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Review Article

The chemical composition of the walnut pellicle and its benefits to health

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Abstract: Walnut pellicle is a layer of film wrapped around the surface of the walnut kernel. The walnut pellicle is rich in tannin compounds, which can react with salivary proteins to produce a bitter and astringent taste, affecting the taste of walnuts. Consequently, the walnut pellicle is typically discarded as waste during food processing. The paper reviews the composition of polyphenolic compounds in the walnut pellicle, mainly including more than 110 kinds of phenolic compounds such as tannins, flavonoids, and phenolic acids; as well as the progress of research on the functional activities in various fields, including inflammation protection, hypolipidemic, antimicrobial, antitumor, and anti-aging, and other five aspects of bioactivity; and finally, elaborates on the interaction between the polyphenolic compounds of the walnut pellicle and walnut proteins, and the possibility that de-phenolization might reduce the structural properties of walnut proteins during processing.

Keywords: walnut pellicle; chemical components; biological activities

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1 Introduction

Walnuts are among the four highly consumed nuts in the world[1]. China is one of the world's largest walnut producers, accounting for around 25% of the world's output[2]. Many clinical studies have shown that eating walnut kernels can reduce the prevalence of cardiovascular diseases, coronary heart disease, hypertension, cognitive impairment, and cancer[3]. Its beneficial biological properties mainly come from the abundance of unsaturated fatty acids[4] and phenolic compounds[5]. Numerous studies have highlighted that the walnut pellicle is a crucial source of phenolic compounds[6].

The thin layer that surrounds the kernel of a walnut is called the walnut pellicle. It typically has a yellow or light yellow color, but can also be purple or other shades. Within this layer, you can find a variety of nutrients including phenolic acids, flavonoids, lipids, tannins, amino acids, organic acids, alkaloids, lignans, and coumarins. Researchers, such as Rong Ruifen and colleagues, have conducted analyses on the nutrients found in walnut pellicles. They discovered that within every 100 g of walnut pellicles, there are 11.03 g of protein, 7.74 g of carbohydrates, and 20.20 g of fats. Mineral elements such as phosphorus, zinc, and calcium are also present in the pellicles with contents of 442.00, 29.20 and 23.20 mg/100 g, respectively. Interestingly, the contents of VB_1, phosphorus, and zinc in pellicles were 1.37, 1.50, and 13.46 times higher than those found in the kernel, respectively. Research shows that over 90% of the polyphenols and flavonoids in walnuts are primarily concentrated in the walnut pellicle[6], and the total phenols and flavonoid contents were 1.038 and 0.744 g/100 g respectively, which is 12.51 and 1.78 times higher than the contents found in the kernel[7]. it is shown that walnut pellicle has high nutritional and health values. Figure 1 shows the main biological activities of the walnut pellicle.

Trandafir *et al*[8]. investigated the antioxidant activity of walnut kernels and walnut pellicles of 12 genotypes and showed that the total phenolic content of the walnut kernel is between 11.31 and 28.92 mg GAE/g, and the total phenolic content of the walnut pellicle is between 115.25 and 338.33 mg GAE/g. Anderson *et al*[9]. demonstrated that the total phenol content of walnut in 50 g was approximately equivalent to 240 mL apple juice, and 150 mL red wine. From this, it can be seen that walnut pellicles are rich in phenolic species and high in content. It is easy to extract phenolics from walnut pellicles. Studies have shown that 80% methanol and 70% ethanol can extract most of the soluble free phenolic acids, soluble esterified phenolic acids, and insoluble bound phenolic acids in the walnut pellicle.

The antioxidant properties and bioactivity of walnut pellicle are widely recognized by researchers. Studies have shown that the polyphenol extract derived from walnut pellicles can aid in the prevention of diseases such as obesity, hepatic steatosis, and diabetes. This is achieved by regulating ecological imbalances, reducing inflammation, and boosting energy expenditure. Moreover, the extract is effective in inhibiting colitis. Seong-Joon Koh's evaluation of the extract, using cellular and mouse models,

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Figure 1 Different parts of the walnut.

demonstrated a significant reduction in NF-κB signaling in two models of colitis. These positive findings indicate that walnut pellicle extract holds immense potential for treating inflammatory bowel disease[10].

During food processing, the unpleasant taste of walnuts is caused by walnut pellicle phenols reacting with salivary proteins which produce a bitter and astringent flavor. As a result, walnut pellicles are often removed as waste material. It is necessary to explore the exploitation of walnut pellicles due to their nutritional activity and extraction efficiency of phenolic substances. However, a comprehensive understanding of the compound makeup and functional properties of walnut pellicles is currently lacking. As a result, this paper seeks to consolidate information on the chemical composition of polyphenols and the associated biological activity of walnut pellicles. By doing so, this paper aims to establish a foundation for the development and proper utilization of walnut pellicle resources.

2 Important compounds present in the walnut pellicle

The walnut pellicle is the part of the walnut with the highest content of phenolic compounds[11]. The total phenolic content in the walnut endodermis is 2–3 times that in the walnut blossoms and partition wood[5]. In addition, Table 1 shows the types of polyphenol compounds contained in the walnut pellicle. Table 2 shows more than 3,000 times that of the walnut shell, more than 140 times that of the walnut green peel, 12 times that of the kernel, and 6 times that of the leaves[12].

The current study identified over 110 phenolic compounds in walnut pellicles[14], including tannins, flavonoids, phenolic acids, and quinones[15]. The extraction method used significantly affects the polyphenolic composition of walnut pellicles, leading to potential deviations in identification results. Table 3 displays the variation in polyphenol and flavonoid levels among various extraction techniques. The data reveals that different organic solvents, material-liquid ratios, and extraction durations can lead to significant differences in the types and amounts of extracted compounds.

2.1 Tannin compound

Tannin compounds in walnut pellicles are classified into condensed and hydrolyzed forms[16]. Hydrolyzed tannins can be classified as gallotannins and ellagitannins. Gallotannins mainly consist of polyols like gallic acid or intermediate metabolites of gallic acid such as catechuic acid and triterpenes; ellagitannins are a type of ester tannins that are created when two gallic acid monomers are linked by a C–C bond and combined with a polyol. Essentially, ellagitannins form when two gallotannic acid monomers combine to create ester tannins, and they have a unique chemical structure that sets them apart from other tannins[15].

The walnut pellicles contain a large amount of ellagitannins, which are a type of tannin compound. Regueiro[16] conducted a study using HPLC to analyze the phenolic compounds found in walnut kernels with pellicles. He identified 120 phenolic compounds and 89 ellagitannins and their glycosides. It was confirmed that ellagitannins are the most abundant compounds found in walnut kernels. Medic[17] used HPLC to identify that the walnut pellicle contained 19 ellagitannins, while the kernel had only one type of condensed tannin.

Qualitative analysis of tannins typically involves identifying neutral losses of glucose and ellagic acid groups[18]. The structures of some ellagitannins are shown in Fig. 2. Zhang *et al.*[5] identified a total of 12 tannins in walnut pellicles by HPLC-MS/MS, and all of them were ellagitannins ((+)-catechin, mono-galloyl-glucose, methylgalloyl-hexyl glucoside, HHDP-glucose isomers, digalloyl galloyl-glucose, epigallocatechin, galloyl-HHDP-glucose, trigalloyl-glucose, bis-HHDP-glucose, tetragalloyl-glucose, galloyl-bis-HHDP-glucose, and trigalloyl-HHDP-glucose); and two species of bis-HHDP-glucose were identified in walnut pellicles by Medic[17], galloyl-HHDP-glucose3, tri-galloyl-HHDP-glucose4, bis-galloyl-HHDP-glucose3, castalagin3, bis-HHDP-glucose derivative, and two galloyl-bis-HHDP-glucose. The results of the characterization of the two individuals in the tannins were different, which may be mainly due to the different extraction conditions. Zhang *et al.*[5] used 70% methanol and ultrasonic enzyme-assisted extraction of the compounds from walnut pellicles, while Medic[17] used pure methanol vortex extraction. From the results, the latter extraction was significantly higher than the former, especially for vescalagin up to 30 mg/g, but with less variety of compounds extracted. The reason for this may be due to the similar solubility of the compounds. The organic solvent selected later is less polar, so it has good solubility for less polar tannins, but large polar tannins may not be solubilized.

Numerous studies have revealed that ellagitannins undergo

Table 1 Phenolic compounds identified and quantified in walnut pellicle

Formula	Measured mass (*m/z*)	Found Retention time (min)	MS/MS fragments	Compound identification	Phenolic contents (μg/g)	Reference
			Phenolic acids			
$C_{10}H_8O_2$	153.019,6	3.15	109.03, 81.03	Protocatehuic acid	4.10	[5]
$C_9H_8O_4$	179.035,2	4.26	135.01, 107.01	Caffeic acid	6.40	[5]
$C_9H_{10}O_5$	197.045,7	8.87	182.02, 167, 153, 138.87, 123.01	Syringic acid	15.48	[5]
$C_{14}H_6O_8$	300.999,3	12.17	300.01, 284.02, 257.03, 179.05, 151	Ellagic acid	1094.96	[5]
$C_7H_6O_5$	168.99	0.939	125, 79.1	Gallic acid	17.67	[6]
$C_8H_8O_4$	163.040,1	9.14	119.05, 93.04	*p*-Coumaric acid	4.51	[5]
$C_{10}H_{10}O_4$	94.057,91	7.03	193.051	Ferulic acid isomer	211.43	[5]
$C_{16}H_{18}O_9$	355.01	1.426	180.9, 145.1	Chlorogenic acid	45.82	[6]
$C_{11}H_{12}O_5$	222.99	1.916	208.1, 149.1	Sinapic acid	2.26	[6]
$C_7H_{12}O_6$	191.056,1	0.79	117.04, 93.03, 85.03	Quinic acid	44.53	[5]
$C_{16}H_{18}O_8$	337.092,7	8.78	173.04, 163.04, 119.05, 93.03	Coumaroylquinic acid	12.11	[5]
$C_{19}H_{14}O_{12}$	433.041,4	11.58	301.02, 300.01, 257.03, 229.03	Ellagic acid pentoside isomer	240.47	[5]
$C_{20}H_{18}O_{14}$	481.062,1	1.02	301, 275.01, 257.07, 229	HHDP-glucose isomer	195.93	[5]
$C_{28}H_{24}O_{16}$	615.099,6	11.48	301, 300.08, 271.04, 169.01, 125.02	Quercetin galloylhexoside isomer	3.36	[5]
$C_8H_8O_4$	167.034,27	1.361	123.044, 139.003	Hydroxymandelic acid	-	[13]
$C_{13}H_{16}O_{10}$	331.067,60	2.073	169.014, 125.023, 271.046, 211.025	Gallic acid hexoside	-	[13]
$C_{13}H_{16}O_9$	315.072,81	2.699	108.021, 152.011	Protocatechuic acid-*O*-hexoside	-	[13]
$C_8H_8O_4$	169.049,51	3.749	111.044, 65.039, 125.060, 169.050, 151.039, 93.034	Isovanillic acid	-	[13]
$C_{16}H_{18}O_9$	353.088,32	3.971	191.056, 135.044, 179.034	Neochlorogenic acid	-	[13]
$C_{13}H_{15}O$	299.077,67	4.470	137.024	Hydroxybenzoyl hexoside	-	[13]
$C_7H_6O_3$	137.023,48	4.911	93.033, 108.021, 137.024	*p*-Hydroxybenzoic acid	-	[13]
$C_{14}H_{10}O_9$	321.025,39	4.930	169.014, 125.024	*M*-Digallate	-	[13]
$C_8H_8O_4$	167.034,27	5.102	152.011, 108.021, 123.044	Vanillic acid	-	[13]
$C_{16}H_{18}O_8$	337.093,44	5.175	163.039, 119.049, 173.045, 93.033	3-*p*-Coumaroylquinic acid	-	[13]
$C_{15}H_{18}O_8$	325.093,41	6.177	265.072, 119.049, 163.039, 205.050, 235.061, 145.029	Coumaric acid hexoside isomer	-	[13]
$C_{10}H_{10}O_2$	163.075,30	6.185	131.049, 103.055, 163.075	Methyl cinnamate	-	[13]
$C_{11}H_{12}O_5$	225.075,91	6.205	175.039, 91.055, 119.049, 147.044, 207.101	Sinapinic acid	-	[13]
$C_{14}H_{18}O_9$	329.088,35	6.571	167.034, 123.044	Vanillic acid hexoside	-	[13]
$C_{20}H_{16}O_{13}$	463.052,98	6.953	300.999, 301.072	Ellagic acid hexoside isomer	-	[13]
$C_9H_{10}O_4$	181.050,02	7.085	166.027, 151.003, 123.008	Syringaldehyde	-	[13]
$C_9H_8O_3$	163.039,32	7.135	119.049, 93.033	*O*-Coumaric acid	-	[13]
$C_{19}H_{14}O_{12}$	433.041,99	7.527	299.992, 301.000, 229.014	Ellagic acid pentoside isomer	-	[13]
$C_9H_{10}O_3$	167.070,22	7.874	167.070, 123.044, 149.096, 121.029	Ethyl paraben	-	[13]
$C_9H_{10}O_3$	165.054,98	7.878	121.029, 108.021	Hydroxyphenylpropionic acid	-	[13]
$C_{10}H_{10}O_4$	193.050,16	8.356	175.039, 147.044, 178.027, 131.049	Ferulic acid	-	[13]
$C_{21}H_{18}O_{13}$	477.060,06	8.502	299.992, 315.016	Methyl ellagic acid hexoside	-	[13]
$C_{15}H_{20}O_{10}$	359.098,94	12.186	197.045	Syringic acid hexoside	-	[13]
-	391		301, 257, 229, 185	Ellagic acid derivative 1	8.4 ± 0.3	[17]
-	907	14.59	783, 301, 257, 229, 185	Ellagic acid derivative 2	20.3 ± 0.1	[17]
-	935	16.9	633, 301, 257, 229, 185	Ellagic acid derivative 3	31.1 ± 0.8	[17]
-	446	17.89	301, 257, 229, 185	Ellagic acid derivative 4	12.1 ± 0.2	[17]
-	485	18.07	301, 257, 229, 185	Ellagic acid derivative 5	15.4 ± 0.3	[17]
-	466	18.58	301, 451, 257, 229, 185	Ellagic acid derivative 6	37.5 ± 0.7	[17]
-	542	21.3	451, 466, 301, 257, 229, 185	Ellagic acid derivative 7	17.9 ± 0.4	[17]
-	529	22.53	467, 457, 391, 301, 257, 229	Ellagic acid derivative 8	14.9 ± 0.5	[17]
-	542	24.19	466, 301, 257, 229, 185	Ellagic acid derivative 9	9.2 ± 0.3	[17]
			Flavonoid			
$C_{45}H_{38}O_{18}$	865.199,52	5.199	125.024, 289.072, 407.078, 151.039	B-type procyanidin trimer isomer	-	[13]

