

REVIEW

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Fermentation as a strategy to improve the bioactive and antioxidant properties of walnuts and their derived products

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Abstract

Oxidative stress is a significant factor in the development of many chronic illnesses, and antioxidants play a vital role in mitigating its effects. The walnut (*Juglans regia* L.) is widely recognized for its diverse range of bioactive compounds, particularly polyphenols, flavonoids, and tocopherols, which exhibit notable antioxidant properties. These beneficial compounds are found in various walnut-based products, including milk, yogurt, cheese substitutes, flowers, protein meals, oils, and extracts from components such as the husk, pellicle, and leaves. Previous studies demonstrated that microbial fermentation using probiotic or non-probiotic starter cultures may significantly enhance the antioxidant capacity of walnut-based products. Outcomes are influenced by factors such as the type of walnut tissue used, the fermentation conditions, the choice of microbial strain, and the extraction method. This review critically summarizes and evaluates current findings on the impact of fermentation on the antioxidant properties of walnuts and walnut-derived products.

Highlights

- Fermentation boosts antioxidant release in walnut products by enhancing phenolic bioavailability.
- Enhanced antioxidant activity varies by walnut tissue, extraction method, microbial strain, and fermentation conditions.
- Findings support developing functional fermented walnut products with enhanced bioactivity.

Keywords Antioxidants, Walnut, Walnut-derived products, Fermentation, Probiotics

1 Introduction

In recent years, antioxidants have garnered increasing attention in the scientific community due to their critical role in combating oxidative stress and maintaining both chemical and biological equilibrium in food systems and living organisms [1]. Oxidative stress is the result of an imbalance between the generation of reactive oxygen species (ROS) and the body's antioxidant defence mechanisms. This imbalance leads to cellular damage and is associated with the progression of various chronic diseases,



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including cancer, cardiovascular disorders, neurodegenerative conditions, and metabolic syndromes [2, 3]. Among natural sources, plant-derived antioxidants have emerged as highly promising due to their effectiveness, accessibility, and favourable safety profiles. Phenolic compounds are recognized for their ability to neutralize free radicals, chelate transition metals, and modulate intracellular signalling pathways that are implicated in oxidative damage [4, 5]. These characteristics make them ideal candidates for applications in nutraceuticals and pharmaceuticals [6]. Walnut (*Juglans regia* L.) is one of the most widely cultivated tree nuts worldwide, with major production in southern Europe, northern Africa, eastern Asia, the United States, western South America, and especially China, the world's leading producer [7, 8]. It is valued for its sensory appeal, nutritional richness, and documented health benefits [9]. Several studies have shown that walnuts contain a diverse array of bioactive compounds, including proteins, unsaturated fatty acids, and plant sterols, which contribute to their antioxidant, anti-inflammatory, and other biological activities [10]. The walnut kernels are particularly rich in proteins, fats, dietary fiber, vitamins, minerals, and phenolic compounds. To improve the functional potential of plant-based products like walnuts, fermentation using probiotics has proven to be a valuable approach [11]. Specific probiotic strains, notably *Lactobacillus* and *Streptococcus thermophilus*, initiate enzymatic processes during fermentation that convert complex plant molecules into simpler, more easily absorbed compounds [12, 13]. This conversion enhances the availability of antioxidant substances, such as flavonoids and phenolics, and alters their molecular structure in ways that increase their solubility and antioxidant performance [14]. Therefore, this review critically summarizes and evaluates current findings on the impact of fermentation on the antioxidant properties of walnuts and walnut-derived products.

