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Research on the Drying Process and Nutritional and Functional Activities of Walnut Meal Powder with High Added Value

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ABSTRACT

The optimal drying process for walnut meal was determined, and the antioxidant and lipid-lowering functions of walnut meal powder extracts were evaluated to achieve high-value utilization of walnut meal. Walnut meal after desalination was dried using low-temperature hot air drying, microwave drying, and vacuum freeze drying, followed by sieving through a 60-mesh sieve. The vacuum freeze drying process was identified as the optimal drying method based on the determination of polyphenol, flavonoid, polysaccharide, and protein contents, with polyphenol content at 22.88 mg/g, flavonoid content at 0.71 mg/g, polysaccharide content at 16.37 mg/g, and protein content at 91.7 mg/g. Using this as the raw material, the antibacterial circles of the water and alcohol extracts of walnut meal powder against Escherichia coli OP50 were determined. The effects of water and alcohol extracts of walnut meal powder at concentrations of 1.25 mg/mL, 2.5 mg/mL, and 5 mg/mL on the malondialdehyde (MDA) content, superoxide dismutase (SOD) activity, glutathione peroxidase (GSH-PX) activity, catalase (CAT) activity, triglyceride (TG) content, oil red O staining, and optical density of Caenorhabditis elegans (C. elegans) were evaluated to assess their antioxidant capacity and lipid-lowering effects. The results showed that within the concentration range of 1.25 mg/mL to 5 mg/mL, both the water and alcohol extracts of walnut meal powder could increase the activities of SOD, CAT, and GSH-PX in C. elegans, reduce the content of the oxidative product MDA ($P<0.05$), and decrease the TG content in C. elegans, thereby reducing fat deposition. Moreover, the antioxidant capacity and lipid-lowering effects increased with increasing dosage, with the alcohol extract of walnut meal powder exhibiting higher activity than the water extract.

KEYWORDS

Drying process; Walnut meal powder; Nutrition; Functional activity; High value utilization

1. INTRODUCTION

Walnut, a plant of significant economic value [1], has been historically utilized in traditional medicine for the treatment of various ailments including microbial infections, cancer, cardiovascular diseases, and sinusitis [2]. Walnut meal, the residue left after the extraction of walnut oil from walnut kernels, yields a minimum of 2.227 million tons annually [3]. Rich in nutrients, walnut meal contains elevated levels of proteins, carbohydrates, polysaccharides, polyphenols, flavonoids, and other health-promoting constituents. Presently, walnut meal remains underutilized and lacks diversified applications, predominantly serving as animal feed or fertilizer. Some efforts have been directed towards extracting active components such as polysaccharides and polyphenols from walnut meal for functional studies [4-6]. Additionally, a small fraction has found application in the fermentation industry due to its high protein content, exemplified by the emergence of walnut meal soy sauce [7,8]. Thus, there exists a pressing need for research into the high-value utilization of walnut meal to enhance the value chain of the walnut industry and better serve public health.

The seed coat of walnuts contains abundant tannins and polyphenolic compounds. Due to the reduction in oil content, walnut meal exhibits an increased proportion of seed coats, leading to a more astringent taste and diminished culinary value. Additionally, after pressing, walnut meal surfaces tend to accumulate oil, making them prone to oxidation. Therefore, a crucial step in walnut meal processing involves weak alkaline washing. Presently, alkaline methods for deseeding and de-oiling have been extensively researched, aiming to retain phenolic compounds to the maximum extent while reducing oxidation of oils, fatty acids, and unsaturated fatty acids, thereby preserving the quality of walnuts [9]. This approach generates no hazardous wastewater and helps maintain a certain level of walnut quality [10].

After washing, walnut meal requires drying to decrease its moisture content and extend its shelf life. Hot air drying, widely used in industrial drying due to its low microbial contamination risk, low investment costs, and better control over drying conditions, is commonly employed for dehydrating food and agricultural products [11]. Vacuum freeze-drying technology, which dehydrates materials directly from a solid to a gas state under low temperature and low oxygen conditions, maximally preserves the original structure and shape of the material [12]. Microwave drying technology, which utilizes electromagnetic waves to induce high-frequency vibration of polar molecules within the drying material, primarily causing rapid internal water molecule movement and frictional heat generation, uniformly heats both the internal and external parts of the material, thus achieving drying [13].These technologies have been extensively applied in oilseed crops such as peanuts [14], soybeans [15], and walnuts [16, 17]. Since the 1960s, the nematode Caenorhabditis elegans, as a model organism, has been found to harbor a wealth of biological and genetic information. In recent years, its applications have become increasingly widespread, including the establishment of disease models to simulate human diseases, drug development, and the investigation of targets for active substances. As one of the important model organisms, C. elegans has attracted growing attention. Consequently, there is a growing trend in utilizing Caenorhabditis elegans as a model organism for research applications. Yet, there is currently no research using Caenorhabditis elegans as a model to investigate the antioxidant and lipid-lowering functions of walnut meal powder.

This study investigates the effects of three different drying methods, namely low-temperature hot air drying, microwave drying, and vacuum freeze-drying, on walnut meal powder after deseeding. Untreated walnut meal serves as the control. The study examines the impact of different drying methods on the nutritional composition of walnut meal powder. Furthermore, water and alcohol extracts of walnut meal powder after vacuum freeze-drying are evaluated for their antioxidant and lipid-lowering functions using Caenorhabditis elegans as a biological model. The optimal drying method for walnut meal is explored, and its functional properties are assessed, aiming to provide theoretical and experimental evidence for the broader application of walnut meal based on this research foundation.