(Continued)

Formula	Measured mass (*m/z*)	Found Retention time (min)	MS/MS fragments	Compound identification	Phenolic contents (μg/g)	Reference
$C_{15}H_{14}O_6$	289.072,24	6.342	123.044, 125.024, 203.071, 137.024, 151.039	(−)-Epicatechin	-	[13]
$C_{21}H_{22}O_{12}$	465.104,03	6.920	285.041	Quercetin-3-ogalactopyranoside isomer	-	[13]
$C_{22}H_{18}O_{10}$	441.083,65	7.384	245.082, 125.024, 203.071, 151.039, 205.050	(−)-Epicatechin 3-*O*-gallate	-	[13]
$C_{21}H_{20}O_{13}$	479.083,34	7.696	316.023, 317.031	Myricetin-*O*-hexoside	-	[13]
$C_{21}H_{20}O_{13}$	481.097,05	7.802	319.045, 153.018, 85.029, 91.039	Myricetin-3-*O*-*β*-*D*-galactopyranoside	-	[13]
$C_{28}H_{24}O_{16}$	615.100,04	8.121	300.028, 301.036, 271.025, 169.014, 125.024	Quercetin galloyl hexoside isomer	-	[13]
$C_{21}H_{20}O_{12}$	463.089,39	8.779	271.025, 151.003, 178.998	Myricitrin	-	[13]
$C_{21}H_{22}O_{11}$	449.109,10	9.040	125.023, 107.013, 303.051, 178.998	Astilbin isomer	-	[13]
$C_{15}H_{10}O_6$	287.054,99	9.116	153.018, 121.029, 241.050	Kaempferol	-	[13]
$C_{15}H_{10}O_6$	285.040,68	9.153	175.039, 133.029, 151.003	Luteolin	-	[13]
$C_{23}H_{22}O_{12}$	489.104,64	9.742	175.039, 169.014, 271.047, 125.023, 313.057, 229.051	3'-*O*-Acetylquercitrin isomer	-	[13]
$C_{21}H_{20}O_{11}$	447.094,02	9.910	300.028, 301.036, 271.025, 255.030, 151.003, 243.030, 178.998	Quercitrin	-	[13]
$C_{21}H_{22}O_{10}$	433.115,14	9.960	271.062, 151.003, 119.049, 177.019	Naringenin-7-oglucoside isomer	-	[13]
$C_{21}H_{22}O_{11}$	449.109,95	10.423	287.057	Eriodictyol-*O*-hexoside	-	[13]
$C_{28}H_{24}O_{14}$	583.110,23	10.676	300.028, 271.025, 255.030, 301.035, 151.003	Quercetin-*O*-(*p*-hydroxy)benzoyl-hexoside	-	[13]
$C_{21}H_{20}O_{12}$	463.089,51	10.904	301.036, 151.003, 178.998	Quercetin-*O*-glucoside	-	[13]
$C_{20}H_{16}O_{11}$	431.099,12	11.015	285.041, 255.030, 284.033, 227.035	Kaempferol-rhamnoside	-	[13]
$C_{37}H_{30}O_{16}$	729.149,72	11.085	125.023, 169.014, 407.078, 289.072	(epi)Catechin-(4, 8')-3'-*O*-galloyl-(epi)catechin	-	[13]
$C_{15}H_{12}O_6$	287.056,40	11.812	135.044, 151.003	Eriodictyol	-	[13]
$C_{16}H_{12}O_6$	299.056,61	14.630	256.039, 227.035, 284.033	Quercetin	-	[13]
$C_{15}H_{12}O_5$	271.061,80	13.474	119.049, 107.013, 151.003	Naringenin	-	[13]
$C_{16}H_{12}O_6$	299.056,61	14.630	256.039, 227.035, 284.033	Kaempferide isomer	-	[13]
$C_{20}H_{20}O_7$	373.128,14	17.493	343.081, 183.029, 271.059, 297.075	Tangeritin	-	[13]
$C_{27}H_{30}O_{16}$	609.510,74	21.734	255.233, 271.047	Rutin	-	[13]
			Hydrolysable tannins			
-	783	8.06	301, 481, 257, 229, 185	Pedunculagin/casuariin isomer (bis-HHDP-glucose) 1	9.5 ± 0.2	[17]
-	783	10.37	301, 481, 257, 229, 185	Pedunculagin/casuariin isomer (bis-HHDP-glucose) 2	4.1	[17]
-	633	9.41	301, 257, 229, 185	Strictinin/isostrictinin isomer (galloyl-HHDP-glucose) 1	1.9 ± 0.0	[17]
-	633	10.08	301, 257, 229 185	Strictinin/isostrictinin isomer (galloyl-HHDP-glucose) 2	7.3 ± 0.3	[17]
-	951	9.65	783, 301, 481, 257, 229, 185	Trigalloyl-HHDP-glucose isomer 1	5.7 ± 0.2	[17]
-	951	11.02	783, 301, 481, 257, 229, 185	Trigalloyl-HHDP-glucose isomer 2	3.0 ± 0.2	[17]
-	951	12.8	783, 301, 481, 257, 229, 185	Trigalloyl-HHDP-glucose isomer 3	3.9 ± 0.1	[17]
-	951	14.3	783, 301, 481, 257, 229, 185	Trigalloyl-HHDP-glucose isomer 4	21.6 ± 0.7	[17]
-	785	11.52	301, 483, 633, 257, 229, 185	Tellimagrandin 1 isomer (digalloyl-HHDP-glucose) 1	6.2 ± 0.2	[17]
-	785	13.64	301, 483, 633, 257, 229, 185	Tellimagrandin 1 isomer (digalloyl-HHDP-glucose) 2	4.3 ± 0.2	[17]
-	785	16.56	301, 483, 633, 257, 229, 185	Tellimagrandin 1 isomer (digalloyl-HHDP-glucose) 3	21.5 ± 0.6	[17]
-	1067	11.81	765, 721, 631	Pterocarinin A isomer	2.8 ± 0.1	[17]
-	933	15.05	451, 631, 301, 257, 229, 185	Castalagin/vescalagin isomer 1	20.7 ± 0.6	[17]
-	933	20.46	451, 631, 301, 257, 229, 185	Castalagin/vescalagin isomer 2	30.6 ± 0.8	[17]
-	933	22.03	451, 631, 301, 257, 229, 185	Castalagin/vescalagin isomer 3	11.7 ± 0.3	[17]
-	860	16.02	783, 301 301, 765, 481, 257, 229 185	bis-HHDP-glucose derivative	22.6 ± 0.5	[17]
-	935	17.39	633, 301, 257, 229, 185	Casuarin/ casuarictin isomer (galloyl-bis-HHDP glucose) 1	30.9 ± 0.4	[17]
-	935	19.59	633, 301, 257, 229, 185	Casuarin/ casuarictin isomer (galloyl-bis-HHDP glucose) 2	13.1 ± 0.1	[17]

(Continued)