2 Antioxidants

ROS are highly reactive molecules containing oxygen that are generated as natural byproducts of cellular metabolism, particularly during mitochondrial respiration [15]. These oxygen-based molecules, though essential in moderate concentrations for signalling and defence mechanisms, become harmful when produced in excess. Elevated ROS levels can disrupt cellular structures by oxidizing nucleic acids, proteins, and lipids, a phenomenon widely recognized as oxidative stress [4, 15, 16]. Persistent oxidative stress is closely associated with the onset and progression of chronic conditions such as atherosclerosis, cancer, neurodegeneration, and metabolic syndromes [2, 3]. The body's defence against this imbalance involves various antioxidants, which function by neutralizing ROS and maintaining redox stability. These antioxidants can be endogenous, such as enzymes like superoxide dismutase and catalase, or exogenous, obtained from diet and supplements [15, 17]. Among dietary antioxidants, those derived from plants are especially valued due to their high bioactivity, natural origin, and wide range of protective functions. Compounds such as polyphenols, flavonoids, and vitamins from fruits, vegetables, nuts, and herbs offer strong defence against oxidative damage [17–19]. Compared to synthetic antioxidants such as butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT), which are often used industrially to preserve food or pharmaceuticals, plant-based antioxidants tend to be safer and offer additional biological benefits, including anti-inflammatory and anti-aging properties [20–22]. To measure and compare antioxidant capacities across different samples, researchers employ a

diverse set of in vitro assays based on distinct reaction mechanisms [17]. Among the most frequently utilized methods are the DPPH•(2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, ABTS•+ (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) assay, ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC), and the Folin–Ciocalteu total phenolic content assay [17–19]. The accuracy and reproducibility of antioxidant assessment heavily rely on standardized protocols and the selection of appropriate methodologies, particularly when comparing isolated compounds to complex plant extracts [20]. Furthermore, external variables such as solvent polarity, pH levels, and reaction temperature can significantly impact antioxidant activity and assay outcomes, reinforcing the need for optimized and consistent testing conditions [23]. In addition to these chemical assays, cell-based antioxidant evaluations and electrochemical or voltammetry-based methods have been increasingly adopted to provide more physiologically relevant and sensitive assessments of antioxidant behavior [24, 25]. Overall, antioxidants represent a vital defence mechanism against oxidative stress and hold substantial therapeutic promise. Their wide-ranging applications in the food, pharmaceutical, and cosmetic industries further highlight the importance of ongoing research into their mechanisms of action and the continued refinement of evaluation methods [15].

3 Probiotics and antioxidants in fermented plant-based foods

Probiotics are live microorganisms that, when ingested in sufficient quantities, offer health benefits to the host by maintaining a balanced gut microbiota and supporting immune function [26]. The most commonly used strains include members of the genera *Lactobacillus* and *Bifidobacterium*. These microbes are frequently incorporated into functional foods due to their proven ability to improve digestive health, inhibit harmful bacteria, and regulate immune responses [27, 28]. Historically, fermented dairy products like yogurt, kefir, and cultured milk have served as the primary delivery systems for probiotics [29]. These products not only provide an environment that supports probiotic viability but also enhance milk's flavor, digestibility, and nutritional value [12]. However, rising consumer interest in plant-based, lactose-free, and allergen-friendly diets has driven the innovation of non-dairy probiotic foods [30]. Probiotic fermentation is now widely applied to plant-based foods, including soy, almond, oat, walnut [31, 32], vegetables (such as kimchi and sauerkraut), legumes (like tempeh), cereal-based foods (including sourdough and fermented grains), and fruit-derived beverages [33, 34]. This process improves their shelf life and sensory qualities while also enriching their nutritional and functional properties. During fermentation, probiotics break down complex nutrients and phytochemicals into simpler, more absorbable forms, such as short-chain fatty acids and phenolic compounds with antioxidant activity [35]. Fermented plant-derived foods are now recognized for their elevated antioxidant capacity, which stems from microbial transformation of phytochemicals into forms that are more easily absorbed and biologically active [36]. This benefit is particularly evident in fermented grains and cereals, including oats, barley, and brown rice foods that naturally contain significant levels of polyphenols, flavonoids, and phenolic acids. Through fermentation, these compounds become more accessible and potent, thereby improving the food's antioxidant profile and its potential in protecting against oxidative stress and chronic disease [37]. Considering this, increasing research attention has been directed toward the antioxidant potential

of fermented walnuts, a nutrient-dense nut that contains high levels of polyunsaturated fats and phenolic compounds. These bioactive compounds can be further released and activated through fermentation, enhancing the walnut's functional value and health-promoting properties [10, 38].