2. MATERIALS AND METHODS

2.1. Materials

The newly squeezed walnut meal was provided by Hebei Lvling Fruit Industry Co., Ltd.

2.2. Experimental

2.2.1. Drying and crushing treatment of walnut meal

Hand-layer the whole walnut meal into single-layer sheets, removing severely oxidized portions. Soak in 0.01% Na_2CO_3 solution at a ratio of 1:8 (meal to solution) for 2 minutes to remove astringency. Then, employ three drying methods: low-temperature hot air drying at 50℃ for 9 hours and 20 minutes (LOW-HAD), microwave drying at 560W for 10 minutes and 45 seconds (MD), and vacuum

freeze-drying for 27 hours (VFD), until the moisture content is below 0.6% to prevent mold growth. Pass the walnut meal dried by the three methods through a 60-mesh sieve. Untreated walnut meal (UT) passed through a 60-mesh sieve serves as the control to investigate the effects of deseeding on its properties.

2.2.2. Analysis and determination of nutritional functional components

(1) Protein content

Following the method described by Di Hongyan et al. [18]. The protein content was determined using the Coomassie Brilliant Blue method. 2g of walnut meal powder was taken, and extraction was performed using a solvent of NaOH solution with a pH of 9, at a liquid-to-material ratio of 1:25. Ultrasonication was carried out under conditions of 270W and 55℃ for 30 minutes. After centrifugation at 5000 rpm for 20 minutes at 4℃, the supernatant was collected. A 1 mL sample of the supernatant, diluted 25 times with distilled water, was mixed with 5 mL of Coomassie Brilliant Blue solution and allowed to stand for 5 minutes. The absorbance was measured at 595 nm. A standard curve was generated using different concentrations of bovine serum albumin standard solution (μg/mL) as the X-axis and the absorbance (A) as the Y-axis. The linear regression equation obtained was: Y = 0.0056X - 0.003, with an R^2 value of 0.9997. Based on the standard curve and the absorbance of the samples, the protein content was calculated using the formula (1).

$$W_1=C_1*V_1*N_1/M_1/1000 \quad (1)$$

In the formula: W_1 is the protein content, mg/g; C_1 is the concentration of the sample, μg/mL; V_1 is the volume of the extraction solution, mL; N_1 is the dilution factor; M_1 is the sample mass, g.

(2) Polyphenol content

Following the method described by Su et al. [19], with appropriate modifications, the total phenolic content in walnut meal was determined using the Folin-Ciocalteu colorimetric method. Briefly, 2 grams of walnut meal were extracted with 50% ethanol solution at a liquid-to-material ratio of 1:20 under ultrasonic conditions (585W, 50℃) for 70 minutes. After centrifugation at 4000 rpm for 20 minutes at 4℃, the supernatant was collected and diluted 25 times with distilled water, from which 1 mL was sampled. Subsequently, 2.5 mL of 10% Folin-Ciocalteu reagent was added to the sample, mixed thoroughly, and allowed to react in the dark for 5 minutes. Following this, 2 mL of 7.5% Na_2CO_3 solution was added, mixed, and incubated in a water bath at 37℃ in the dark for 1 hour. The absorbance was measured at 765 nm. A standard curve was constructed using different concentrations of gallic acid standard solutions (μg/mL) as the X-axis and absorbance (A) as the Y-axis. The linear regression equation obtained was Y=0.0168X+0.0194, with an R^2 value of 0.9993. Based on the standard curve and the absorbance of the samples, the polyphenol content was calculated using the formula (2).

$$W_2=C_2*V_2*N_2/M_2/1000 \quad (2)$$

In the formula: W_2 is the content of polyphenols, mg/g; C_2 is the concentration of the sample, μg/mL; V_2 is the volume of the extraction solution, mL; N_2 is the dilution factor; M_2 is the sample mass, g.

(3) Flavonoids content

Referring to Shi, Y. et al. [20], the determination of flavonoid content was conducted using the sodium nitrite-aluminum nitrate method. Two grams of walnut meal powder were extracted with a solvent consisting of a 60% ethanol solution at a ratio of 1:30 (meal to solvent). The extraction was performed under ultrasonic conditions at 630W and 40℃ for 40 minutes. After centrifugation at 4000 rpm for 20 minutes at 4℃, the supernatant was collected. One milliliter of the supernatant was taken and diluted to 5.0 mL with a 60% ethanol solution. Subsequently, 0.5 mL of 5% NaNO2 solution was

added and mixed well, followed by 6 minutes of incubation. Then, 0.5 mL of 10% Al $(NO_3)_3$ solution was added, mixed well, and incubated for another 6 minutes. Afterward, 4 mL of 4% NaOH solution was added, mixed well, and incubated for 15 minutes. The absorbance was measured at 510 nm. A linear regression equation was obtained by plotting the absorbance (A) against different concentrations of rutin standard solution (mg/mL), yielding the equation Y = 20.374X - 0.0045, with an R^2 value of 0.9998. Based on the standard curve and the absorbance of the samples, the flavonoids content was calculated using the formula (3).

$$W_3=C_3*V_3*N_3/M_3 \quad (3)$$

In the formula: W_3 represents the flavonoid content, mg/g; C_3 is the concentration of the sample, mg/mL; V_3 is the volume of the extraction solution, mL; N_3 is the dilution factor; M_3 is the sample mass, g.