Formula	Measured mass (*m/z*)	Found Retention time (min)	MS/MS fragments	Compound identification	Phenolic contents (μg/g)	Reference
$C_{15}H_{14}O_6$	289.072,1	6.31	245.01, 203.05, 151.09, 125.8, 109.75	(+)-Catechin	1094.96	[5]
$C_{16}H_{18}O_8$	337.092,7	8.78	173.04, 163.04, 119.05, 93.03	Monogalloyl-glucose	17.94	[5]
$C_{20}H_{18}O_{14}$	481.062,1	1.02	301, 275.01, 257.07, 229	HHDP-glucose isomer	259.17	[5]
$C_{20}H_{20}O_{14}$	483.078,2	6.59	313.02, 271.09, 211.06, 169.08, 125.04	Digalloyl-glucose	16.80	[5]
$C_{27}H_{22}O_{18}$	633.073,7	5.19	301.04, 275.04, 257, 229, 125	Galloyl-HHDP-glucose	23.93	[5]
$C_{27}H_{24}O_{18}$	635.089,5	10.47	313.04, 169.05, 125.08	Trigalloyl-glucose	44.42	[5]
$C_{34}H_{24}O_{22}$	783.069,6	3.43	300.1, 275.02, 257.01, 229.01	bis-HHDP-glucose	380.87	[5]
$C_{34}H_{28}O_{22}$	787.100,5	12.81	617.08, 465.07, 313.06, 169.01, 125.02	Tetragalloyl-glucose	3.36	[5]
$C_{41}H_{28}O_{26}$	935.080,3	7.99	301.00, 275.02, 257.02, 229.01, 125.01	Galloyl-bis-HHDP-glucose	5.54	[5]
$C_{41}H_{28}O_{27}$	951.075,4	5.78	301.00, 275.02, 257.01, 229.01	Trigalloyl-HHDP-glucose	18.03	[5]
$C_{13}H_{16}O_{10}$	331.067,60	1.162	169.014, 271.046, 211.025, 125.023	Monogalloyl-glucose isomer	-	[13]
$C_{15}H_{20}O_{10}$	359.098,97	4.270	197.045, 153.055	Dimethyl galloyl hexoside	-	[13]
$C_{20}H_{20}O_{14}$	483.078,86	5.099	169.014, 125.024, 271.047, 211.025, 313.057, 331.068	Digalloyl-glucose isomer	-	[13]
$C_{21}H_{22}O_{13}$	481.099,24	6.387	169.014, 125.024, 300.999	Galloyl methylgalloyl dexoyhexoside isomer	-	[13]
$C_{27}H_{22}O_{19}$	649.069,03	7.299	300.999	Valoneoyl-glucose	-	[13]
$C_{27}H_{24}O_{18}$	635.090,70	7.460	483.079, 313.057, 465.068, 211.025	Trigalloyl-glucose isomer	-	[13]
$C_{34}H_{28}O_{22}$	787.102,97	8.718	617.080, 465.069, 313.057	Tetragalloyl-glucose isomer	-	[13]
$C_{48}H_{32}O_{31}$	551.039,61	10.131	301.000, 169.014, 125.023, 275.020	Calamanin A isomer	-	[13]
			Quinones			
$C_{10}H_6O_3$	173.023,76	4.930	173.024, 145.029, 117.034	2-Hydroxy-1, 4-naphthoquinone	-	[13]
$C_{10}H_{10}O_3$	179.070,21	5.266	133.065, 147.044	Juglanoside isomer	-	[13]
$C_{10}H_8O_3$	177.054,47	5.951	131.049, 121.065, 159.044	5-Hydroxy-1, 4-naphthaoquinon	-	[13]
$C_{16}H_{18}O_8$	339.107,48	6.400	177.055, 145.028, 321.097, 117.034	Trihydroxynaphthaline glucoside	-	[13]
$C_{10}H_6O_3$	175.075,20	6.546	119.086, 147.080, 129.070	Juglone	-	[13]
$C_{10}H_8O_2$	161.059,65	8.704	133.065, 105.070, 115.054, 91.055	Naphthalened iol isomer	-	[13]
$C_{25}H_{26}O_{12}$	517.136,23	10.909	175.039	Dihydroxynaphthol-*O*[*O*-(dimethoxyhydroxybenzoyl)] glucopyranoside	-	[13]
$C_{23}H_{20}O_{12}$	489.102,69	11.014	327.050, 265.049, 237.055, 309.039	Jugnaphthalenoside A	-	[13]
$C_{16}H_{20}O_9$	355.104,28	11.231	175.040, 134.036, 160.016	Juglanoside D isomer	-	[13]
$C_{10}H_6O_4$	189.018,77	23.493	161.024, 117.034	Dihydroxynaphthoquinone isomer	-	[13]
			Other phenolic			
$C_9H_6O_3$	163.038,88	4.090	163.039, 107.049, 95.050	7-Hydroxycoumarine	-	[13]
$C_8H_8O_5$	183.029,30	4.448	168.006, 124.016	Methyl gallate	-	[13]
$C_{15}H_{22}O_5$	281.139,80	5.887	237.150, 171.118, 123.080, 189.129	Dihydrophaseic acid	-	[13]
$C_{10}H_{10}O_3$	177.055,04	5.927	177.055, 159.044, 149.060, 131.049	Isosclerone isomer	-	[13]
$C_8H_8O_3$	151.039,22	6.344	136.016, 108.021, 123.045	Vanillin	-	[13]
$C_{21}H_{10}O_{13}$	469.005,40	6.743	425.016, 301.000	Valoneic acid dilactone	-	[13]
$C_9H_{16}O_4$	187.097,17	10.167	125.096, 97.065, 168.888	Azelaic acid	-	[13]
$C_{28}H_{35}NO_{13}$	592.205,20	11.312	241.108, 403.162, 343.140	Glansreginin A	-	[13]
$C_{21}H_{24}O_{10}$	435.130,80	11.813	123.044, 273.078, 119.049	Phlorizin	-	[13]
$C_{15}H_{14}O_5$	273.077,48	14.008	125.024, 167.034, 123.044, 119.049	Phloretin	-	[13]
$C_9H_{10}O_2$	149.059,92	14.024	149.009, 105.070	Hydrocinnamic acid	-	[13]
$C_{10}H_{12}O$	149.096,18	14.582	149.096, 105.070, 79.055, 65.039	Cuminaldehyde	-	[13]

continuous hydrolysis via lipid bonds to produce ellagic acid under acidic conditions in the stomach and small intestine[19–21]; Ellagic acid is not easily absorbed and utilized in intestinal tissues[22–24]. However, it can be utilized by colonic microorganisms such as Elagibacter and Enterocloster in colonic tissues to decompose into the bioactive substance, urolithin[25,26]. Studies showed that urolithin is the main active component of ellagitannins after metabolism *in vivo*[27]. Urolithin plays important roles in antioxidant[28], anti-inflammatory[29,30], antibacterial[31], anticancer[32,33], anti-obesity[34] and anti-aging. The biological activity and mechanism of action of ellagitannins will be specifically described in Section 3.

Table 2 Contents of polyphenols and flavonoids in different parts of walnut

Extraction part	Extraction mode	Solid-liquid ratio (*m*/*V*)	Extraction time (min)	Total phenolic compounds	Flavonoids (mg/g)	Reference
Walnut pellicle	85% ethanol	1:30	55	536.80 mg/g	282.18	[12]
Walnut leaf	85% ethanol	1:30	55	(189.33 ± 13.76) mg/g	189.33	[12]
Shell of walnut	50% methanol	1:20	45	0.54–1.33 mg/g	0.34–1.01	[13]
Defatted walnut flour With pellicles	70% ethanol	1:20	60	4.62 g/100 g	No	[14]
Defatted walnut flour without pellicles	70% ethanol	1:20	60	0.19 g/100 g	No	[14]
Walnut husk	85% ethanol	1:30	55	21.71 mg/g	22.16	[12]

Table 3 Contents of polyphenols and flavonoids in walnut pellicles by different extraction style

Extraction part	Extraction mode	Solid-liquid ratio (*m*/*V*)	Extraction time (min)	Total phenolic compounds	Flavonoids	Reference
Walnut pellicle	85% ethanol	1:30	55	536.80 mg/g	282.18 mg/g	[12]
Walnut pellicle	70% ethanol	1:20	60	4.6 g/100 g	No	[14]
Walnut pellicle	50% methanol	1:20	45	26.02 mg/g	No	[13]
Walnut with red pellicle	Methanol	1:6.67	60	1,709.52 mg/100 g	115.62 mg/100 g	[8]
Walnut with yellow pellicle	Methanol	1:6.67	60	1,861.11 mg/100 g	119.80 mg/100 g	[8]

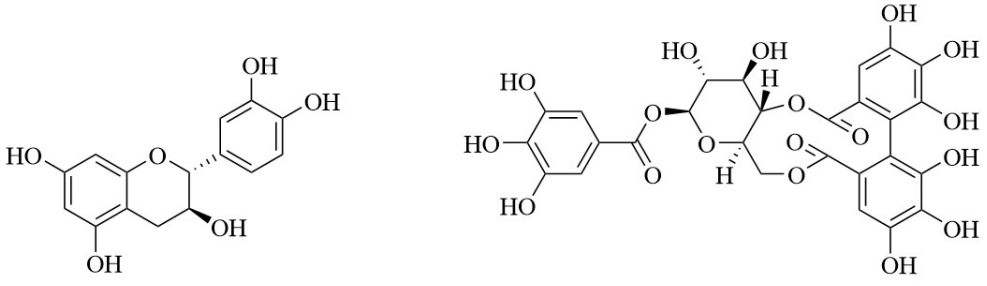

Figure 2 Typical tannin compounds.

2.2 Flavonoids

The pellicle is rich in flavonoids derived from the walnut. The ultra-high performance liquid chromatography-quadrupole-orbitrap technique was used by Sheng *et al.*[13] to identify 110 phenolic constituents in walnut pellicles, of which a total of 28 flavonoids were identified. Shen *et al.*[6] determined the contents of phenolic compounds in the walnut kernel and its pellicle using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), and the results showed that dihydroquercetin was the highest content of all flavonoids in the walnut pellicle (112.76 μg/g), and the remaining fractions, in order of priority, were 4, 5, 7-trihydroxyflavanone (108.33 μg/g), dihydrosorbenzoin (12.47 μg/g), syringic acid (12.09 μg/g), quercetin (9.69 μg/g), trichothecenes (2.52 μg/g), kaempferol (2.44 μg/g), and rutin (0.19 μg/g). Figure 3 shows the structural formula of the major flavonoid compounds.

A variety of plants have been implicated in the formation of their pellicle color concerning the synthesis of flavonoid compounds[35,36]. Flavonoids are the key compounds affecting the color of the walnut pellicle[36,37]. A recent investigation by Wang *et al.*[38] delved into the diverse hues of walnut pellicles (ranging from light to dark) originating from the same area and measured the primary substances present within them. The results of the study showcased that dark walnut pellicles possessed an abundance of flavanols, flavonoids, and flavanols compounds, specifically catechins and epigallocatechins. These two compounds were found to be present in extraordinary amounts, with 40.7 and 25.8 μg/g, respectively, which exceeded the levels of other free phenolic compounds by 2–153 times. Fang *et al.*[36] analyzed the compositional differences of walnut pellicles with varying colors using broadly targeted metabolomics. The study found that the flavonoid biosynthesis pathway, as well as the flavonoid and flavonol biosynthesis pathway, plays a significant role in determining the color of walnut pellicles. The study also suggests that a higher content of anthocyanin metabolites, which exhibit greater variation, could be one of the key factors affecting the color of walnut pellicles.

The color of the outer covering of walnuts, known as pellicle, varies greatly among different varieties of walnuts[38]. The inconsistent expression of key genes in the synthesis pathways of flavonoids, anthocyanins, and other compounds is the main reason, and two authors. A study was conducted by Zeng *et al.*[37] and Persic *et al.*[39] to compare the color variations of the outer layer of five types of walnuts: Shajiwuren, Hongguowuren, Songguowuren, Lara, and Fernor. The researchers analyzed the color variations in Fernor's pellicle and delved into the synthesis pathways of related compounds as well as the expression of key genes. The study's findings suggest that the biosynthetic pathways of phenylpropanes and flavonoids are closely linked to the

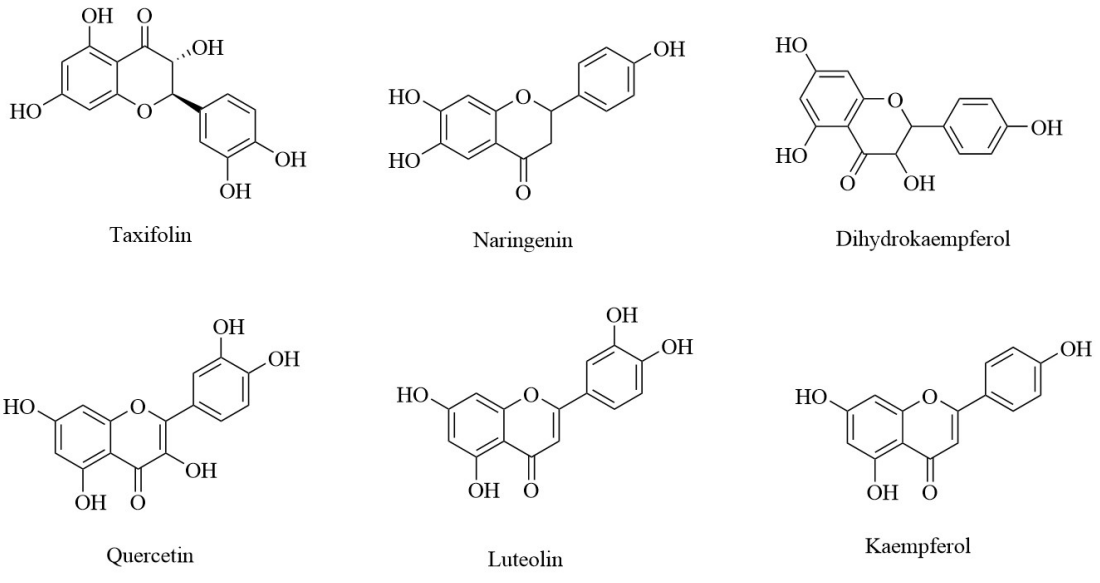

Figure 3 Typical flavonoid compounds.

differences in color between different types of walnut pellicles.

2.3 Phenolic Acids

A total of 11 phenolic acids were identified in walnut pellicles in Zhang's study[5], ellagic acid, quinic acid, coumaroylquinic acid, azelaic acid, *p*-coumaric acid, hydroxymandelic acid, ferulic acid isomer, protocatechuic acid, caffeic acid, and two types of hydroxybenzoic acid, *p*-hydroxybenzoic acid and butyric acid. The walnut pellicles had the highest levels of ellagic acid, with concentrations reaching 934.59–1,094.96 μg/g, and relatively high concentrations of ferulic acid ranging from 204.93 μg/g to 211.43 μg/g, with similar results identified by Regueiro[16], Medic[17], Shen *et al*[6]. Figure 4 shows the structural formula of the major phenolic acid compounds.

