0.01$), respectively. As shown in Fig. 6D, treatment with 20 mg mL⁻¹ FOs significantly reduced oxidative product accumulation: LFOs and HFOs lowered MDA content by 27.78% ($P < 0.01$) and 20.20% ($P < 0.05$), respectively.

FOs enhanced antioxidant defence and reduced oxidative damage, thereby preserving cellular function and extending lifespan (Wang et al., 2025). LFOs showed stronger effects than HFOs in upregulating enzyme activities and reducing MDA levels, indicating the superior potency of lower-DP fractions. This advantage stems from their smaller size, which improves absorption and bioactivity (Zeng et al., 2021). In summary, LFOs outperformed HFOs by

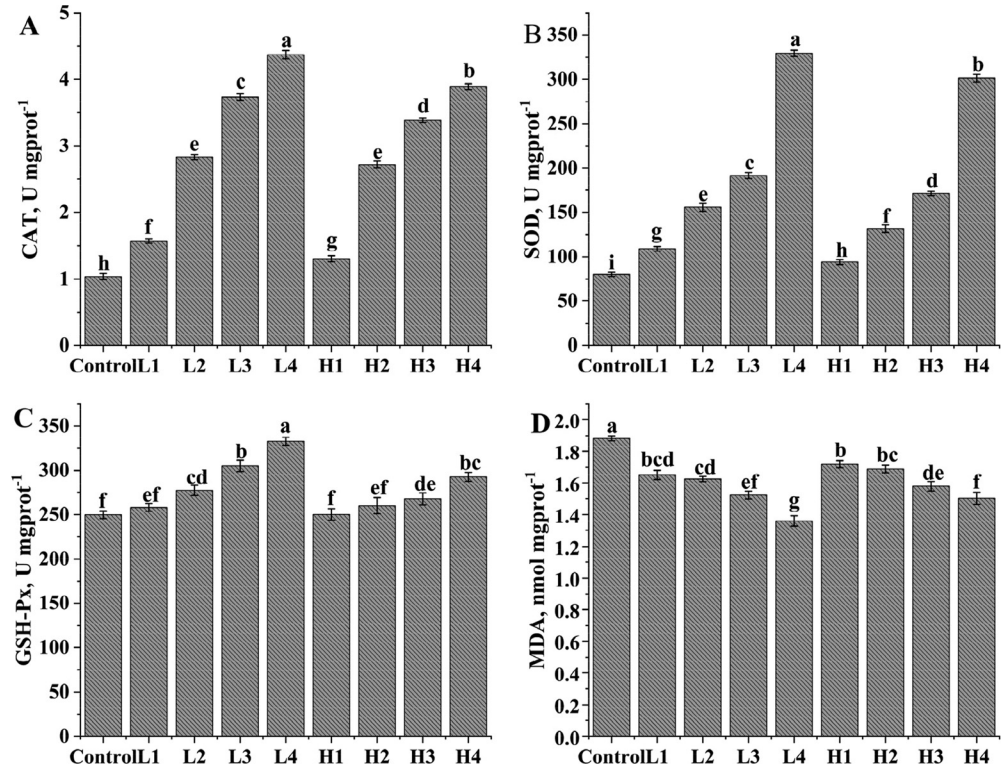


Fig. 6. Effects of feruloylated oligosaccharides LFOs and HFOs on antioxidant enzyme activities and MDA content of *C. elegans*. (A) CAT activity, (B) SOD activity, (C) GSH-Px activity, and (D) MDA content. For the same parameter, identical labelled letters indicate non-significant differences ($P > 0.05$), and different labelled letters indicate significant differences ($P < 0.05$)

dose-dependently enhancing the endogenous antioxidant system and inhibiting lipid peroxidation, demonstrating their greater potential to delay ageing and mitigate oxidative damage.

4. CONCLUSIONS

This study prepared two FOs with distinct DPs and examined their antioxidant and anti-ageing effects. LFOs consisted mainly of DP 2–5, while HFOs were primarily DP 6. *In vitro*, LFOs showed better electron transfer ability, due to their smaller size and greater active-site exposure. In *C. elegans*, LFOs showed better electron transfer ability. This enhanced capacity is likely attributable not only to their smaller size and greater active-site exposure, but potentially also to the differences in their specific monosaccharide compositions and linkage patterns. In *C. elegans*, LFOs and HFOs extended lifespan, promoted growth, and improved stress tolerance. LFOs more effectively activated the endogenous antioxidant system, elevating SOD, CAT, and GSH-Px activities and reducing MDA. These results suggest that a lower DP, alongside corresponding structural features, may play a crucial role in facilitating bioavailability and bioactivity, supporting the potential of low-DP FOs in developing anti-ageing functional foods.