(4) Polysaccharide content

Referring to Yan, X. et al. [21], the determination of polysaccharide content was carried out using the anthrone-sulfuric acid method. Two grams of walnut meal powder were extracted with water as the solvent at a ratio of 1:25 (meal to solvent). The extraction was performed under ultrasonic conditions at 558W and 58 ℃ for 60 minutes. After centrifugation at 5000 rpm for 20 minutes at 4 ℃, the supernatant was collected. One milliliter of the sample, diluted 15-fold with distilled water, was taken and mixed with 0.2% anthrone-sulfuric acid. The mixture was heated in a boiling water bath for 5 minutes and then cooled in an ice water bath for 5 minutes. The absorbance was measured at 620 nm. By plotting the absorbance (A) against different concentrations of glucose standard solution (μg/mL), a linear regression equation was obtained: Y = 0.0288X + 0.1018, with an R^2 value of 0.999. Based on the standard curve and the absorbance of the samples, the polysaccharide content was calculated using the formula (4).

$$W_4=C_4*V_4*N_4/M_4/1000 \quad (4)$$

In the formula: W_4 is the polysaccharide content, mg/g; C_4 is the concentration of the sample, μg/mL; V_4 is the volume of the extraction solution, mL; N_4 is the dilution factor; M_4 is the sample mass, g.

2.2.3. Preparation of water and alcohol extracts of walnut meal powder

The walnut meal powder was extracted at a ratio of 1:40 (meal to solvent) under the following conditions: ultrasonic temperature of 50 ℃, ultrasonic power of 234 W, and using either distilled water or a 50% ethanol solution as the solvent for a duration of 60 minutes. After extraction, the extraction solution was centrifuged at 8000 rpm for 15 minutes to collect the supernatant, discarding the precipitate. The supernatant was then subjected to vacuum concentration and freeze-drying at approximately -80 ℃ for 50 hours, followed by overnight freezing at -20 ℃ until complete freezing. The dried powder was collected from the centrifuge tubes and stored in a -20 ℃ freezer for further use.

2.2.4. Determination of antibacterial zone diameter of freeze-dried walnut meal extract

The freeze-dried water extract of walnut meal powder (WEW) and alcohol extract of walnut meal powder (AEW) were serially diluted with sterile physiological saline to concentrations of 80, 40, 20, 10, 5, 2.5, 1.25, and 0.625 mg/mL. The diluted solutions were then filtered through a 0.45 μm aqueous phase filter membrane. Ampicillin was diluted with sterile physiological saline to a concentration of 0.1 mg/mL. The solutions were stored at 4 ℃ in the refrigerator and used immediately upon preparation.

Using the agar diffusion method as previously described [22], with some modifications, activated OP50 was diluted with sterile water to a 0.5 McFarland standard turbidity, resulting in a bacterial

concentration of approximately 1.5×10^8 CFU/mL. In each 250 mL of sterilized TSA agar medium, 2 mL of the bacterial suspension was added to prepare the inoculated agar plates, with approximately 15-20 mL poured into each 90 mm diameter Petri dish. Blank control plates without bacterial suspension were also prepared. Filter paper discs (6 mm in diameter) containing 5 μL of the sample solution were placed on the solidified TSA agar surface. Sterile PBS served as the negative control, while ampicillin at a concentration of 0.1 mg/mL served as the positive control. The inoculated plates were then inverted and incubated at 37 ℃ for 18-24 hours. After incubation, plates displaying zones of inhibition were singled out, and the diameter of the inhibition zones was measured using a cross technique.

2.2.5. Simultaneous cultivation of Caenorhabditis elegans

Approximately 30 nematodes in the egg-laying stage were selected under a dissecting microscope and transferred using a platinum wire pick to another NGM agar plate seeded with OP50. The time and number of transferred nematodes were recorded. After egg laying for 1-2 hours under 20 ℃ conditions, the nematodes were removed. These picked nematodes could either be transferred back to their original plate or continue to be transferred to new plates to repeat the process. The agar plates containing eggs were then inverted and placed in a 20 ℃ constant temperature and humidity incubator for approximately 3 days, with regular observations. After about 3 days of incubation, the eggs developed into the L4 larval stage. At this stage, the nematodes were synchronized in terms of growth and were ready for experimental use.

2.2.6. Administration route

Take 100 μL of the walnut meal extract solution at the corresponding concentration and filter it through a 0.45 μm sterile water membrane filter to remove bacteria. Prior to seeding with OP50 Escherichia coli, evenly spread the filtered solution onto NGM agar plates with a diameter of 90 mm using a spreader. Under these conditions, the sample solution will be ingested by the nematodes during their crawling and feeding process.

2.2.7. Dose grouping of walnut meal extract

Combining with the inhibition zone experiment, grouping was determined within a concentration range (0.125-5 mg/mL) that would not inhibit OP50. The experiment was divided into four groups: blank control, low dose (1.25 mg/mL), medium dose (2.5 mg/mL), and high dose (5 mg/mL). After pouring NGM agar into each group's petri dishes, sterile water or WEW or AEW at the three concentrations were evenly spread onto the surface of the agar using a spreader with 100 μL each. After the liquid had penetrated the agar, 100 μL of OP50 Escherichia coli was uniformly spread onto the agar surface. Before starting the experiment, the required agar plates could be prepared in bulk 1-2 days in advance according to the daily consumption, and stored in a refrigerator at 4 ℃ for subsequent use.