3 Metabolism of ellagitannins in the intestines

Ellagitannins are the most abundant polyphenolic compounds present in walnut pellicles[17]. After oral administration, ellagitannins can be first hydrolyzed by tannin acyl hydrolases in the stomach and small intestine to produce ellagic acid[29, 40]. Ellagic acid is not easily absorbed by the human gastrointestinal tract, making its bioavailability very low.

It has been found through various studies that certain microorganisms present in the colon, such as Gordonibacter and Ellagicobacter, can break down and use ellagic acid to produce a highly active and easily absorbed metabolite called urolithin[29, 41–43]. However, the efficiency of this conversion process can be affected by the levels of individual Gordonibacter and Ellagicobacter present[44].

Ellagic acid undergoes a multi-step metabolic process in the colon. First, it removes a lactone ring to form Uro-M5. Then, a hydroxyl group is removed from a different position to produce tetrahydroxyuranocortin (Uro-D, Uro-M6, Uro-E, and Uro-M6 R). This is further metabolized to trihydroxyuranocortin (Uro-C, Uro-M7, Uro-M7 R, and Uro-CR) and undergoes a dihydroxylation reaction to produce dihydroxyuranolites (Uro-A, iso-Uro-A, and Uro-AR). Finally, iso-Uro-A (or Uro-A) undergoes keto-enol interconversion isomerization to yield secondary alcohols and quinones, which drive dehydration and lead to monohydroxy-urolithin (Uro-B). The urolithin metabolite is absorbed and utilized through the colonic mucosa and undergoes phase II metabolism (methylated glucuronide and sulfonation) via human cellular enzymes to produce glucuronic anhydride and sulfate conjugates. These conjugates reach the plasma and systemic tissues within 3–4 days of ingestion to produce biological functions. Ultimately, they bind to the liver and are excreted in the urine[45].

Several studies have indicated that urolithins and their derivatives possess significant antioxidant, anticancer, anti-inflammatory, and bacteriostatic effects[46–49]. Urolithin A is commonly regarded as the most biologically potent among the urolithins[50]; In recent years, there has been a lot of emphasis on the anti-aging effects of urolithin A. The ability of intestinal microorganisms to break down ellagic acid has been closely associated with its anti-aging effects[51, 52].

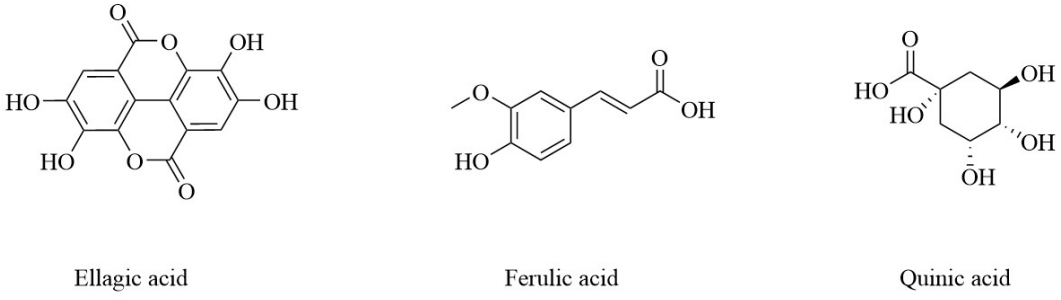

Figure 4 Typical phenolic acid compounds.

The composition of intestinal flora varies between individuals, resulting in unique metabolites and these phenotypic differences may be closely related to individual differences as well as to the composition of the intestinal flora[53].

In summary, the impact of ellagitannins on the body is influenced by the presence of colonic microorganisms. If walnut pellicles contain colonic microorganisms that can break down ellagic acid, individuals may experience enhanced bioactivity after consumption. Furthermore, the amount of Gordonibacter present is linked to the level of ellagic acid converted to urolithin A. As such, adjusting the composition of intestinal flora can alter the metabolism of ellagic acid, resulting in improved bioactivation of ellagitannin-rich substances.

4 The potential health benefits of walnut pellicle

The paper provides a comprehensive review of the bioactivities of walnut pellicles in five areas: anti-inflammatory effect, hypolipidemic activities, antimicrobial, antitumor, and antiaging, and were briefly explained in Fig. 5. The studies reported in the literature are summarized to present a clear understanding of the potential health benefits of walnut pellicles.

4.1 Anti-inflammatory effect

Several studies have demonstrated that polyphenolic compounds present in plants have remarkable protective effects against inflammation[54]. Kim *et al.*[55] conducted a study to investigate the protective effects of walnut polyphenol extract (WPE) on neuroinflammation induced by Aβ1−42 in mice. The results of the study showed that WPE improved behavioral and memory deficits, regulated related enzyme activities and protein expression, improved mitochondrial function abnormalities, and alleviated neuroinflammatory symptoms in mice.

The protective effects of walnut polyphenolic compounds against inflammation are influenced by multiple pathways. Extracts from the endocotyledon of walnuts can reduce neuroinflammation caused by oxidative stress by upregulating PKA/CREB/BDNF signaling. Additionally, walnut pellicle endocotyledon polyphenol extracts can also regulate the expression of proteins called zona occludens band-1 (ZO-1) and occludin, which are important for the function of the blood-brain barrier (BBB). These effects are achieved through the Akt pathway, which is activated by Aβ. Overall, walnut polyphenols have anti-inflammatory effects that can be beneficial for health[56].

The anti-inflammatory effect of walnut polyphenolic compounds may depend on the ellagitannin metabolite urolithin. It can significantly inhibit the levels of inflammatory factors such as anti–tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interleukin-12 (IL-12)[56]; and enhance the production of the anti-inflammatory factor IL-10[30,57–59]. A large number of studies have shown that the conversion of ellagitannins to urolithins can exert their anti-inflammatory effects through a variety of pathways.

Xian *et al.*[51] investigated the effects of ellagic acid on *D*-galactose-induced senescence in rats, in this study, ellagic acid ameliorated cognitive deficits and hippocampal damage, significantly increased the levels of two inhibitory neurotransmitters, γ-aminobutyric acid (GABA) and 5-hydroxytryptamine (5-HT), as well as suppressed inflammation and oxidative stress in senescent rats, in addition to the finding that the intestinal *Clostridium strictoides*, *Lactobacillus* and Zoarotrichum flora abundance was significantly increased.

Urolithin A reduces inflammation in pro-inflammatory cells by altering the production of inflammatory arachidonic acid from leukocytes. This is achieved through the modulation of the 5-lipoxygenase and cyclooxygenase-2 pathways[60].

In a study conducted by Xu *et al.*[61], the anti-inflammatory effects of urolithins A and B on lipopolysaccharide (LPS)-induced microglia were examined. The results of the study demonstrated that both urolithins A and B effectively reduced the levels of NO in LPS-treated microglial cells. Moreover, these compounds were found to inhibit the mRNA levels of various pro-inflammatory genes, including TNF-α, IL-6, IL-1β, iNOS, and COX-2, thus corroborating the findings of a prior investigation. The compounds Urolithins A and B have been discovered to reduce the levels of phosphorylation in Erk1/2, p38 MAPK, and Akt, prevent the phosphorylation and degradation of IκB-α, and hinder the phosphorylation and nuclear translocation of the NF-κB p65 subunit in LPS-stimulated microglia. These findings indicate that Urolithin A and B could potentially have an anti-inflammatory

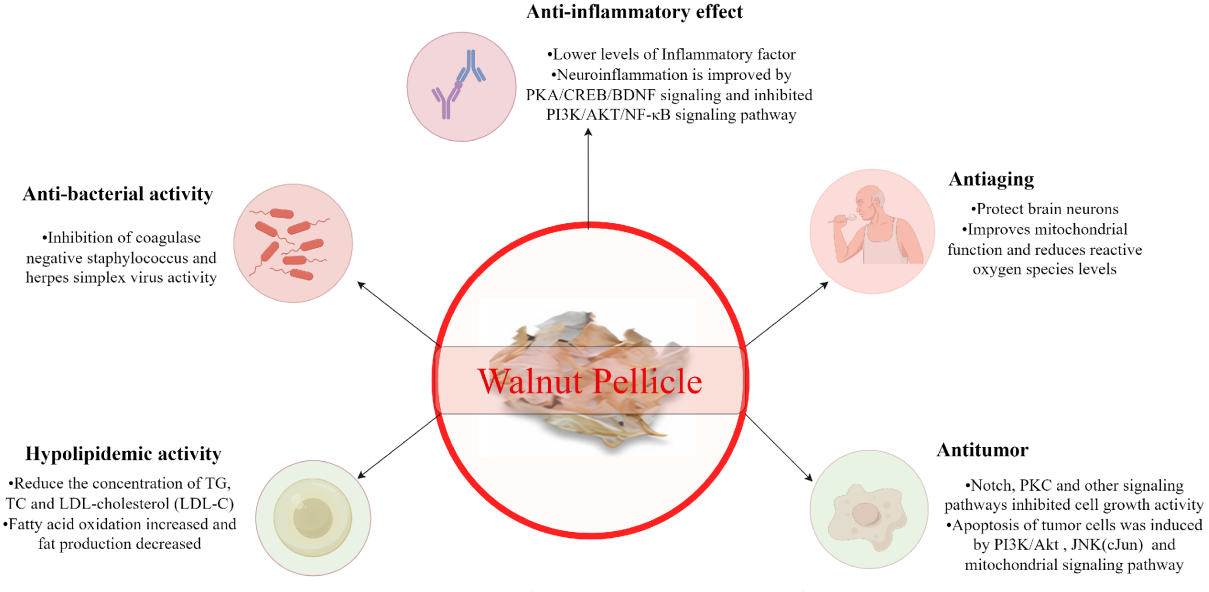

Figure 5 Schematic diagram of the potential biological activities of walnut pellicle.

effect by impeding the activation of NF-κB, MAPKs (p38 and Erk1/2), and Akt signaling pathways[61]. From the results, both urolithin A and B showed significant anti-inflammatory effects, but the levels of inflammatory factors in 3 μm urolithin A were almost the same as in 30 μm urolithin B, and the higher concentration of urolithin A showed better anti-inflammatory activity, so the anti-inflammatory effect of urolithin A is significantly better than that of urolithin B. Therefore, we can increase the level of Gordonella. by regulating the intestinal flora, increasing the level of urolithin A, and better utilizing the anti-inflammatory activity of walnut pellicle.

4.2 Hypolipidemic activity

Excessive energy intake can cause an excessive accumulation of fat or abnormal body distribution[62]. This can lead to high blood pressure, high cholesterol, and other related illnesses, which can negatively impact quality of life and increase the risk of mortality[63]. Polyphenolic compounds have functional activities that help regulate lipid synthesis and metabolism.

It was shown that walnut pellicle polyphenol extract has potent anti-obesity activity in high fat feed (HFD)-induced obese mice[64,65], Shi *et al.*[65] conducted a study to examine the impact of WPE on porcine pancreatic lipase. They utilized a fluorescence burst method along with an enzyme assay and discovered that the intensity of fluorescence emitted by porcine pancreatic lipase decreased. This confirmed that walnut polyphenols can inhibit lipase activity; in addition, the authors investigated the WPEs on the therapeutic effects on high-fat feed-fed obese mice, and the results showed that WPEs, significantly reduced triglyceride(TG), total serum cholesterol (TC), and low density lipoprotein cholesterol (LDL-C) concentrations, and elevated high density lipoprotein cholesterol (HDL-C).

Palabıyık *et al.*[66] conducted an experiment on male rats to study hyperlipidemia. The rats were given Triton WR-1339 through intraperitoneal injection to induce hyperlipidemia. Then, they were treated with ethanol extract from the walnut pellicle at doses of 150 and 300 mg/kg for five days. The results showed that the walnut pellicle extract effectively reduced hyperlipidemia and decreased the elevated levels of aldose reductase (AR), sorbitol dehydrogenase (SDH), butyryl cholinesterase (BChE), recombinant paraoxonase 1 (PON1), glutathione reductase (GR), acetylcholinesterase (AChE), and glutathione transferase (GST) enzyme activities, which were induced by the Triton WR-1339 injection. Additionally, the extract attenuated oxidative stress in cardiac, renal, and hepatic tissues.