ACKNOWLEDGEMENTS

This research was financed under the National Natural Science Foundation of China (grant number 32402007) and the Natural Science Foundation of Jiangsu Province for Youths (grant number BK20241081).

LIST OF ABBREVIATIONS

ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FOs	Feruloyl oligosaccharides
FA	Ferulic acid
DP	Degree of polymerisation
HFOs	High-degree of polymerisation feruloyl oligosaccharides
LFOs	Low-degree of polymerisation feruloyl oligosaccharides
SOD	Superoxide dismutase
MDA	Malondialdehyde
TP	Total protein
GSH-Px	Glutathione peroxidase
CAT	Catalase
ROS	Reactive oxygen species
<i>C. elegans</i>	<i>Caenorhabditis elegans</i>
FUDR	5-Fluoro-2'-deoxyuridine
<i>E. coli</i> OP50	<i>Escherichia coli</i> OP50
CGC	Caenorhabditis Genetics Center

ET	Electron transfer
Gal	Galactose
GlcA	Glucuronic acid
Glu	Glucose
Man	Mannose
Rha	Rhamnose
Xyl	Xylose
Ara	Arabinose
FT-IR	Fourier transform infrared spectroscopy
HPLC	High-performance liquid chromatography
MS	Mass spectrometry
PMP	1-Phenyl-3-methyl-5-pyrazolone
UV-Vis	Ultraviolet–visible spectroscopy
NGM	Nematode growth medium
HAT	Hydrogen atom transfer

REFERENCES

Bian, Z., Li, C., Peng, D., Wang, X., and Zhu, G. (2022). Use of steaming process to improve biochemical activity of *Polygonatum sibiricum* polysaccharides against D-galactose-induced memory impairment in mice. *International Journal of Molecular Sciences*, 23(19): 11220.

Chen, K., Shi, L., Ren, Z., and Weng, W. (2023). Antioxidant characteristics of hydrolysate from low-value sea cucumber: *in vitro* and *in vivo* activities of *Caenorhabditis elegans*. *Food Chemistry: X*, 19: 100836.

Chu, Q., Xie, S., Wei, H., Tian, X., Tang, Z., Li, D., and Liu, Y. (2024). Enzyme-assisted ultrasonic extraction of total flavonoids and extraction polysaccharides in residue from *Abelmoschus manihot* (L). *Ultrasonics Sonochemistry*, 104: 106815.

Colquhoun, I.J., Ralet, M.-C., Thibault, J.-F., Faulds, C.B., and Williamson, G. (1994). Structure identification of feruloylated oligosaccharides from sugar-beet pulp by NMR spectroscopy. *Carbohydrate Research*, 263(2): 243–256.

Gao, F., Zhang, Z., Xue, N., Ma, Y., Jiao, J., Wang, C., Zhang, K., Lin, Y., Li, S., and Guo, Z. (2024). Identification of a novel oligopeptide from defatted walnut meal hydrolysate as a potential neuro-protective agent. *Food & Function*, 15(10): 5566–5578.

Gong, L., Feng, D., Liu, J., Yu, Y., and Wang, J. (2022). Ionic liquid depolymerize the lignocellulose for the enzymatic extraction of feruloylated oligosaccharide from corn bran. *Food Chemistry: X*, 15: 100381.

He, Z., Liu, S., Zhao, W., Yin, M., Jiang, M., and Bi, D. (2022). Comparative assessment of proportions of urea in blend for nitrogen-rich pyrolysis: characteristics and distribution of bio-oil and biochar. *ACS Omega*, 8(1): 1232–1239.

Kwon, H.C. and Lee, S.-J.V. (2025). Brief guide to *Caenorhabditis elegans* survival assays. *Molecules and Cells*, 48(8): 100232.

Lessel, C.E., Parkes, T.L., Dickinson, J., and Merritt, T.J. (2017). Sex and genetic background influence superoxide dismutase (cSOD)-Related phenotypic variation in *Drosophila melanogaster*. *G3 Genes|Genomes|Genetics*, 7(8): 2651–2664.