2.2.8. Determination of antioxidant capacity in walnut meal extract

The experiment was conducted over a period of 3 days with daily transfer of nematodes to fresh plates. After the third transfer, nematodes from each group on the agar plates were washed three times with ice-cold sterile M9 buffer and collected into EP tubes. The collected nematodes in the EP tubes were centrifuged at 1500 rpm for 3 minutes at 4 ℃, resulting in nematode pellets at the bottom of the tubes. After carefully removing the supernatant, the nematode pellets were weighed. The nematode pellets were resuspended in sterile PBS at a ratio of 1:9 (nematode mass: PBS volume), and grinding beads were added. The suspension was homogenized using a low-temperature tissue homogenizer, followed by centrifugation at 2500 rpm for 10 minutes. The supernatant was collected and stored at 4 ℃, constituting a 10% volume fraction nematode homogenate. Enzyme activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), as well as malondialdehyde (MDA) content in the nematode homogenate, were determined according to the instructions provided with the assay kits for C. elegans. Additionally, protein quantification was standardized using a BCA

assay kit. The nematode homogenate prepared on the same day was used for experiments, and protein content was re-measured before each assay to ensure data accuracy.

2.2.9. Determination of lipid-lowering ability of walnut meal extract in vivo

Triglyceride (TG) determination: The pre-treatment is the same as above, and the TG content in the homogenate of Caenorhabditis elegans was determined according to the instructions.

Oil Red O Dyeing [23] and Optical Density Determination: Nematodes were transferred from M9 buffer to centrifuge tubes, followed by centrifugation and removal of the supernatant. Then, 500 μL of 60% isopropanol was added, and centrifugation was performed at 4000 rpm for 3 minutes. After discarding the supernatant, 200 μL of Oil Red O staining solution was added, and the reaction was carried out in the dark at room temperature (25 ℃) for 10 minutes. Subsequently, the tubes were centrifuged, the supernatant was discarded, and the nematodes were washed with M9 buffer. This washing process was repeated three times. Next, 250 μL of 0.01% Triton-X100 was added, and images were captured under a microscope.

The stained nematode suspension was centrifuged, the supernatant was discarded, and M9 buffer was added to a new centrifuge tube. Centrifugation was performed at 5000 rpm for 1 minute, repeated three times, and the lower nematode bodies were collected. These nematode bodies were treated with 200 μL of absolute ethanol for decolorization, with gentle shaking for 5 minutes. After centrifugation for 1 minute, the supernatant was collected, and absorbance was measured at 510 nm to assess lipid-lowering effects, using the OD value of decolorized Oil Red O dye in nematodes as an indicator.

2.3. Statistical Analysis

All experimental data were collected in triplicate and expressed as mean ±standard deviation. A one-way variance analysis (ANOVA) was performed using SPSS Statistics 21.0 (IBM Corp., NY, USA) to compare the mean values. Statistical significance was considered at the $P < 0.05$ probability level. The drawing software uses Origin 2023b.

3. RESULTS

3.1. The effect of Different Drying Methods on the Nutritional and Functional Components of Walnut Meal Powder

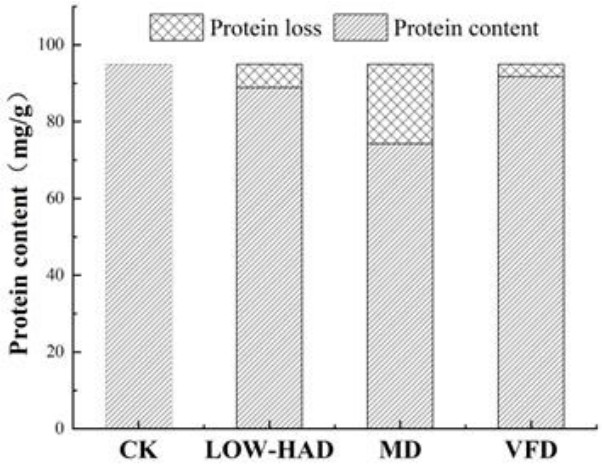

Figure 3-1. Protein content and loss of walnut meal powder under different drying methods

The protein content of walnut meal powder dried by different methods was not significantly reduced compared to (UT) samples ($P < 0.05$), as shown in Figure 3-1. The protein content of walnut meal powder dried by three different methods was as follows: LOW-HAD, 88.89 mg/g; MD, 74.24 mg/g;

VFD, 91.70 mg/g. The protein content ranked from highest to lowest was VFD > LOW-HAD > MD, with protein loss as follows: LOW-HAD, 6.04 mg/g; MD, 20.69 mg/g; VFD, 3.23 mg/g. The protein loss ranked from highest to lowest was MD > LOW-HAD > VFD. Different drying methods had varying effects on the protein content of walnut meal powder. LOW-HAD resulted in compact tissue and protein denaturation, leading to lower protein content. The high-temperature treatment during MD drying induced Maillard reaction in walnut meal, imparting a distinct roasted walnut aroma, and causing protein denaturation and subsequent protein loss, with MD exhibiting the highest protein loss.