There has been limited research conducted on the lipid-lowering mechanism of walnut pellicle polyphenol extracts. However, it is believed that they may help by enhancing mitochondrial activity and activating AMPK, reducing hypertrophy and macrophage infiltration of subcutaneous and visceral adipocytes, and enhancing signaling of hepatic lipid metabolism[65]. Many studies have investigated the lipid-lowering of urolithiomelanin, a metabolite of cholesterol. Lü *et al.*[67] used ellagic acid, a metabolite of pomegranate polyphenol, to establish an *in vitro* model of L-02 hepatocyte steatosis. The authors investigated the protein expression of the cholesterol metabolism pathway and found that PPARγ, ABCA1, and CYP7A1 protein expression were up-regulated. Therefore, it was suggested that the molecular mechanism may be through the activation of the target gene *PPARγ*, which then up-regulates the expression of the downstream genes *ABCA1* and *CYP7A1* through the PPARγ-ABCA1/CYP7A1 cellular signaling pathway, resulting in L-02 cell cholesterol metabolism. PPARγ was also shown to be an important component of cholesterol metabolism in L-02 hepatocytes. The activation of PPARγ is the most common pathway that regulates lipids. In addition, ellagic acid promotes adipocyte browning by overstimulating β3-adrenergic receptors and activating p38-MAPK. It also regulates lipid metabolism through an AMPK-dependent mechanism that leads to a decrease in TG accumulation, an increase in fatty acid oxidation, and a decrease in adipogenesis[68]. Each of these pathways may be relevant to the lipid-regulating activity of walnut pellicles.

4.3 Anti-bacterial activity

As previously mentioned, walnut pellicles are rich in polyphenolic compounds. Several studies have explored the antimicrobial properties of walnut pellicles due to this reason. Coagulase-negative staphylococci (CNS) are gram-positive cocci that grow in clusters, and this genus has a high capacity to form biofilms on both living and non-living surfaces. This makes them more prone to causing infections linked with catheters, prostheses, and other devices[69]. Acquaviva *et al.*[70] conducted a study to evaluate the effectiveness of walnut pellicle extract against CNS. They found that the extract demonstrated promising antibacterial activity, with a minimum inhibitory concentration (MIC) ranging from 3.60 μg/mL to 461.75 μg/mL, and a minimum bactericidal concentration (MBC) ranging from 461.75 μg/mL to > 461.75 μg/mL. Additionally, the extract was found to significantly reduce the biomass of CNS biofilms and cell viability in a dose-dependent manner.

Herpes simplex virus (HSV) is a common infectious virus that causes severe nerve pain. A study conducted by D'Angeli *et al.*[71] found that walnut pellicle polyphenol extracts have antibacterial and antifungal properties. These extracts were effective in inhibiting the replication of HSV, as well as the growth of medically important bacterial and fungal strains. The study used microdilution to determine the effectiveness of the extracts.

The two authors analyzed the polyphenol and flavonoid content in ethanolic extracts of walnut pellicles. They found high levels of these compounds in the endodermis, which led them to conclude that the antimicrobial activities observed could be attributed to the elevated levels of phenolic and flavonoid compounds present in the walnut pellicle contents.

There are various ways in which walnut pellicles exhibit antimicrobial activity. Research studies have reported that the main compound present in walnut pellicles, ellagitannin, is responsible for its antimicrobial activity. Virtanen *et al.*[72] conducted a study to investigate the interaction between 12 different ellagitannins and pentagalloylglucose with lipid extracts of *Escherichia coli* using high resolution magic angle spinning nuclear magnetic resonanc spectroscopy. The study suggested that the antimicrobial properties of ellagitannin may be due to its interaction with lipid membranes.

Ellagitannins showed good antimicrobial activity against *Clostridium perfringens*, *E. coli*, *Lactobacillus plantarum*, and *Staphylococcus aureus*, and their antimicrobial activity may depend on the molecular weight size of the ellagitannins, and ellagitannins with small molecular weights may have better antimicrobial properties[31]. In the previous paper, in the study of Zhang *et al.*[5], it was found that bis-HHDP-dextrose and HHDP-dextrose were the two highest contents of ellagitannins in the walnut pellicle. The walnut pellicle's antimicrobial activity was likely related to the small-molecule ellagitannins it contained, as the molecular weights were on the lower side.

4.4 Anti-tumor activity

Numerous studies have linked walnut consumption with being beneficial for several chronic diseases such as cancer, mainly due to the high amount of bioactive substances contained in the walnut pellicle. For example, walnut polyphenols such as walnut glycosides, walnut ketones, and the ellagitannin metabolite urolithin are considered to be the compounds with the walnut glycosides, walnut ketones, e most antitumor potential[73].

The inactivation of tumor-infiltrating lymphocytes (TIL) is one mechanism that weakens anti-tumor immunity during tumor development and progression[74]. Yang *et al.*[75] conducted a study on the effects of WPE on the immunotoxicity induced by 4-pentylphenol and 3-methyl-4-nitropheno in splenic lymphocytes of mice. The study found that the WPE significantly enhanced the proliferation of splenic lymphocytes that were exposed to PP or PNMC, reduced the activities of oxidative stress enzymes, and effectively addressed the immunotoxicity of the cells.

The anti-cancer mechanisms of plant polyphenols are complex and varied, and the current research on the anti-tumor mechanisms of walnut polyphenols is still in a scarce stage, so we can only speculate on the possible anti-tumor mechanisms based on the composition of their main compounds. Inhibition of tumor cell proliferation is an important way to play an anticancer role, it has been reported that ellagic acid can inhibit cell growth activity by inducing VEGF-A and programmed cell death ligand 1PD-L1 down-regulation of VEGFR-2 expression[76], Notch signaling pathway[77], PKC[78, 79] and other signaling pathways; in addition, ellagic acid can also play an anticancer role by promoting apoptosis of cancer cells. In addition, ellagic acid can also play an anticancer role by promoting cancer cell apoptosis. Ellagic acid can induce apoptosis and block the epithelial mesenchymal transition process in tumor cells through the PI3K/Akt signaling pathway[80, 81], the JNK (cJun) signaling pathway[82, 83], the mitochondrial signaling pathway[84–86], the Bcl-2/Bax signaling pathway[87], and the TGF-β/Smad 3 signaling pathway[88]. The polyphenolic compounds of walnut pellicles are rich in ellagitannins, ellagic acid, and other compounds, so we hypothesized that the anti-tumor mechanism of walnut pellicles may be related to these pathways.

4.5 Anti-aging activity

As society ages, there is a growing focus on researching anti-aging substances. Research suggests that aging is often accompanied by chronic diseases, including but not limited to osteoporosis, Alzheimer's disease, and cardiovascular disease[89].

Superoxide produced by the mitochondrial respiratory chain generates many reactive free radicals, which leads to the stoichiometric oxidation of a large number of macromolecular compounds[90] and causes oxidative damage at the site of free radical production, thus accelerating the rate of senescence[91]. Polyphenolic compounds, however, can directly scavenge reactive oxygen species through the provision of electrons or hydrogen atoms to reduce oxidative stress-induced damage to neurons and so on, and so are thought to have good bioactivity in delaying the rate of senescence. It has good biological activity[92].

Tian *et al.*[93] conducted a study on aging mice induced by *D*-galactose to investigate the protective effects of polyphenol extracts from defatted walnut kernels. The study found that the WPEs were effective in reducing inflammation levels in the tissues and serum of aging mice, ultimately slowing down the rate of aging. Emil Rusu is covered that extracts from walnut polyphenols protected rat neurons from the effects of *D*-galactose-induced senescence. Furthermore, it has been observed that the colonic infusion of Uro-A (a metabolite of urolithin A) has anti-aging effects. The study investigated the quantitative relationship between colonic infusion of Uro-A and its anti-aging effects. The results showed that both doses (3.0 and 15.0 mg/kg) of Uro-A were effective in inhibiting *D*-galactose-induced increases in oxidative stress levels, muscle atrophy, and neuronal apoptosis *in vivo*. Colonic infusion of Uro-A could also attenuate *D*-galactose-induced senescence in mice by inhibiting NF-κB and mTOR targets, providing significant protection against motor and cognitive function[94]. Rahimi *et al.*[95] investigated the *D*-galactose-induced SH-SY5Y human adult neuronal cell tumor cell model. The results showed that a low concentration (0.1–1 μmol/L) of ellagic acid may offer greater protection against aging than high concentrations of Uro-A. This is achieved through the PPAR-γ/HO-1 signaling pathway, and the anti-aging properties provided by this method were superior to those of high concentrations (10 μmol/L) and metformin (2.5 mmol/L).

Ryu *et al.*[96] conducted an experiment where nematodes were fed with standard concentrations of Urolithin A, Urolithin B, Urolithin C, or Urolithin D from their egg stage until their death. The lifespan of the nematodes was prolonged by 45.4%, 36.6%, 36.0%, and 19.0%, respectively, when treated with UA, UB, UC, or UD. However, ellagic acid treatments at the same concentrations did not affect the lifespan of the nematodes. The experiment showed that there was a significant dose-response effect on the nematode lifespan when the UA concentration was increased from 10 μmol/L to 50 μmol/L. Moreover, UA helped to maintain the normal activities of nematodes during senescence, including motility and pharyngeal twitching, and preserved the ability of mitochondrial respiration. A team of experts conducted a comprehensive study on diverse strains with the capability of generating urolithin A. They utilized ellagitannins as substrates to examine the impacts of the fermentation products on nematode models' anti-aging properties. The results showed that the metabolites could extend lifespan by enhancing mitochondrial function and reducing reactive oxygen species levels, leading to an increase in lifespan of up to 46.33%. These findings align with earlier research in the field[97]. This result suggests that the anti-aging activity of walnut pellicle polyphenol extract is most likely dependent on the action of its metabolite urolithin.

Ryu *et al.*[96] conducted two studies using mice models to explore the impact of UA treatment. In the first study, he focused on the preventive effect of UA treatment on age-related muscle decline in male C57BL/6J mice who were fed a HFD and aged 16 months. The findings showed that after eight months of UA treatment, there were notable improvements in muscle function observed at 22 and 24 months of age. These included increased grip strength and spontaneous exercise levels. In the second study, aged male C57BL/6J mice (22.5 months) on a normal food diet (NCD) were given UA treatment for six weeks. The study revealed that UA significantly increased running endurance by 42%.

In conclusion, our research has revealed that the walnut pellicle extract has various anti-aging benefits, including its ability to scavenge free radicals, protect neurons, reduce inflammation levels, and enhance muscle function. Additionally, our hypothesis suggests that urolithin A, a metabolite present in the extract, may play a pivotal role in producing these effects and potentially extending lifespan.

5 Interaction of walnut pellicle polyphenolic compounds with walnut proteins

The defatted walnut meal, a by-product of walnut oil extraction, typically contains over 40% protein, including the main nutrient found in walnuts[98]. A multitude of studies have demonstrated that walnut proteins and peptides possess a diverse range of physiological functions, including but not limited to antioxidant, antidiabetic, blood pressure reducing, and anti-fatigue effects[99–102]. In the process of manufacturing walnut oil, the elevated temperatures utilized can trigger the oxidation of numerous phenolic compounds that exist. Consequently, semiquinones and quinones are formed, which have the capacity to interact with different nucleophilic reagents. The particular protein side chains that are most often impacted by this reaction are lysine and cysteine[103]. These reactions may cause changes in the secondary and tertiary structures of walnut proteins, as well as some of their physicochemical properties[104].

Understanding the interplay between walnut protein and polyphenol is essential for advancing the field of walnut-based cuisine. The dynamic interaction of these components can yield varying outcomes on protein structure and physicochemical properties depending on the pH level. Research by Labuckas *et al.*[105] has revealed that phenolic compounds inherent in walnuts can impede the solubility of walnut proteins under neutral conditions. Yet, when exposed to alkaline conditions, the solubility of both de-phenolized and un-phenolized walnut proteins proved comparable. In addition to this, many scholars have studied the emulsifying and foaming properties of dephenolized and unphenolized walnut proteins, as well as the solubility of these proteins.