4 Walnut and walnut-based products

Walnuts (*Juglans regia* L.) rank among the most extensively cultivated and consumed tree nuts worldwide, appreciated for their distinct flavor, nutritional richness, and recognized health-promoting attributes. Originally native to regions of Central Asia and the Mediterranean, they are now grown extensively, with top producers including China, the United States, and Iran [7, 8]. From a nutritional standpoint, walnuts are a notable source of alpha-linolenic acid (ALA), a plant-derived omega-3 fatty acid, alongside high-quality proteins, dietary fiber, vitamin E, magnesium, and a diverse array of polyphenolic compounds [39]. These nutrients collectively contribute to their antioxidant, anti-inflammatory, cardioprotective, and neuroprotective potential [10]. To meet the increasing interest in plant-based and health-oriented diets, a diverse selection of walnut-based innovations has entered the market [40]. These include walnut milk, which serves as a nutrient-rich dairy alternative; fermented walnut beverages enriched with probiotics to enhance antioxidant capacity; walnut oil valued for its unsaturated fatty acid profile and phenolic content; and walnut flour and protein isolates, both widely used in gluten-free, high-protein, or therapeutic food formulations [41–43]. Moreover, previously overlooked parts of the walnut tree, such as the husk, skin (pellicle), and leaves, are now gaining attention for their antioxidant and antimicrobial functions, finding new applications in food preservation, skincare, and pharmaceuticals [38, 44]. Advancing the use of walnut-derived ingredients not only enhances consumer health outcomes but also promotes resource efficiency and sustainability within the walnut industry, positioning the nut as a core element in the future of functional food design [32].

5 Antioxidants in fermented and non-fermented walnuts and walnut-based products

Walnuts are highly valued for their dense nutritional composition and notable antioxidant capacity [45]. They are especially rich in bioactive molecules such as polyphenols, flavonoids, and tocopherols, which contribute significantly to the scavenging of reactive oxygen species (ROS) and the mitigation of oxidative stress within biological systems [10, 38]. Notably, among various types of nuts, walnuts rank among the highest in overall antioxidant content. This property is largely attributed to the phenolic compounds concentrated in the walnut's outer layer, known as the pellicle or seed coat [46]. The total phenolic in 50 g of walnuts (about a handful) is higher than a glass of apple juice (240 mL), 43 g of milk chocolate, or a glass of red wine (150 mL [47]).

Multiple studies have demonstrated that fermentation significantly enhances the antioxidant properties of walnut-derived products (Table 1). For instance, Popovici et al. [48] reported that fermenting walnut cheese with 1.5% instant yeast elevated the DPPH radical scavenging activity from a low 2.24% in the control sample to an impressive 94.47% after 12 h of fermentation. Similarly, Zhong et al. [43] utilized *Lactobacillus paracasei* to ferment walnut milk, resulting in the production of antioxidant peptides. The diprotein extracts obtained exhibited DPPH and ABTS scavenging activities of 77.63% and

Table 1 Antioxidant activities of fermented and non-fermented walnuts and walnut-based products