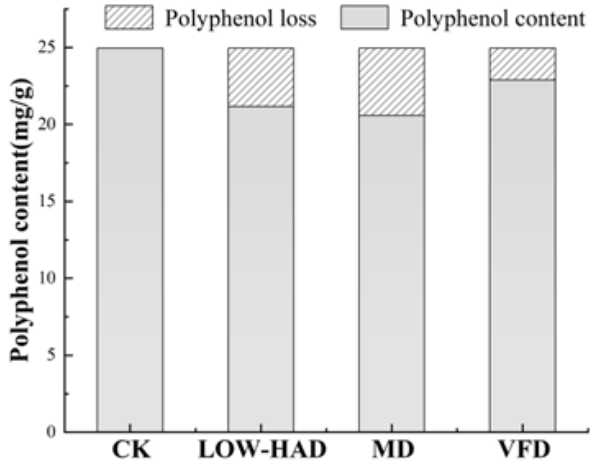

Figure 3-2. Polyphenol content and loss of walnut meal powder under different drying methods

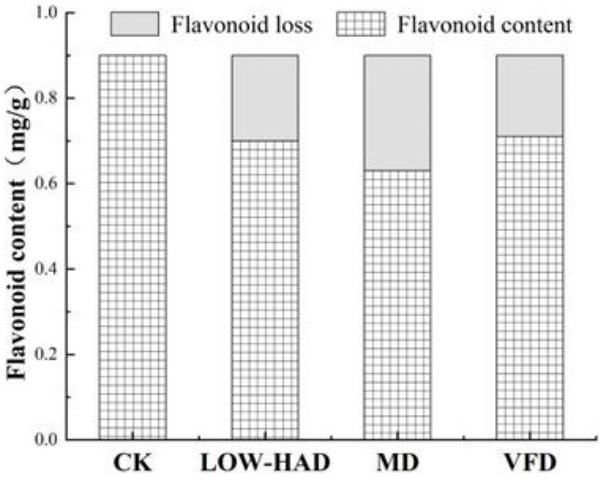

Figure 3-3. Flavonoids content and loss of walnut meal powder under different drying methods

The polyphenol and flavonoid contents of walnut meal powder dried by different methods were significantly reduced compared to untreated samples ($P < 0.05$), as shown in Figures 3-2 and 3-3. This reduction is attributed to the removal of some polyphenolic substances during the cleaning process. The polyphenol contents of walnut meal powder dried by three different methods were as follows: LOW-HAD, 21.15 mg/g; MD, 20.59 mg/g; VFD, 22.88 mg/g. The corresponding losses of polyphenols were LOW-HAD, 3.81 mg/g; MD, 4.37 mg/g; VFD, 2.08 mg/g. The flavonoid contents were LOW-HAD, 0.7 mg/g; MD, 0.63 mg/g; VFD, 0.71 mg/g, with losses of flavonoids of LOW-HAD, 0.2 mg/g; MD, 0.27 mg/g; VFD, 0.19 mg/g. The order of polyphenol and flavonoid contents from highest to lowest was VFD > LOW-HAD > MD, while the order of losses was MD > LOW-HAD > VFD. The differences in polyphenol and flavonoid contents resulting from different drying methods can be attributed to the low temperature and vacuum conditions of VFD, which effectively inhibit their oxidation, reduce the conversion of polyphenols and flavonoids, and mitigate their degradation.

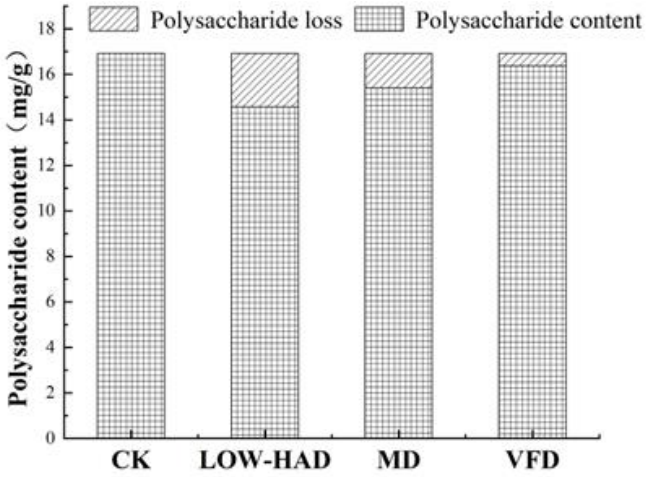

Figure 3-4. Polysaccharide content and loss of walnut meal powder under different drying methods

The polysaccharide content of walnut meal powder dried by different methods, except for VFD, was significantly reduced compared to untreated samples ($P < 0.05$), as shown in Figure 3-4. The polysaccharide contents of walnut meal powder dried by three different methods were as follows: LOW-HAD, 14.56 mg/g; MD, 15.4 mg/g; VFD, 16.37 mg/g. The order of contents from highest to lowest was VFD > MD > LOW-HAD, while the losses of polysaccharides were LOW-HAD, 2.36 mg/g; MD, 1.52 mg/g; VFD, 0.55 mg/g. The order of losses from highest to lowest was LOW-HAD > MD > VFD. The loss of polysaccharide content in walnut meal powder is related to the Maillard reaction occurring during the drying process, influenced by factors such as temperature and time. The low temperature and vacuum conditions of VFD still contribute to reducing the loss of polysaccharide content.

The three drying methods, LOW-HAD, MD, and VFD, exert varying effects on the content of polyphenols, flavonoids, polysaccharides, and proteins within walnut meal powder. VFD demonstrates the highest retention and lowest loss of nutritional components in walnut meal powder due to its low temperature and vacuum conditions. Following VFD, LOW-HAD exhibits relatively favorable preservation of nutritional constituents, while MD results in the most pronounced loss of nutritional content post-drying. Additionally, MD treatment leads to particularly severe losses in protein content, making it unsuitable for materials with higher protein content.