Wu *et al.*[106] utilized ultrasound-assisted ethanol extraction (UAE) to remove phenols from defatted walnut powder and then compared the impact of this treated powder and untreated powder on the solubility, emulsification, and foaming of walnut isolate protein. The results revealed that the functionality of the walnut protein isolate after UAE dephenolization was notably enhanced, surpassing that of the untreated isolate. At pH 5, both walnut proteins demonstrated the least functionality, with solubility of 5.31% and 4.86%, and emulsification activity indices (EAIs) of 24.95 and 19.91 m^2/g, respectively. Similarly, at pH 11, solubility was 82.35% and 73.55%, EAI was 46.35 and 37.28 m^2/g, and foaming power was 35.85% and 18.87%, respectively.

Wang *et al.*[107] used ultra-performance liquid chromatography-Q-time of flight mass spectrometry (UPLC-Q-TOF-MS) to establish the phenolic compound profiles of walnut meal and walnut meal isolate. The functional properties of the walnut meal isolates were evaluated under acidic environment after removing phenolic compounds. It was found that phenol removal significantly improved the water-holding capacity, oil-absorbing capacity, foaming capacity, foaming stability, emulsification stability index, and *in vitro* gastric digestibility of the isolates.

Researchers led by Su *et al.*[108] conducted a study examining the impact of walnut pellicle polyphenols on walnut proteins. Their findings indicated that these polyphenols can alter the proteins' secondary structure and amino acid composition, resulting in enhanced inhibition of hydrolase and acetylcholinesterase (AChE) enzymes.

Research suggests that in acidic conditions, there is a noteworthy interaction between the polyphenols and proteins found in walnut pellicles. This interaction impacts several important indices, such as the water-holding capacity, oil-absorbing capacity, foaming capacity, foaming stability, and emulsion stability of proteins. Additionally, it may even inhibit certain enzyme activities.

Numerous researchers have delved into the relationship between walnut pellicle extracts and proteins in alkaline environments. Their findings suggest that walnut pellicle extracts have the potential to improve the solubility of walnut protein in such environments. Huang *et al.*[109] conducted a study to explore the impact of ellagic acid or epigallocatechin-3-gallate (EGCG) conjugates on the emulsification properties and solubility of alkali-extracted walnut isolate proteins. The study revealed that the combination of walnut isolate protein with polyphenols significantly reduced its surface hydrophobicity, increased its surface charge, and enhanced its solubility.

A study conducted by Kong *et al.*[110] delved into the interaction between walnut proteins and walnut pellicle phenolic extracts. Their objective was to enhance protein solubility under neutral conditions by examining their interaction under alkaline conditions. Through the use of SDS-PAGE and size exclusion chromatography analyses, the researchers discovered that the covalent interaction between walnut proteins and polyphenols led to the creation of larger soluble protein aggregates. These aggregates, in turn, boosted protein solubility. Additionally, the study revealed that the primary covalent binding site in walnut protein was lysine.

Under alkaline conditions, the interaction between the proteins found in walnuts and polyphenolic compounds present in its pellicle forms protein aggregates. These aggregates exhibit a higher surface charge and lower surface hydrophobicity, resulting in enhanced solubility of the walnut proteins.

Recent research has indicated that the use of walnut pellicles can have a significant impact on the interaction between polyphenolic compounds and proteins. As walnut oil is rich in unsaturated fatty acids and is typically processed in an acidic environment, high temperatures and acid levels can negatively affect the solubility and foaming properties of walnut proteins. To avoid this adverse effect, incorporating walnut pellicles into the process can lead to the production of higher-quality walnut proteins.

6 Conclusion and prospects

Walnut pellicle is a by-product of the walnut processing industry that has good biological activity. However, it affects the taste of walnuts during consumption, which is why many enterprises choose to remove it during production and processing. Unfortunately, this results in a waste of walnut pellicle resources. There are a few reasons why walnut pellicles aren't more commonly used. Firstly, the technology used to separate them from the seed kernels is often flawed and reliant on manual labor. Secondly, there's no complete industrial supply chain in place to collect and make use of these resources, leading to pellicles being discarded in the initial separation phase. Thirdly, there's a lack of available products on the market, which means that consumers aren't aware of the benefits of walnut pellicles. To make the most of this resource, it's important to develop an industrialized process for the production of walnut pellicles and create products that are both bioactive and appetizing.

Data availability

The data used to support this study are available within the article.

Conflicts of interest

All authors declare that there are no conflicts of interest regarding this study.

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References

[1] Fordos, S., Abid, N., Gulzar, M., et al. Recent development in the application of walnut processing by-products (walnut shell and walnut husk). *Biomass Conversion and Biorefinery*, **2023**, 13: 14389–14411. https://doi.org/10.1007/s13399-023-04778-6

[2] Martínez, M. L., Maestri, D. M. Oil chemical variation in walnut (*Juglans regia* L.) genotypes grown in Argentina. *European Journal of Lipid Science and Technology*, **2008**, 110: 1183–1189. https://doi.org/10.1002/ejlt.200800121

[3] Alasalvar, C., Salvadó, J. S., Ros, E. Bioactives and health benefits of nuts and dried fruits. *Food Chemistry*, **2020**, 314: 126192. https://doi.org/10.1016/j.foodchem.2020.126192

[4] Song, H., Cong, Z., Wang, C., et al. Research progress on walnut oil: bioactive compounds, health benefits, extraction methods, and medicinal uses. *Journal of Food Biochemistry*, **2022**, 46: 14504. https://doi.org/10.1111/jfbc.14504

[5] Zhang, Y. G., Kan, H., Chen, S. X., et al. Comparison of phenolic compounds extracted from *Diaphragma juglandis* fructus, walnut pellicle, and flowers of *Juglans regia* using methanol, ultrasonic wave, and enzyme assisted-extraction. *Food Chemistry*, **2020**, 321: 126672. https://doi.org/10.1016/j.foodchem.2020.126672

[6] Shen, D., Yuan, X., Zhao, Z., et al. Determination of phenolic compounds in walnut kernel and its pellicle by ultra-high-performance liquid chromatography-tandem mass spectrometry. *Food Analytical Methods*, **2021**, 14: 2408–2419. https://doi.org/10.1007/s12161-021-02069-2

[7] Rong, R. F., Li, Z. X., Liu, X. Z., et al. Primary determination study on contents of nutritional and functional components of walnut kernel pellicle. *Food Science*, **2008**, 29: 541–543.

[8] Trandafir, I., Cosmulescu, S., Botu, M., et al. Antioxidant activity, and phenolic and mineral contents of the walnut kernel (*Juglans regia* L.) as a function of the pellicle color. *Fruits*, **2016**, 71: 177–184. https://doi.org/10.1051/fruits/2016006

[9] Wu, S. T., Shen, D. Y., Wang, R. H., et al. Phenolic profiles and antioxidant activities of free, esterified and bound phenolic compounds in walnut kernel. *Food Chemistry*, **2021**, 350: 129217. https://doi.org/10.1016/j.foodchem.2021.129217

[10] Koh, S. J., Choi, Y. I., Kim, Y., et al. Walnut phenolic extract inhibits nuclear factor kappaB signaling in intestinal epithelial cells, and ameliorates experimental colitis and colitis-associated colon cancer in mice. *European Journal of Lipid Science and Technology*, **2019**, 58: 1603–1613. https://doi.org/10.1007/s00394-018-1704-3

[11] Colaric, M., Veberic, R., Solar, A., et al. Phenolic acids, syringaldehyde, and juglone in fruits of different cultivars of *Juglans regia* L. *Journal of Agricultural and Food Chemistry*, **2005**, 53: 6390–6396. https://doi.org/10.1021/jf050721n

[12] Zhao, X. D. Study on extraction, isolation and activity of antioxidant components from walnut kernel pellicle, Xianyang, China: Northwest A&F University, **2022**.

[13] Sheng, F., Hu, B., Jin, Q., et al. The analysis of phenolic compounds in walnut husk and pellicle by UPLC-Q-Orbitrap HRMS and HPLC. *Molecules*, **2021**, 26: 3013. https://doi.org/10.3390/molecules26103013

[14] Yan, C., Zhou, Z. Walnut pellicle phenolics greatly influence the extraction and structural properties of walnut protein isolates. *Food Research International*, **2021**, 141: 110163. https://doi.org/10.1016/j.foodres.2021.110163

[15] Ekambaram, S. P., Perumal, S. S., Balakrishnan, A. Scope of hydrolysable tannins as possible antimicrobial agent: scope of hydrolysable tannins as antimicrobial agents. *Phytotherapy Research*, **2016**, 30: 1035–1045. https://doi.org/10.1002/ptr.5616

[16] Regueiro, J. Comprehensive identification of walnut polyphenols by liquid chromatography coupled to linear ion trap–orbitrap mass spectrometry. *Food Chemistry*, **2014**, 152: 340–348. https://doi.org/10.1016/j.foodchem.2013.11.158

[17] Medic, A. Identification and quantification of the major phenolic constituents in *Juglans regia* L. peeled kernels and pellicles, using HPLC–MS/MS. *Food Chemistry*, **2021**, 352: 129404. https://doi.org/10.1016/j.foodchem.2021.129404

[18] Chang, Z., Zhang, Q., Liang, W., et al. A comprehensive review of the structure elucidation of tannins from *Terminalia* Linn. *Evidence-Based Complementary and Alternative Medicine*, **2019**, 2019: 8623909. https://doi.org/10.1155/2019/8623909

[19] Macierzyński, J., Sójka, M., Kosmala, M., et al. Transformation of oligomeric ellagitannins, typical for *Rubus* and *Fragaria* genus, during strong acid hydrolysis. *Journal of Agricultural and Food Chemistry*, **2020**, 68: 8212–8222. https://doi.org/10.1021/acs.jafc.0c02674

[20] Buenrostro-Figueroa, J., Miereles, M., Ascacio-Valdés, J. A., et al. Enzymatic Biotransformation of pomegranate ellagitannins: initial approach to reaction conditions. *Iranian Journal of Biotechnology*, **2020**, 18: 2305. https://doi.org/10.30498/ijb.2020.137202.2305

[21] Brück, W. M., Díaz Escobar, V. D., Droz-dit-Busset, L., et al. Fermentative liberation of ellagic acid from walnut press cake ellagitannins. *Foods*, **2022**, 11: 3102. https://doi.org/10.3390/foods11193102

[22] Cerdá, B., Espín, J. C., Parra, S., et al. The potent *in vitro* antioxidant ellagitannins from pomegranate juice are metabolised into bioavailable but poor antioxidant hydroxy-6*H*-dibenzopyran-6-one derivatives by the colonic microflora of healthy humans. *European Journal of Nutrition*, **2004**, 43: 205–220. https://doi.org/10.1007/s00394-004-0461-7

[23] Cerdá, B., Llorach, R., Cerón, J. J., et al. Evaluation of the bioavailability and metabolism in the rat of punicalagin, an antioxidant polyphenol from pomegranate juice. *European Journal of Nutrition*, **2003**, 42: 18–28. https://doi.org/10.1007/s00394-003-0396-4

[24] Cerdá, B., Tomás-Barberán, F. A., Espín, J. C. Metabolism of antioxidant and chemopreventive ellagitannins from strawberries, raspberries, walnuts, and oak-aged wine in humans: identification of biomarkers and individual variability. *Journal of Agricultural and Food Chemistry*, **2005**, 53: 227–235. https://doi.org/10.1021/jf049144d

[25] Iglesias-Aguirre, C. E., González-Sarrías, A., Cortés-Martín, A., et al. *In vivo* administration of gut bacterial consortia replicates urolithin metabotypes A and B in a non-urolithin-producing rat model. *Food & Function*, **2023**, 14: 2657–2667. https://doi.org/10.1039/D2FO03957E

[26] Heilman, J., Andreux, P., Tran, N., et al. Safety assessment of urolithin A, a metabolite produced by the human gut microbiota upon dietary intake of plant derived ellagitannins and ellagic acid. *Food and Chemical Toxicology*, **2017**, 108: 289–297. https://doi.org/10.1016/j.fct.2017.07.050

[27] Garcia-Muñoz, C., Vaillant, F. Metabolic fate of ellagitannins: implications for health, and research perspectives for innovative functional foods. *Critical Reviews in Food Science and Nutrition*, **2014**, 54: 1584–1598. https://doi.org/10.1080/10408398.2011.644643

