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Upcycling walnut (*Juglans regia* L.) by-products: characterisation of nutritionally relevant bioactive compounds

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Walnuts (*Juglans regia* L.) are attracting increasing interest from consumers, industries, and the healthcare community due to their high content of bioactive compounds, including (poly)phenols, carotenoids, tocopherols, and omega-3 fatty acids. Despite the continued growth in global walnut production, the effective valorisation of walnut processing by-products remains a challenge. While most research has focused on the edible kernel, emerging evidence suggests that underutilised walnut fractions may also represent valuable sources of compounds with antioxidant and anti-inflammatory properties. This study aimed to characterise the bioactive compound profiles of green walnuts, defective walnuts, and walnut shells in comparison with commercial whole walnuts and kernels. Samples collected from an orchard located in Huesca (Spain) were homogenized and analysed in triplicate. Proximate composition was determined using official methods, the fatty acid profile was determined by GC-FID, the phenolic profile by UHPLC-ESI-MS/MS, and carotenoids and tocopherols by UPLC-DAD. Green walnuts contained exceptionally high concentrations of carotenoids, especially β-carotene (629.7 ± 39.4 mg kg^{-1}). Walnut shells were the richest source of phenolic compounds, notably ellagic acid (713.3 ± 7.6 mg kg^{-1}) and catechin (71.9 ± 1.2 mg kg^{-1}). Tocopherols were mainly concentrated in the kernel, dominated by γ-tocopherol (226.9 ± 8.1 mg kg^{-1}), while defective walnuts showed a compositional profile similar to commercial walnuts. This comparative analysis highlights the significant potential of walnut processing by-products as sources of nutritionally relevant bioactive compounds. These findings support their valorisation as functional ingredients for agro-food and cosmetic applications within a circular economy framework.

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1. Introduction

Walnuts (*Juglans regia* L.) are increasingly recognised for their nutritional value and versatile applications, attracting growing interest from consumers, the healthcare community, and the food industry. The health-promoting effects of regular walnut consumption are well documented in epidemiological studies, particularly regarding improvements in cognitive performance and sleep quality,[1,2] as well as associations with a reduced risk of cardiovascular and neurodegenerative diseases.[3,4] These benefits are largely attributed to the diverse nutrients present in walnuts, including omega-3 fatty acids (notably α-linolenic acid)[5,6] and antioxidant bioactive compounds such as (poly) phenols, carotenoids, and tocopherols.[7–9]

In response to growing demand, global walnut production has risen steadily, reaching 3.9 million tonnes in 2023 according to the Food and Agriculture Organization of the United Nations. As walnuts are commercialized as whole fruits or isolated kernels, their processing generates various by-products. Consequently, increasing attention is being directed toward the effective upcycling and valorisation of these materials due to their economic and nutritional value.[10,11] These by-products are predominantly the green husk and the walnut shell, which together account for approximately 60–70% of the fresh walnut weight,[12] and also include defective whole walnuts,[13] walnut meal, and the walnut septum.[14]

While the edible kernel has been the main focus of previous research on walnut composition,[15] emerging evidence indicates that processing by-products may represent rich and underexploited sources of bioactive compounds.[14] For example, the walnut septum shows anti-inflammatory and anti-aging properties with potential cosmetic applications,[16] while walnut shells are reported to be abundant in phenolic

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compounds such as ellagic acid and catechins.[17] The green husk, traditionally used in several cultures for medicinal purposes, has been studied for its antibacterial, antioxidant, and anti-inflammatory properties,[18] as recently reviewed by Mukarram *et al.* (2024).[18]

Beyond their direct benefits for human health, these materials may also serve as valuable functional ingredients in food processing, contributing to improved oxidative stability and extended shelf life.[14,19] Walnut shells, for example, have been investigated for their physicochemical properties and potential application in sausage formulations.[17]

Against this background, the objective of the present study is to characterise the bioactive compound profiles of underutilised fractions generated during walnut processing and assess their valorisation potential. To this end, the composition of green walnuts, defective whole walnuts, and walnut shells was analysed and compared with that of commercially marketed whole walnuts and walnut kernels.

2. Experimental

2.1 Chemicals and reagents

Milli-Q water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). Acetonitrile, acetone, formic acid, and *n*-hexane (purity > 99.9%) were sourced from Sigma-Aldrich® (Madrid, Spain). LC-MS grade methanol, ethanol, and *tert*-butyl methyl ether were purchased from Merck® (Darmstadt, Germany).

The standards used for analyte identification and quantification were obtained from various suppliers: quercetin, naringenin, hesperidin, ellagic acid, gallic acid, catechin, epicatechin, and chlorogenic acid standards from Sigma-Aldrich®; α-carotene, β-carotene, α-tocopherol, γ-tocopherol, and δ-tocopherol standards from Niagen Bioscience®; and lutein from Extrasynthese®.