3.2. The Inhibitory Effect of Walnut Meal Alcohol Extract on OP50 Escherichia Coli

Table 3-1. Size of Antibacterial Circle for Water and Alcohol Extracts of Walnut Meal Powder (mm)

Concentration(mg/mL)	water extract of walnut meal powder	alcohol extract of walnut meal powder	ampicillin
0.625	-	-	
1.25	-	-	
2.5	-	-	
5	-	-	
10	-	7.53±0.21	
20	-	8.95±0.3	
40	-	10.17±0.61	
80	-	12.95±0.73	
1			12.46±1.99
Notes:"-" represents "no visible antibacterial zone or antibacterial zone diameter ≤ 7mm.			

Based on the analysis of the nutritional content of walnut meal powder with different drying methods in the early stage, the walnut meal powder with the highest nutritional content after vacuum freeze-drying was selected for subsequent functional evaluation experiments.

In this experiment, the inhibitory effects of WEW and AEW on OP50 were observed to determine the suitability of the extracts for use. Strong inhibitory effects indicate a limitation in the growth of OP50, potentially affecting various lifespan-related indicators during the growth process of the nematode due to food scarcity. As shown in Table 3-1, the diameter of the antibacterial zone is dependent on the concentration of the samples, with higher concentrations resulting in stronger antibacterial effects. The antibacterial zone of the 80 mg/mL AEW is slightly larger than that of the 1 mg/mL ampicillin positive control, indicating good antibacterial efficacy. Through antibacterial experiments on OP50, it was determined that the AEW exhibits no inhibitory effect on Escherichia coli OP50 within the concentration range of 0.625-5 mg/mL, while the WEW exhibits no inhibitory effect within the concentration range of 0.625-80 mg/mL. To compare the effects of WEW and AEW, walnut meal powder extracts of the same concentration were selected for subsequent experiments.

3.3. Evaluation of Antioxidant Function of Walnut Meal Extract

Based on the size of the antibacterial zone and inhibitory effect on OP50 of the WEW and AEW in the early stage, concentrations of 1.25 mg/mL, 2.5 mg/mL, and 5 mg/mL were selected for the evaluation of the antioxidant and lipid-lowering functions of nematodes.

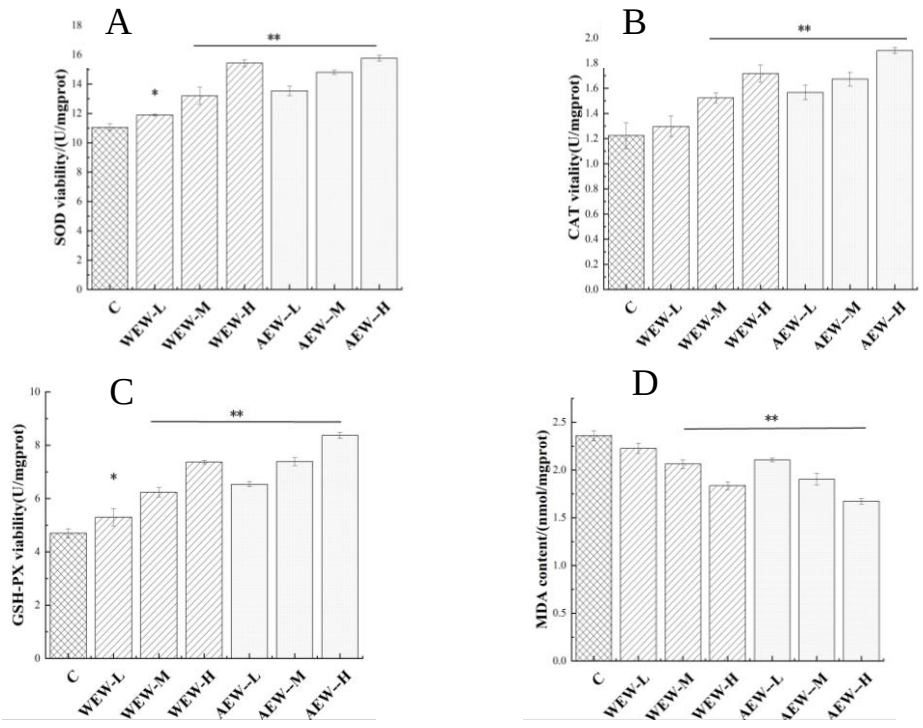

Figure 3-5. Effects of water and alcohol extracts from walnut meal powder on SOD(A), CAT(B), GSH Px(C), and MDA(D) in nematodes

The determination results of SOD activity in nematodes are depicted in Figure 3-5A. Comparatively, both WEW and AEW treatments exhibited higher SOD enzyme activity compared to the control group. Specifically, in the WEW-L, WEW-M, and WEW-H groups, SOD enzyme activity increased by 7.61% (P <0.05), 19.37% (P <0.01), and 39.61% (P <0.01), respectively. Correspondingly, in the AEW-L, AEW-M, and AEW-H groups, SOD enzyme activity increased by 22.50% (P <0.01), 33.99% (P <0.01), and 42.65% (P <0.01), respectively. The enhancement of SOD enzyme activity in the three dose groups of AEW was 12.15%, 10.91%, and 2.13%, respectively, higher than that in WEW.