[28] Al-Sayed, E., Korinek, M., Esmat, A., et al. Anti-inflammatory, hepatoprotective and antioxidant activity of ellagitannin isolated from *Melaleuca styphelioides*. *Phytochemistry*, **2020**, 177: 112429. https://doi.org/10.1016/j.phytochem.2020.112429

[29] Garcia, G., Pais, T. F., Pinto, P., et al. Bioaccessible raspberry

extracts enriched in ellagitannins and ellagic acid derivatives have anti-neuroinflammatory properties. *Antioxidants*, **2020**, 9: 970. https://doi.org/10.3390/antiox9100970

[30] Piazza, S., Martinelli, G., Fumagalli, M., et al. Ellagitannins from *Castanea sativa* Mill. leaf extracts impair H. pylori viability and infection-induced inflammation in human gastric epithelial cells. *Nutrients*, **2023**, 15: 1504–1506. https://doi.org/10.3390/nu15061504

[31] Puljula, E., Walton, G., Woodward, M. J., et al. Antimicrobial activities of ellagitannins against *Clostridiales perfringens, Escherichia coli, Lactobacillus plantarum* and *Staphylococcus aureus*. *Molecules*, **2020**, 25: 3714. https://doi.org/10.3390/molecules25163714

[32] Cai, Y., Zhang, J., Chen, N. G., et al. Recent advances in anticancer activities and drug delivery systems of tannins. *Medicinal Research Reviews*, **2017**, 37: 665–701. https://doi.org/10.1002/med.21422

[33] Senobari, Z., Karimi, G., Jamialahmadi, K. Ellagitannins, promising pharmacological agents for the treatment of cancer stem cells. *Phytotherapy Research*, **2022**, 3: 231–242. https://doi.org/10.1002/ptr.7307

[34] Wang, S., Liu, S., Liu, C., et al. Affinity screening of potential anti-obesity and anti-diabetic component from pomegranate peel by co-immobilization of lipase and α-amylase using carbon nanotube and hydrogel. *Process Biochemistry*, **2023**, 126: 51–60. https://doi.org/10.1016/j.procbio.2023.01.002

[35] Li, H. Abnormal expression of bHLH3 disrupts a flavonoid homeostasis network, causing differences in pigment composition among mulberry fruits. *Horticulture Research*, **2020**, 7: 83. https://doi.org/10.1038/s41438-020-0302-8

[36] Fang, X. S., Wu, T., Liang, L. J. Broadly targeted metabolomics analysis of differential metabolites between light yellow and purple walnut endocarps. *Food Science*, **2021**, 42: 215–221

[37] Zeng, Y., Liu, H., Chen, S., et al. Transcriptome analysis of walnut quality formation and color change mechanism of pellicle during walnut development. *Gene Expression Patterns*, **2022**, 45: 119260. https://doi.org/10.1016/j.gep.2022.119260

[38] Wang, R., Tian, X., Li, Q., et al. Walnut pellicle color affects its phenolic composition: free, esterified and bound phenolic compounds in various colored-pellicle walnuts. *Journal of Food Composition and Analysis*, **2022**, 109: 104470. https://doi.org/10.1016/j.jfca.2022.104470

[39] Persic, M., Mikulic-Petkovsek, M., Halbwirth, H., et al. Red walnut: characterization of the phenolic profiles, activities and gene expression of selected enzymes related to the phenylpropanoid pathway in pellicle during walnut development. *Journal of Agricultural and Food Chemistry*, **2018**, 66: 2742–2748. https://doi.org/10.1021/acs.jafc.7b05603

[40] Akter, R., Chan Ahn, J., Nahar, J., et al. Pomegranate juice fermented by tannin acyl hydrolase and *Lactobacillus vespulae* DCY75 enhance estrogen receptor expression and anti-inflammatory effect. *Frontiers in Pharmacology*, **2022**, 13: 1010103. https://doi.org/10.3389/fphar.2022.1010103

[41] Henning, S. M., Summanen, P. H., Lee, R. P., et al. Pomegranate ellagitannins stimulate the growth of *Akkermansia muciniphila in vivo*. *Anaerobe*, **2017**, 43: 56–60. https://doi.org/10.1016/j.anaerobe.2016.12.003

[42] García-Villalba, R., Tomás-Barberán, F. A., Iglesias-Aguirre, C. E., et al. Ellagitannins, urolithins, and neuroprotection: human evidence and the possible link to the gut microbiota. *Molecular Aspects of Medicine*, **2023**, 89: 101109. https://doi.org/10.1016/j.mam.2022.101109

[43] Selma, M. V., Espín, J. C., Tomás-Barberán, F. A. Interaction between phenolics and gut microbiota: role in human health. *Journal of Agricultural and Food Chemistry*, **2009**, 57: 6485–6501. https://doi.org/10.1021/jf902107d

[44] Romo-Vaquero, M., García-Villalba, R., González-Sarrías, A., et al. Interindividual variability in the human metabolism of ellagic acid: contribution of gordonibacter to urolithin production. *Journal of Functional Foods*, **2015**, 17: 785–791. https://doi.org/10.1016/j.jff.2015.06.040

[45] Zhang, M., Cui, S., Mao, B., et al. Ellagic acid and intestinal microflora metabolite urolithin A: a review on its sources, metabolic distribution, health benefits, and biotransformation. *Critical Reviews in Food Science and Nutrition*, **2023**, 63: 6900–6922. https://doi.org/10.1080/10408398.2022.2036693

[46] Lin, I. C., Wu, J. Y., Fang, C. Y., et al. Absorption and metabolism of urolithin A and ellagic acid in mice and their cytotoxicity in human colorectal cancer cells. *Evidence-Based Complementary and Alternative Medicine*, **2023**: 1–11. https://doi.org/10.1155/2023/8264716

[47] Al-Harbi, S. A., Abdulrahman, A. O., Zamzami, M. A., et al. Urolithins: the gut based polyphenol metabolites of ellagitannins in cancer prevention, a review. *Frontiers in Nutrition*, **2021**, 8: 647582. https://doi.org/10.3389/fnut.2021.647582

[48] Yu, Z., Cao, M., Wang, Y., et al. Urolithin A attenuates helicobacter pylori-induced damage *in vivo*. *Journal of Agricultural and Food Chemistry*, **2022**, 70: 11981–11993. https://doi.org/10.1021/acs.jafc.2c03711

[49] Wang, L., Chen, J., Chen, Q., et al. The gut microbiota metabolite urolithin B prevents colorectal carcinogenesis by remodeling microbiota and PD-L1/HLA-B. *Oxidative Medicine and Cellular Longevity*, **2023**: 1–16. https://doi.org/10.1155/2023/6480848

[50] Kujawska, M., Jodynis-Liebert, J. Potential of the ellagic acid-derived gut microbiota metabolite–urolithin A in gastrointestinal protection. *World Journal of Gastroenterology*, **2020**, 26: 3170–3181. https://doi.org/10.3748/wjg.v26.i23.3170

[51] Xian, W., Deng, Y., Yang, Y., et al. Ameliorative effect of ellagic acid on aging in rats with the potential mechanism relying on the gut microbiota and urolithin A-producing ability. *Journal of Agricultural and Food Chemistry*, **2023**, 71: 7396–7407. https://doi.org/10.1021/acs.jafc.3c00960

[52] Cortés-Martín, A., García-Villalba, R., González-Sarrías, A., et al. The gut microbiota urolithin metabotypes revisited: the human metabolism of ellagic acid is mainly determined by aging. *Food & Function*, **2018**, 9: 4100–4106. https://doi.org/10.1039/C8FO00956B

[53] Selma, M. V., Romo-Vaquero, M., García-Villalba, R., et al. The human gut microbial ecology associated with overweight and obesity determines ellagic acid metabolism. *Food & Function*, **2016**, 7: 1769–1774. https://doi.org/10.1039/C5FO01100K

[54] Carregosa, D., Carecho, R., Figueira, I., et al. Low-molecular weight metabolites from polyphenols as effectors for attenuating neuroinflammation. *Journal of Agricultural and Food Chemistry*, **2020**, 68: 1790–1807. https://doi.org/10.1021/acs.jafc.9b02155

[55] Kim, J. M., Lee, U., Kang, J. Y., et al. Anti-amnesic effect of walnut via the regulation of BBB function and neuro-inflammation in Aβ1-42-induced mice. *Antioxidants*, **2020**, 9: 976. https://doi.org/10.3390/antiox9100976

[56] Abdelazeem, K. N. M., Kalo, M. Z., Beer-Hammer, S., et al. The gut microbiota metabolite urolithin A inhibits NF-κB activation in LPS stimulated BMDMs. *Scientific Reports*, **2021**, 11: 7117. https://doi.org/10.1038/s41598-021-86514-6

[57] Lee, G., Park, J. S., Lee, E. J., et al. Anti-inflammatory and antioxidant mechanisms of urolithin B in activated microglia. *Phytomedicine*, **2019**, 55: 50–57. https://doi.org/10.1016/j.phymed.2018.06.032

[58] Yu, Q. J., He, J. W., Liu, F., et al., Ellagic acid (EA), a tannin was isolated from *Eucalyptus citriodora* leaves and its anti-inflammatory activity. *Medicinal Chemistry Research*, **2021**, 30: 2277–2288. https://doi.org/10.1007/s00044-021-02806-6

[59] Piwowarski, J. P., Granica, S., Zwierzyńska, M., et al. Role of human gut microbiota metabolism in the anti-inflammatory effect of traditionally used ellagitannin-rich plant materials. *Journal of Ethnopharmacology*, **2014**, 155: 801–809. https://doi.org/10.1016/j.jep.2014.06.032

[60] Giménez-Bastida, J. A., González-Sarrías, A., Espín, J. C., et al.

Inhibition of 5-lipoxygenase-derived leukotrienes and hemiketals as a novel anti-inflammatory mechanism of urolithins. *Molecular Nutrition & Food Research*, **2020**, 64: 2000129. https://doi.org/10.1002/mnfr.202000129

[61] Xu, J., Yuan, C., Wang, G., et al. Urolithins attenuate LPS-induced neuroinflammation in BV2Microglia via MAPK, Akt, and NF-κB signaling pathways. *Journal of Agricultural and Food Chemistry*, **2018**, 66: 571–580. https://doi.org/10.1021/acs.jafc.7b03285

[62] Zhou, X. H., Qiao, Q., Zethelius, B., et al. Diabetes, prediabetes and cancer mortality. *Diabetologia*, **2010**, 53: 1867–1876. https://doi.org/10.1007/s00125-010-1796-7

[63] Li, W., Zhang, X., Sang, H., et al. Effects of hyperglycemia on the progression of tumor diseases. *Journal of Experimental & Clinical Cancer Research*, **2019**, 38: 327. https://doi.org/10.1186/s13046-019-1309-6

[64] Delgadillo-Puga, C., Torre-Villalvazo, I., Noriega, L. G., et al. Pecans and its polyphenols prevent obesity, hepatic steatosis and diabetes by reducing dysbiosis, inflammation, and increasing energy expenditure in mice fed a high-fat diet. *Nutrients*, **2023**, 15: 2591. https://doi.org/10.3390/nu15112591

[65] Shi, D., Chen, C., Zhao, S., et al. Walnut polyphenols inhibit pancreatic lipase activity *in vitro* and have hypolipidemic effect on high-fat diet-induced obese mice. *Journal of Food and Nutrition Research*, **2014**, 2: 757–763. https://doi.org/10.12691/jfnr-2-10-16

[66] Palabıyık, E., Sulumer, A. N., Uguz, H., et al. Assessment of hypolipidemic and anti-inflammatory properties of walnut (*Juglans regia*) seed coat extract and modulates some metabolic enzymes activity in triton WR-1339-INDUCED hyperlipidemia in rat kidney, liver, and heart. *Journal of Molecular Recognition*, **2023**, 36: e3004. https://doi.org/10.1002/jmr.3004

[67] Lü, O., Wang, L., Li, J., et al. Effects of pomegranate peel polyphenols on lipid accumulation and cholesterol metabolic transformation in L-02 human hepatic cells via the PPARγ-ABCA1/CYP7A1 pathway. *Food & Function*, **2016**, 7: 4976–4983. https://doi.org/10.1039/C6FO01261B