Sample	Starter culture	Extraction	Method	Controls values	Treatments values	Fermentation (temp./time) & storage (temp./period)	Significant level (p)	Citation
Walnut cheese	Instant yeast (1.5%)	Water	DPPH	Walnut milk 2.24%	94.47%	NM	(p<0.05)	[48]
Fermented walnut milk	<i>Lactobacillus paracasei</i> (SMN-LBK)	Diprotein antioxidant peptides	DPPH ABTS Metal ion chelating	Single protein antioxidant peptides: DPPH=21.90% ABTS=57.02% Metal ion=25.40%	DPPH=77.63% ABTS=93.31% Metal ion=79.37%	4 °C/12 h 37 °C/12 h –	(p<0.05)	[43]
Walnut yogurt	<i>Lactiplantibacillus plantarum</i> LP16	Water	DPPH ABTS	Unfermented walnut milk: DPPH=87.34% ABTS=92.98%	DPPH=94.61% ABTS=93%	37 °C/ 11.36 h 4 °C/24 h	(p<0.05)	[49]
Fermented walnut flower	<i>Lactiplantibacillus plantarum</i> (SWFU) <i>Lactiplantibacillus plantarum</i> (ATCC) <i>Lactiplantibacillus casei</i> (ATCC) <i>Lactiplantibacillus bulgaricus</i> (CICC) <i>Lactiplantibacillus rhamnosus</i> (ATCC)	Water	DPPH ABTS FRAP	Fermented walnut flower + carbon + nitrogen DPPH:	DPPH	37 °C/ 7 day –	(p<0.05)	[38]

Table 1 (continued)

Sample	Starter culture	Extraction	Method	Controls values	Treatments values	Fermentation (temp./time) & storage (temp./period)	Significant level (p)	Citation
Walnut protein meal	<i>Bacillus subtilis</i>	Water	DPPH Fe ²⁺ ion chelating	SWFU= ~95%	SWFU= ~99%	Day 4		
				ATCC8014= ~111%	ATCC8014= ~123%	Day 1		
				CICC= ~45%	CICC= ~95%	Day 1		
				ATCC334= ~197%	ATCC334= ~95%	Day 1		
				ATCC53013= ~ 53%	ATCC53013= ~115%	Day 1		
				ABTS:	ABTS:			
				SWFU = ~ 55%	SWFU= ~85%	Day 2		
				ATCC8014 = ~100%	ATCC8014= ~124%	Day 1		
				CICC = ~80%	CICC= ~85%	Day 3		
				ATCC334 = ~ 92%	ATCC334= ~95%	Day 1		
Whey-based beverage + walnut oil	<i>Streptococcus thermophilus</i> , <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	Isopropanol	DPPH	ATCC53013= ~105%	ATCC53013= ~122%	Day 7		
				FRAP:	FRAP:			
				SWFU= ~72%	SWFU= ~115%	Day 1		
				ATCC8014= ~126%	ATCC8014= ~128%	Day 1		
				CICC= ~55%	CICC= ~85%	Day 1		
				ATCC334= ~98%	ATCC334= ~85%	Day 1		
				ATCC53013= ~49%	ATCC53013= ~115%	Day 6		
				DPPH:	DPPH= 87.79% at 1.0	37 °C/ 82.01 h	(p<0.05)	[50]
				VC= ~72%	mg/ml concentration	–		
				GSH= ~65%	Fe ²⁺ ion= 87% at 1.4-2.0			
Walnut meal + soy sauce	<i>Aspergillus oryzae</i>	Water	DPPH	Fe ²⁺ ion:	mg/ml concentration			
				EDTA= ~95–96%				
				GSH= ~65–70%				
				Non-lipolyzed	Lipolyzed oil	42 °C/ 24 h	(p<0.05)	[41]
				DPPH = 37.5%	DPPH = 40.4%	–		
Walnut meal + soy sauce	<i>Aspergillus oryzae</i>	Water	DPPH	VC= ~95–100%	DPPH= ~75–80% at 0.35	45 °C/ 6 days	(p<0.05)	[42]
				BHT= ~85–90%	mg/ml concentration	4 °C/ 8 weeks		

Table 1 (continued)