The determination results of CAT activity in nematodes are presented in Figure 3-5B. Compared to the control group, the treatment groups exhibited higher CAT enzyme activity. Specifically, in the WEW-L, WEW-M, and WEW-H groups, CAT enzyme activity increased by 5.99%, 24.52% (P <0.01), and 40.33% (P <0.01), respectively. Similarly, in the AEW-L, AEW-M, and AEW-H groups, CAT enzyme activity increased by 28.07% (P <0.01), 36.78% (P <0.01), and 55.31% (P <0.01), respectively. When comparing the same concentrations, the enhancement of CAT enzyme activity in the three dose groups of AEW was 17.23%, 8.96%, and 9.65%, respectively, higher than that in WEW.

The determination results of GSH-Px activity in nematodes are illustrated in Figure 3-5C. Compared to the control group, the treatment groups exhibited higher GSH-Px enzyme activity. Specifically, in the WEW-L, WEW-M, and WEW-H groups, GSH-Px enzyme activity increased by 12.85% (P <0.05), 32.72% (P <0.01), and 56.92% (P <0.01), respectively. Similarly, in the AEW-L, AEW-M, and AEW-H groups, GSH-Px enzyme activity increased by 39.18% (P <0.01), 57.20% (P <0.01), and 78.14% (P <0.01), respectively. When comparing the same concentrations, the enhancement of GSH-Px enzyme activity in the three dose groups of AEW was 18.92%, 15.58%, and 11.91%, respectively, higher than that in WEW.

The determination results of MDA content in nematodes are depicted in Figure 3-5D. Compared to the control group, the treatment groups exhibited lower MDA content. Specifically, in the WEW-L, WEW-M, and WEW-H groups, MDA content decreased by 5.65% (P <0.05), 12.57% (P <0.01), and 22.18% (P <0.01), respectively. Similarly, in the AEW-L, AEW-M, and AEW-H groups, MDA content decreased by 10.73% (P <0.01), 29.35% (P <0.01), and 29.10% (P <0.01), respectively. When comparing the same concentrations, the reduction in MDA content in the three dose groups of AEW was 5.39%, 7.75%, and 8.89%, respectively, higher than that in WEW.

The above data indicate that both WEW and AEW can enhance the activity of antioxidative enzymes to some extent in nematodes, thereby strengthening the organism's resistance to oxidative damage, reducing peroxidation products, alleviating oxidative stress, and possessing the potential to activate the antioxidative defense system in nematodes. Within the concentration range of 1.25-5 mg/mL, the antioxidative activity increases with increasing concentration, with AEW exhibiting effects exceeding 10% of the aqueous extract.

3.4. Evaluation of lipid-lowering function of walnut meal extract

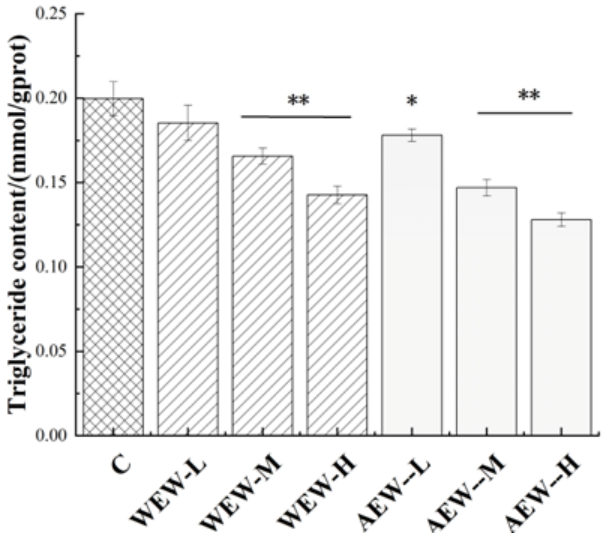

Figure 3-6. Effects of water and alcohol extracts from walnut meal powder on TG in nematodes

The main component in nematodes is TG, which can serve as an important indicator of lipid-lowering effects. As illustrated in Figure 3-6, compared to the control group, the TG content in the WEW-L, WEW-M, and WEW-H groups decreased by 7.18%, 17.03% (P <0.01), and 28.55% (P <0.01),

respectively. Similarly, in the AEW-L, AEW-M, and AEW-H groups, the TG content decreased by 10.85% (P <0.05), 26.38% (P <0.01), and 35.89% (P <0.01), respectively. When comparing the same concentrations, AEW exhibited reductions in TG content of 3.96%, 11.27%, and 10.28% higher than WEW, indicating that both WEW and AEW can decrease TG content in nematodes, with AEW demonstrating superior lipid-lowering effects.

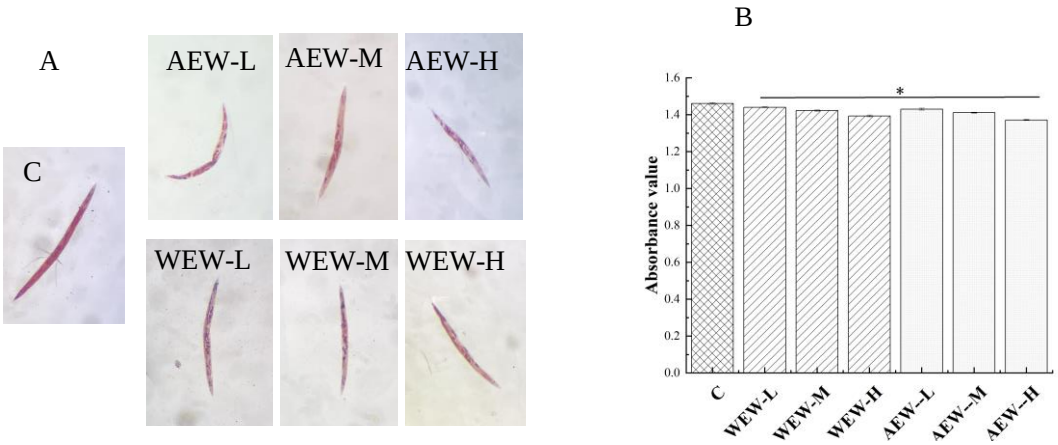

Figure 3-7. Oil Red O Staining of Nematodes (A) and absorbance values of nematode oil red O staining (B)