[68] Kang, I., Kim, Y., Tomás-Barberán, F. A., et al. Urolithin A, C, and D, but not iso-urolithin A and urolithin B, attenuate triglyceride accumulation in human cultures of adipocytes and hepatocytes. *Molecular Nutrition & Food Research*, **2016**, 60: 1129–1138. https://doi.org/10.1002/mnfr.201500796

[69] Heilmann, C., Ziebuhr, W., Becker, K., Are coagulase-negative staphylococci virulent? *Clinical Microbiology and Infection*, **2019**, 25: 1071–1080. https://doi.org/10.1016/j.cmi.2018.11.012

[70] Acquaviva, R., D'Angeli, F., Malfa, G. A., et al. Antibacterial and anti-biofilm activities of walnut pellicle extract (*Juglans regia* L.) against coagulase-negative staphylococci. *Natural Product Research*, **2021**, 35: 2076–2081. https://doi.org/10.1080/14786419.2019.1650352

[71] D'Angeli, F., Malfa, G. A., Garozzo, A., et al. Antimicrobial, antioxidant, and cytotoxic activities of *Juglans regia* L. pellicle extract. *Antibiotics*, **2021**, 10: 159. https://doi.org/10.3390/antibiotics10020159

[72] Virtanen, V., Räikkönen, S., Puljula, E., et al. Ellagitannin-lipid interaction by HR-MAS NMR spectroscopy. *Molecules*, **2021**, 26: 373. https://doi.org/10.3390/molecules26020373

[73] Catanzaro, E., Greco, G., Potenza, L., et al. Natural products to fight cancer: a focus on *Juglans regia*. *Toxins*, **2018**, 10: 469. https://doi.org/10.3390/toxins10110469

[74] Lee, M., Li, J., Li, J., et al. Tet2 inactivation enhances the antitumor activity of tumor-infiltrating lymphocytes. *Cancer Research*, **2021**, 81: 1965–1976. https://doi.org/10.1158/0008-5472.CAN-20-3213

[75] Yang, L., Ma, S., Han, Y., et al. Walnut polyphenol extract attenuates immunotoxicity induced by 4-pentylphenol and 3-methyl-4-nitrophenol in murine splenic lymphocyte. *Nutrients*, **2016**, 8: 287. https://doi.org/10.3390/nu8050287

[76] Ceci, C., Tentori, L., Atzori, M., et al. Ellagic acid inhibits bladder cancer invasiveness and *in vivo* tumor growth. *Nutrients*, **2016**, 8: 744. https://doi.org/10.3390/nu8110744

[77] Zhao, M., Tang, S. N., Marsh, J. L., et al. Ellagic acid inhibits human pancreatic cancer growth in BALB/c nude mice. *Cancer Letters*, **2013**, 337: 210–217. https://doi.org/10.1016/j.canlet.2013.05.009

[78] Mishra, S., Vinayak, M. Ellagic acid checks lymphoma promotion via regulation of PKC signaling pathway. *Molecular Biology Reports*, **2013**, 40: 1417–1428. https://doi.org/10.1007/s11033-012-2185-8

[79] Mishra, S., Vinayak, M. Role of ellagic acid in regulation of apoptosis by modulating novel and atypical PKC in lymphoma bearing mice. *BMC Complementary and Alternative Medicine*, **2015**, 15: 281. https://doi.org/10.1186/s12906-015-0810-5

[80] Vanella, L., Di Giacomo, C., Acquaviva, R., et al. Apoptotic markers in a prostate cancer cell line: effect of ellagic acid. *Oncology Reports*, **2013**, 30: 2804–2810. https://doi.org/10.3892/or.2013.2757

[81] Eskandari, E., Heidarian, E., Amini, S., et al. Evaluating the effects of ellagic acid on pSTAT3, pAKT, and pERK1/2 signaling pathways in prostate cancer PC3 cells. *Journal of Cancer Research and Therapeutics*, **2016**, 12: 1266. https://doi.org/10.4103/0973-1482.165873

[82] Wang, D., Chen, Q., Liu, B., et al. Ellagic acid inhibits proliferation and induces apoptosis in human glioblastoma cells. *Acta Cirurgica Brasileira*, **2016**, 31: 143–149. https://doi.org/10.1590/S0102-865020160020000010

[83] Xu, W., Xu, J., Wang, T., et al. Ellagic acid and sennoside B inhibit osteosarcoma cell migration, invasion and growth by repressing the expression of c-Jun. *Oncology letters*, **2018**, 16: 898–904. https://doi.org/10.3892/ol.2018.8712

[84] Duan, J., Li, Y., Gao, H., et al. Phenolic compound ellagic acid inhibits mitochondrial respiration and tumor growth in lung cancer. *Food & Function*, **2020**, 11: 6332–6339. https://doi.org/10.1039/D0FO01177K

[85] Ho, C. C., Huang, A. C., Yu, C. S., et al. Ellagic acid induces apoptosis in tsgh8301 human bladder cancer cells through the endoplasmic reticulum stress- and mitochondria-dependent signaling pathways: ellagic acid induces apoptosis in TSGH8301 cells. *Environmental Toxicology*, **2013**, 29: 1262−1274. https://doi.org/10.1002/tox.21857

[86] Edderkaoui, M., Odinokova, I., Ohno, I., et al. Ellagic acid induces apoptosis through inhibition of nuclear factor κB in pancreatic cancer cells. *World Journal of Gastroenterology*, **2008**, 14: 3672. https://doi.org/10.3748/wjg.14.3672

[87] Naiki-Ito, A., Chewonarin, T., Tang, M., et al. Ellagic acid, a component of pomegranate fruit juice, suppresses androgen-dependent prostate carcinogenesis via induction of apoptosis: PFJ and EA prevent prostate cancer. *The Prostate*, **2015**, 75: 151–160. https://doi.org/10.1002/pros.22900

[88] Chen, H. S., Bai, M. H., Zhang, T., et al. Ellagic acid induces cell cycle arrest and apoptosis through TGF-β/Smad3 signaling pathway in human breast cancer MCF-7 cells. *International Journal of Oncology*, **2015**, 46: 1730–1738. https://doi.org/10.3892/ijo.2015.2870

[89] Zhang, Y., Li, Q., Niu, Y., et al. Research progress on aging mechanism and drugs and the role of stem cells in anti-aging process. *Experimental Gerontology*, **2023**, 179: 112248. https://doi.org/10.1016/j.exger.2023.112248

[90] de Grey, A. D. N. J. Free radicals in aging: causal complexity and its biomedical implications. *Free Radical Research*, **2006**, 40: 1244–1249. https://doi.org/10.1080/10715760600913176

[91] Perez-Campo, R., López-Torres, M., Cadenas, S., et al. The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, **1998**, 168: 149–158. https://doi.org/10.1007/s003600050131

[92] Luo, J., Si, H., Jia, Z., et al. Dietary anti-aging polyphenols and potential mechanisms. *Antioxidants*, **2021**, 10: 283. https://doi.org/

10.3390/antiox10020283

[93] Tian, W., Wu, B., Sun, L., et al. Protective effect against *D*-gal-induced aging mice and components of polypeptides and polyphenols in defatted walnut kernel during simulated gastrointestinal digestion. *Journal of Food Science*, **2021**, 86: 2736–2752. https://doi.org/10.1111/1750-3841.15744

[94] Zhang, M., Tang, X., Mao, B., et al. Inhibition of the NF-κB and mTOR targets by urolithin A attenuates *D*-galactose-induced aging in mice. *Food & Function*, **2023**, 14: 10375–10386. https://doi.org/10.1039/D3FO03847E

[95] Rahimi, V. B., Askari, V. R., Mousavi, S. H. Ellagic acid reveals promising anti-aging effects against *D*-galactose-induced aging on human neuroblastoma cell line, SH-SY5Y: a mechanistic study. *Biomedicine & Pharmacotherapy*, **2018**, 108: 1712–1724. https://doi.org/10.1016/j.biopha.2018.10.024

[96] Ryu, D., Mouchiroud, L., Andreux, P. A., et al. Urolithin A induces mitophagy and prolongs lifespan in *C. elegans* and increases muscle function in rodents. *Nature Medicine*, **2016**, 22: 879–888. https://doi.org/10.1038/nm.4132

[97] Zhang, M., Cui, S., Mao, B., et al., Urolithin A produced by novel microbial fermentation possesses anti-aging effects by improving mitophagy and reducing reactive oxygen species in *Caenorhabditis elegans*. *Journal of Agricultural and Food Chemistry*, **2023**, 71: 6348–6357. https://doi.org/10.1021/acs.jafc.3c01062

[98] Mao, X., Hua, Y. Composition, structure and functional properties of protein concentrates and isolates produced from walnut (*Juglans regia* L.). *International Journal of Molecular Sciences*, **2012**, 13: 1561–1581. https://doi.org/10.3390/ijms13021561

[99] Gu, M., Chen, H. P., Zhao, M. M., et al. Identification of antioxidant peptides released from defatted walnut (*Juglans Sigillata Dode*) meal proteins with pancreatin. *LWT-Food Science and Technology*, **2015**, 60: 213–220. https://doi.org/10.1016/j.lwt.2014.07.052

[100] Wang, J., Wu, T., Fang, L., et al. Anti-diabetic effect by walnut (*Juglans mandshurica* Maxim.)-derived peptide LPLLR through inhibiting α-glucosidase and α-amylase, and alleviating insulin resistance of hepatic HepG2 cells. *Journal of Functional Foods*, **2020**, 69: 103944. https://doi.org/10.1016/j.jff.2020.103944

[101] Wu, W., Zhao, S., Chen, C., et al. Optimization of production conditions for antioxidant peptides from walnut protein meal using solid-state fermentation. *Food Science and Biotechnology*, **2014**, 23: 1941–1949. https://doi.org/10.1007/s10068-014-0265-3

[102] Liu, R., Wu, L., Du, Q., et al. Small molecule oligopeptides isolated from walnut (*Juglans regia* L.) and their anti-fatigue effects in mice. *Molecules*, **2018**, 24: 45. https://doi.org/10.3390/molecules24010045

[103] Rohn, S. Possibilities and limitations in the analysis of covalent interactions between phenolic compounds and proteins. *Food Research International*, **2014**, 65: 13–19. https://doi.org/10.1016/j.foodres.2014.05.042

[104] Shen, P., Gao, Z., Xu, M., et al. The impact of hempseed dehulling on chemical composition, structure properties and aromatic profile of hemp protein isolate. *Food Hydrocolloids*, **2020**, 106: 105889. https://doi.org/10.1016/j.foodhyd.2020.105889

[105] Labuckas, D. O., Maestri, D. M., Perelló, M., et al. Phenolics from walnut (*Juglans regia* L.) kernels: antioxidant activity and interactions with proteins. *Food Chemistry*, **2008**, 107: 607–612. https://doi.org/10.1016/j.foodchem.2007.08.051

[106] Wu, Q., Lü, D., Mao, X. Polyphenol removal with ultrasound-assisted ethanol extraction from defatted walnut powder: optimization of conditions and effect on functional properties of protein isolates. *Journal of Agricultural and Food Chemistry*, **2023**, 103: 6995–7005. https://doi.org/10.1002/jsfa.12782

[107] Wang, Y., Tan, B., Chen, C., et al. The phenolic profile of walnut meal protein isolate and interaction of phenolics with walnut protein. *Food Research International*, **2023**, 170: 113042. https://doi.org/10.1016/j.foodres.2023.113042

[108] Su, G., Chen, J., Huang, L., et al. Effects of walnut seed coat polyphenols on walnut protein hydrolysates: structural alterations, hydrolysis efficiency, and acetylcholinesterase inhibitory capacity. *Food Chemistry*, **2024**, 437: 137905. https://doi.org/10.1016/j.foodchem.2023.137905

[109] Huang, X., Yan, C., Lin, M., et al. The effects of conjugation of walnut protein isolate with polyphenols on protein solubility, antioxidant activity, and emulsifying properties. *Food Research International*, **2022**, 161: 111910. https://doi.org/10.1016/j.foodres.2022.111910

[110] Kong, X., Huang, Z., Zhang, C., et al. Phenolic compounds in walnut pellicle improve walnut (*Juglans regia* L.) protein solubility under pH-shifting condition. *Food Research International*, **2023**, 163: 112156. https://doi.org/10.1016/j.foodres.2022.112156