2.2 Walnut samples

Walnut samples evaluated in the present study are presented in Scheme 1. All walnut samples were kindly supplied by FINCLARA S.L. and were cultivated in Belver de Cinca, Huesca, Spain (41°41′00″N, 0°12′50″E) at an altitude of 200 meters above sea level. The crop cycle spanned from winter 2023 to autumn 2024, during which the region experienced very low annual precipitation (<200–300 mm), exceptionally hot summer temperatures frequently exceeding 40 °C, and cold winter periods with recurrent sub-zero events.

The walnut harvest was conducted in October 2024 using a fully mechanized system. A harvesting machine equipped with a vibration clamp shook each tree to dislodge the nuts, which were then collected from the ground by additional machinery and transported to the processing facility for cleaning, drying, and size grading. Commercial walnuts were subsequently cracked to obtain the walnut kernels, consisting of the edible cotyledons together with the pellicle, with walnut shells generated as a by-product of the cracking process. The defective walnuts corresponded to fruits with visibly darker shells compared to those classified as 'commercial' or 'intact', and/or presenting shell imperfections such as fractured areas or darker surface markings. In addition, immature green walnuts were manually harvested directly from the trees in June 2024, four months prior to the regular harvest period, and shipped fresh. Upon reception, all walnut samples were stored at −80 °C until analysis to prevent post-harvest degradation.

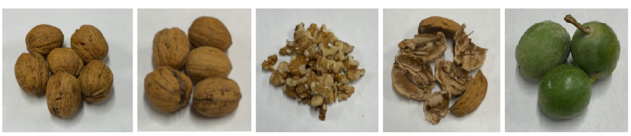
Green walnuts, commercial walnuts, and defective walnuts were collected from multiple trees within the orchard, covering different spatial orientations and positions. This sampling strategy was implemented to capture the inherent variability of the raw material, considering potential differences in environmental exposure, tree physiology, and microclimatic conditions.

2.2.1 Sample preparation. Upon receipt, 200 g of each sample type were weighed and homogenized for five minutes using a commercial Moulinex® grinder. The resulting homogenates (Scheme 2) were transferred to tubes and stored at −20 °C for subsequent analysis. This procedure was performed in triplicate, yielding a total of 600 g of homogenized material per sample type.

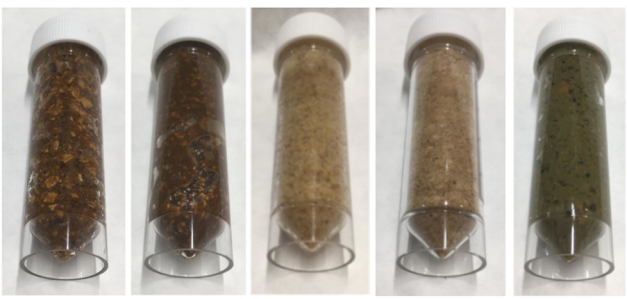
2.3 Analytical determinations

The homogenized samples were analysed for proximate composition, fatty acid profile, phenolic profile, tocopherols, and carotenoids.

2.3.1 Proximate composition. The content of moisture and volatile matter was determined gravimetrically in accordance with Regulation (EC) No. 152/2009.[20] Total protein was quantified by combustion following the UNE-EN ISO 16634-



Scheme 1 Walnut samples evaluated in the present study. From left to right: whole commercial walnuts, defective walnuts, walnut kernels, walnut shells, and green walnuts.



Scheme 2 Homogenates of walnut samples. From left to right: whole commercial walnut, defective walnut, walnut kernel, walnut shell, and green walnut.

1:2009 method.[21] Total fat was determined by acid hydrolysis and gravimetry, as specified in Regulation (EC) No. 152/2009.[20] Soluble and insoluble dietary fibres were analysed gravimetrically using the Association of Official Analytical Chemists (AOAC) 993.19 method.[22] The total ash content was determined gravimetrically in accordance with Regulation (EC) No. 152/2009.[20] Total carbohydrates were calculated by difference, subtracting the sum of the other components from the sample weight. Finally, total sugars were measured volumetrically using the Luff-Schoorl method as outlined in Regulation (EC) No. 152/2009.[20]

2.3.2 Fatty acid profile. Fatty acids were extracted using the Soxhlet method, followed by transesterification according to the official American Oil Chemists' Society (AOCS) Ce 2-66 method.[23] Identification and quantification (g per 100 g of fat) were performed by gas chromatography with flame ionization detection (GC-FID).

2.3.3 Phenolic profile. Phenolic compounds were extracted in triplicate using acetone/water (60 : 40, v/v) as described by Regueiro *et al.* (2014).[15] The phenolic profile (catechin, epicatechin, chlorogenic acid, ellagic acid, quercetin, naringenin, hesperidin, and gallic acid) of the extracts was analysed by ultra-high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UHPLC-ESI-MS/MS). Chromatographic separation was performed on a Waters Acquity UHPLC system (Waters, Milford, MA, USA) equipped with an Atlantis T3 C18 column (100 × 2.1 mm, 3 µm) from Waters (Milford, MA, USA) maintained at 40 °C. The mobile phases consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The linear gradient and flow rate increased linearly as follows, considering time (min), %B, and flow rate (µL min^{-1}): (0, 0, 250), (1, 0, 250), (4, 8, 250), (24, 20, 250), (26, 30, 250), (28, 100, 250), (34, 100, 250), (36, 0, 250) and (41, 0, 250). The injection volume was 10 µL.