Oil red O is a commonly used lipophilic dye with specific affinity for neutral fats, forming distinctive red complexes. Therefore, oil red O staining enables the observation of neutral fat deposition in C. elegans. This staining method allows for the visualization of fat storage within the nematode, particularly the distribution of fat droplet-forming lipids in the intestine and subcutaneous tissues [24]. Images of oil red O staining of nematodes captured using a stereomicroscope are depicted in Figure 3-7A, where nematodes in the control group exhibit the deepest red color and largest area, while the stained area of nematodes in the experimental groups gradually decreases and the red color becomes lighter with increasing concentrations of WEW and AEW. As shown in Figure 3-7B, the absorbance values of the WEW-L, WEW-M, and WEW-H groups decreased by 1.39% ($p<0.05$), 2.60% ($p<0.05$), and 4.61% ($p<0.05$), respectively; while the absorbance values of the AEW-L, AEW-M, and AEW-H groups decreased by 2.10% ($p<0.05$), 3.38% ($p<0.05$), and 6.12% ($p<0.05$), respectively. Hence, AEW-H exhibits the most optimal lipid-lowering activity within the nematodes.

4. DISCUSSION

Among the three drying methods, vacuum freeze-drying has the least impact on the nutritional functional components in the raw materials, preserving them well and forming a porous structure. Previous studies on freeze-dried Cordyceps militaris [25], dried apples [26] and licorice [27] have confirmed this. However, freeze-drying is time-consuming, energy-intensive, and limited by the amount of material dried per cycle. Microwave drying stands out for its rapid speed compared to other drying methods. For instance, it takes 7 to 16 minutes to dry 5mm thick walnut meal, and the resulting material undergoes a certain degree of loosening, making it easy to crush, which is superior to hot air drying. However, it is not suitable for heating materials with a certain thickness due to uneven heating of thick materials by microwaves. Considering large-scale industrial production, energy efficiency, and convenience, HAD is the optimal drying method with wide applicability.

Given that Caenorhabditis elegans, as a functional evaluation biological model, has a short life cycle and features similar to human genes, this experiment utilized C. elegans as the model to assess the in vivo antioxidant and lipid-lowering functions of walnut meal powder. Walnut meal powder obtained through vacuum VFD served as the raw material, and three different doses (1.25 mg/mL, 2.5 mg/mL, 5 mg/mL) were used to investigate the antioxidant and lipid-lowering effects of WEW and AEW. It

was confirmed that both polyphenol WEW and AEW activated multiple antioxidant enzyme systems in vivo and reduced the content of oxidative metabolites such as MDA.

In studies utilizing C. elegans as the model organism, various concentrations of jujube water extracts were found to enhance the antioxidant activity of C. elegans. For instance, jujube water extract at 0.5 mg/mL reduced the release of reactive oxygen species (ROS) and reactive nitrogen species (RNS) by 44% and 22%, respectively, while increasing the activities of antioxidant enzymes such as SOD by 18% and CAT by 55% [28].Both WEW and AEW exhibited lipid-lowering effects, with similar efficacy observed between the two. These findings are consistent with previous studies on alcohol extracts of Allium chinense [29], water extracts of different concentrations of Ganoderma lucidum tea [30] and alcohol extracts of different doses of Panax notoginseng [31] demonstrating lipid-lowering effects. In conclusion, the results of this study align with previous findings, indicating that WEW and AEW exert antioxidant and lipid-lowering effects by scavenging free radicals, enhancing antioxidant enzyme activity, and reducing lipid content.

5. CONCLUSIONS

(1) Different drying methods for walnut meal powder show varying effects on the preservation of nutritional components and quality. Vacuum freeze-drying maintains the highest nutritional functionality and optimal quality, followed by low-temperature hot air drying. Microwave drying results in the greatest loss of nutritional quality, particularly in terms of protein content. Vacuum freeze-drying requires the longest time and consumes the most energy, making it suitable for drying high-quality medicinal materials but unsuitable for large-scale production. Low-temperature hot air drying, characterized by its short duration, low energy consumption, and ability to dry multiple batches of materials simultaneously, is more practical for large-scale production and is widely used in industrial settings.

(2) At concentrations of 1.25 mg/mL, 2.5 mg/mL, and 5 mg/mL, both aqueous and alcohol extracts of walnut meal powder enhance the activities of three antioxidant enzymes SOD, CAT, and GSH-PX within the nematode Caenorhabditis elegans, while concurrently reducing the levels of the oxidative byproduct MDA. Moreover, these extracts mitigate fat deposition within the nematode. Notably, the antioxidant activity of alcohol extracts of walnut meal powder surpasses that of its aqueous counterparts.

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