Sample	Starter culture	Extraction	Method	Controls values	Treatments values	Fermentation (temp./time) & storage (temp./period)	Significant level (p)	Citation
Walnut pellicle extract	–	Ethanol	DPPH	–	DPPH = 48.35 ± 1.7 µg/ml	–	(p < 0.05)	[46]
Green walnut husk	–	Ethanol	DPPH ABTS FRAP	–	DPPH = 0.40 mgTE/g ABTS = 0.90 mgTE/g FRAP = 0.61 mgTE/g	–	(p < 0.05)	[51]
Walnut leaves genotypes	–	Methanol	DPPH FRAP	–	Suleiman DPPH = 75.50% Tuttle FRAP = 148.1 µM Fe ²⁺ /g DW	–	(p < 0.05)	[52]
<i>Juglans regia</i> and <i>J. australis</i> walnut shells	–	Methanol (MeOH) Ethyl acetate (AcOEt)	DPPH FRAP	–	DPPH (mM Trolox /100 g DW) <i>J. australis</i> MeOH = 9.7 AcOEt = 2.06 <i>J. regia</i> MeOH = 11.5 AcOEt = 2.0 FJ. australisRAP (mM Trolox /100 g DW) MeOH = 56.8 AcOEt = 5.1 <i>J. regia</i> MeOH = 43.1 AcOEt = 13.8	–	(p < 0.05)	[53]

DPPH: 2,2-Diphenyl-1-picrylhydrazyl, ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), FRAP: Ferric Reducing Antioxidant Power, NM: Not mention, -: Not Applicable, Fe²⁺: Ferrous ion, GSH: Glutathione, VC: Vitamin C, EDTA: Ethylenediaminetetraacetic acid, BHT: butylated hydroxyl toluene, MeOH: methanol, mg/ml: milligrams per milliliter, µg/ml: micrograms per milliliter, mgTE/g: Milligrams Trolox Equivalent per gram, µM Fe²⁺/g DW: micromolar ferrous iron per gram dry weight, mM: millimolar, µmol: micromole, mmol Fe²⁺/g DE: millimole ferrous iron gram of dry extract

93.31%, respectively, along with strong metal ion chelating ability (79.37%), indicating that the combination of protein fermentation and bioactive peptide extraction can produce potent antioxidant effects (Table 1). Further supporting these findings, Zhai et al. [49] demonstrated the beneficial impact of fermentation on walnut yogurt. The DPPH scavenging activity increased from 87.34% in unfermented milk to 94.61% in yogurt fermented with *Lactiplantibacillus plantarum* LP16. ABTS activity remained high at approximately 93%, suggesting that fermentation not only preserves but may slightly improve antioxidant efficacy by facilitating the release or transformation of phenolic compounds into more active or bioavailable forms (Table 1).

The influence of different probiotic strains on the antioxidant capacity of fermented walnut flowers has been investigated by Ru et al. [38]. They found that *Lactiplantibacillus plantarum* ATCC8014 yielded the highest antioxidant values with DPPH (123%), ABTS (124%), and FRAP (128%). Other strains, such as ATCC53013 and SWFU, also produced consistently elevated antioxidant activities, whereas the CICC strain showed significantly lower results across all assays. These observations highlight that the antioxidant potential of fermented walnut flowers is highly dependent on the microbial strain used, emphasizing the importance of strain selection in the development of functional foods. In addition, walnut protein meal (WPM) fermented with *Bacillus subtilis* has been reported to have DPPH activity reaching 87.79%, alongside metal ion chelation of 87% at optimal concentrations [50]. These antioxidant levels were comparable to or exceeded those of common antioxidants such as vitamin C and glutathione. Conversely, Moslemi et al. [41] observed only modest improvements in DPPH activity (40.4%) when walnut oil was lipolyzed compared to the control (37.5%) within a whey matrix fermented by *Streptococcus thermophilus* and *Lactobacillus delbrueckii*, suggesting that fermentation of protein-based substrates yields more pronounced antioxidant enhancements than oil-based systems (Table 1).