- Liu, H., Sun, X.-Y., Wang, F.-X., and Ouyang, J.-M. (2020). Regulation on calcium oxalate crystallization and protection on HK-2 cells of tea polysaccharides with different molecular weights. *Oxidative Medicine and Cellular Longevity*, 2020(1): 5057123.
- López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., and Kroemer, G. (2023). Hallmarks of aging: an expanding universe. *Cell*, 186(2): 243–278.
- Lu, X., Liu, L., Zhang, H., Lu, H., Tian, T., Du, B., Li, P., Yu, Y., Zhou, J., and Lu, H. (2024). High-yield expressed human ferritin heavy-chain nanoparticles in *K. marxianus* for functional food development. *Foods*, 13(18): 2919.
- Sissi, S., Di Giacomo, S., Ferrante, C., Angelini, P., Macone, A., Giusti, A.M., Toniolo, C., Vitalone, A., Abdellah, A., and Larhsini, M. (2022). Characterisation of the phytochemical composition and bioactivities of *Anacyclus maroccanus* Ball. and *Anacyclus radiatus* Loisel aerial parts: preliminary evidence for the possible development of Moroccan plants. *Molecules*, 27(3): 692.
- Wang, M., Yu, J., Xu, H., Xu, K., Sohail, M., Wang, J., Ning, H., Zhang, J., and Yu, B. (2025). Recent research advances in multi-functional diallyl trisulfide (DATS): a comprehensive review of characteristics, metabolism, pharmacodynamics, applications, and drug delivery systems. *International Journal of Nanomedicine*, 2025(20): 4381–4398.
- Wang, S., Chen, C., and Lu, Y. (2023). Epigallocatechin-3-gallate reduces Cd-induced developmental toxicity of bodysize in *Caenorhabditis elegans* via the REK-1/eIF-2 α /ATF-4 pathway. *Molecules*, 28(17): 6344.
- Wang, W., Zhao, Y., He, L., Song, Z., Shi, C., Jia, P., Yu, Q., and Han, L. (2024). Cowhide gelatin peptide as a source of antioxidants for inhibiting the deterioration of pudding quality during storage. *Food Chemistry: X*, 22: 101327.
- Wei, Q., Zhong, X., Haruna, M.H., Liu, S., Zhou, F., and Chen, M. (2022). Evaluation of different agricultural wastes for the production of polysaccharides from *Oudemansiella raphanipes* and its antioxidant properties. *Food Science & Nutrition*, 10(10): 3444–3452.
- Wu, Q., Wang, Q., Luo, X., Jin, P., Jin, M., Hussain, S., Qi, Y., Mo, J., Yu, Y., and Shao, H. (2025). Glucosamine activates intestinal P-glycoprotein inhibiting drug absorption. *Nature Communications*, 16(1): 6100.
- Yin, Z., Liu, M., Wang, B., Zhao, D., Li, H., and Sun, J. (2024). Extraction, identification, and *in vitro* anti-inflammatory activity of feruloylated oligosaccharides from Baijiu distillers' grains. *Foods*, 13(8): 1283.
- Yu, X., Zhu, X., Lin, X., Li, F., and Gu, Z. (2016). Effects of two-stage controlled pH and temperature vs. one-step process for hemicellulase biosynthesis and feruloyl oligosaccharide fermentation using *Aureobasidium pullulans*. *Bioresources*, 11(2): 5113–5123.
- Zeng, F., Lai, M., Li, Q., Zhang, H., Chen, Z., Gong, S., Liu, X., and Liu, B. (2023). Anti-oxidative and anti-aging effects of mannoprotein-rich yeast cell wall enzymatic hydrolysate by modulating gut microbiota and metabolites in *Caenorhabditis elegans*. *Food Research International*, 170: 112753.
- Zeng, L., Li, J., Cheng, Y., Wang, D., Gu, J., Li, F., and Han, W. (2021). Comparison of biochemical characteristics, action models, and enzymatic mechanisms of a novel exolytic and two endolytic lyases with mannuronate preference. *Marine Drugs*, 19(12): 706.
- Zhang, G., Zhong, Y., Zhang, X., Wang, Y., Sun, Y., Li, X., Liu, Z., and Liang, J. (2024a). Flavor characteristics, antioxidant activity and *in vitro* digestion properties of bread with large-leaf yellow tea powder. *Foods*, 13(5): 715.
- Zhang, X., Chen, Q., Chen, L., Chen, X., and Ma, Z. (2024b). Anti-aging in *Caenorhabditis elegans* of polysaccharides from *Polygonatum cyrtoneura* Hua. *Molecules*, 29(6): 1276.

- Zhao, H., Li, J., Zhang, J., Wang, X., Hao, L., and Jia, L. (2017). Purification, *in vitro* antioxidant and *in vivo* anti-aging activities of exopolysaccharides by *Agrocybe cylindracea*. *International Journal of Biological Macromolecules*, (102): 351–357.
- Zhao, W., Chen, H., Wu, L., Ma, W., and Xie, Y. (2018). Antioxidant properties of feruloylated oligosaccharides of different degrees of polymerisation from wheat bran. *Glycoconjugate Journal*, 35(6): 547–559.

Open Access statement. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium for non-commercial purposes, provided the original author and source are credited, a link to the CC License is provided, and changes – if any – are indicated.