Walnut meal fermented with soy sauce using *Aspergillus oryzae*, reported DPPH values between 75% and 80% at 0.35 mg/mL concentration [42]. Although lower than the vitamin C or BHT controls, these results still indicate substantial antioxidant capacity. On the other hand, alcohol-based extraction from walnut pellicle showed moderate DPPH activity (48.35 µg/mL; [46]), whereas green walnut husk extracts showed higher antioxidant activities, with DPPH (0.40 mg TE/g), ABTS (0.90 mg TE/g), and FRAP (0.61 mg TE/g), emphasizing the variability in antioxidant potential depending on the walnut part and extraction methodology [51]. Ara et al. [52] examined walnut leaves and reported genotype-dependent variations in antioxidant capacity. For example, genotypes Suleiman and Tuttle reached DPPH values of 75.50% and FRAP levels of 148.1 µM Fe²⁺/g DW, respectively. Similarly, Orce et al. [53] found species-related differences between *Juglans regia* and *J. australis*, where methanol and ethyl acetate extracts of walnut shells exhibited moderate DPPH and FRAP activities. These findings highlight that both genetic factors and extraction solvents substantially influence antioxidant capacity (Table 1).

Fermented walnut-based products exhibit superior antioxidant activity compared with non-fermented matrices. This effect is largely mediated by microbial enzymatic processes during fermentation that release bound phenolics and generate bioactive antioxidant peptides [38]. By contrast, non-fermented walnut tissues and industrial by-products (e.g., green husk, pellicle, and leaves) display variable and generally lower

antioxidant capacities that depend strongly on tissue type and extraction solvent [44, 46, 51]. Nevertheless, accumulating evidence demonstrated that fermentation is markedly more effective in enhancing both antioxidant concentration and bioaccessibility [51].

6 Conclusion

Walnut-based products, particularly walnut milk, are recognized for their high antioxidant potential due to the presence of bioactive constituents such as polyphenols, flavonoids, and tocopherols. The antioxidant performance of these products is significantly influenced by the walnut part utilized, the extraction method applied, and the incorporation of fermentation. Employing specific probiotic strains during fermentation enhances the release and bioavailability of antioxidant compounds, contributing to improved functional characteristics. Furthermore, variations in antioxidant levels among different walnut tissues and cultivars emphasize the importance of selecting appropriate raw materials. Prospective research should aim to identify probiotic strains with strong enzymatic potential that can convert complex phenolic compounds in walnut matrices into simpler, more effective forms. Integrative techniques such as metagenomics and metabolomics could provide insights into microbial interactions during fermentation and help pinpoint the bacterial species responsible for improved antioxidant activity. Exploring the fermentation behaviour of individual strains to specific walnut types may offer strategies to boost antioxidant yield. Moreover, advancements in synthetic biology and precision fermentation may allow for the design of microbial communities tailored to enhance targeted antioxidant pathways. These developments could support the creation of fortified or fermented walnut beverages with advanced health-promoting properties, maximizing the functional value of walnut-derived foods. As a limitation, most studies rely predominantly on in vitro assays, with limited in vivo or clinical validation, leaving the physiological relevance of observed improvements uncertain. Moreover, the precise mechanisms by which fermentation enhances the bioavailability and activity of phenolics, peptides, and other bioactive compounds remain incompletely understood, and interactions between specific probiotic strains and walnut compounds are largely unexplored. Collectively, these limitations underscore the need for standardized fermentation protocols, integrative multi-omics analyses, and in vivo or clinical studies to elucidate mechanisms, optimize functional properties, and validate potential health benefits.

Acknowledgements

Acknowledgements This project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant No. (IPP: 77-247-2025). The authors, therefore, gratefully acknowledge the DSR technical and financial support.

Author contributions

Fai Abdulhadi Al-Subaie : Resources, Formal analysis, Data curation, Validation, Software, Writing – original draft. Amal Bakr Shori: Conceptualization, Visualization, Project administration, Supervision, Data curation, Writing – original draft, Writing – review & editing.

Funding

This work was supported by the King Abdulaziz University (grant number IPP: 77-247-2025).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable. This review does not contain any identifiable personal data.

Competing interests

The authors declare no competing interests.

Received: 23 June 2025 / Accepted: 23 December 2025

Published online: 05 January 2026

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