Identification and quantification were carried out using an API 4000 triple quadrupole mass spectrometer (Sciex, Framingham, MA, USA) equipped with an electrospray ionization source operating in negative mode. Mass spectrometric parameters were based on previous work by the group.[24] For quantification, multiple reaction monitoring (MRM) transitions were optimized for each analyte using commercial standards prior to analysis (detailed conditions in SI2 – MRM). Quantification was performed by constructing calibration curves with the standards, achieving >80% accuracy and linearity >0.99. Data were processed using MultiQuant MD software from Sciex® and results were expressed in mg per 100 g of sample.

2.3.4 Quantification of tocopherols and carotenoids. Each homogenized sample underwent solid–liquid extraction in triplicate using an *n*-hexane : ethanol (4 : 3, v/v) solution. To prevent analyte oxidation, all extractions were carried out under filtered light in a dark room, following a previously described method.[25]

Identification and quantification of the major carotenoids and tocopherols were performed using an Acquity™ UPLC system (Waters, Milford, MA, USA) equipped with a quaternary pump, autosampler, and diode array detector (DAD G1315B). Analytes were separated on a YMC™ C30 column (250 × 4.6 mm, 5 µm) from Waters (Milford, MA, USA) maintained at 25 °C with a constant flow rate of 600 µL min^{-1}. The mobile phase composition, elution gradient, and other chromatographic details are provided in Rinaldi de Alvarenga *et al.* (2019).[25]

The DAD was set to scan from 350 to 600 nm, with chromatograms recorded at 450 nm for carotenoids and 295 nm for tocopherols. Compounds were identified by comparing retention times and UV/VIS absorption spectra (λ_{max}) with those of authentic standards. Quantification was performed using Waters Empower software (Waters Corporation, Milford, MA, USA), based on external calibration curves of lutein, γ-tocopherol, δ-tocopherol, α-carotene, and β-carotene. All calibration curves showed excellent linearity, with correlation coefficients (R^2) > 0.99. Results were expressed in mg per kg of sample.

2.4 Data analysis

All statistical analyses and visualizations were performed in R version 4.4.3 (R Core Team, 2025) using RStudio (version 2024.12.1.563). Statistical differences between walnut samples were evaluated using a one-way permutation ANOVA (10 000 permutations), a non-parametric method selected to avoid assumptions of normality and homogeneity of variances. Pairwise comparisons were then performed using t-tests with Bonferroni correction to account for multiple testing. Statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1 Proximate composition

The proximate composition of the walnut samples (moisture and volatile matter, crude protein, crude fat, soluble and insoluble dietary fibre, ash, carbohydrates, and total sugars) is presented in SI1 – Table S1 and summarized graphically in Fig. 1.

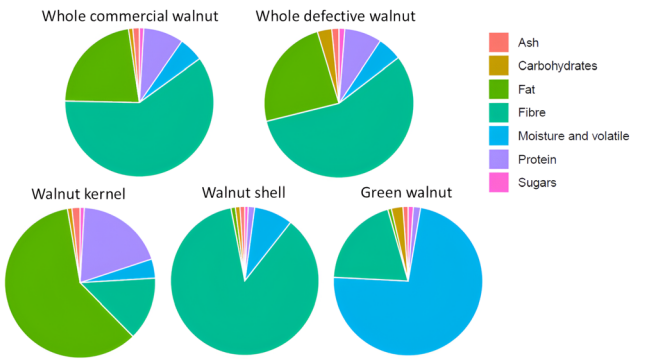


Fig. 1 Graphical representation of the proximate composition of walnut samples. From top left to bottom right: whole commercial walnut, whole defective walnut, walnut kernel, walnut shell and green walnut. Data are expressed as percentages of total weight.

Defective walnuts exhibited a proximate composition closely resembling that of whole commercial walnuts. Given their high fibre content, caloric value, and non-negligible protein fraction, defective walnuts represent a promising ingredient for human and animal nutrition following appropriate processing, such as oil extraction or the separation of protein and fibre fractions.[14]

In contrast to whole walnuts (including the edible kernel), the caloric value of the shell was very low, consistent with its minimal protein, lipid, and carbohydrate content.[17,26] It was, however, characterised by a high proportion of insoluble structural fibre (87.10%), mainly composed of lignin, cellulose, and hemicellulose. Consequently, the shell fraction requires specific treatments, such as extrusion or fermentation,[27,28] to improve its technological functionality for applications in food, cosmetics, or biomaterials. In this context, its incorporation into packaging formulations has been explored.[29] These fibres may also confer physiological benefits in human and animal diets, including improved intestinal transit and increased faecal bulk.[30]

Green walnuts contained substantially higher moisture than the other samples (73.94%). Drying of the green husk may therefore be necessary for valorisation, as it concentrates key components and facilitates downstream processing.[31] Dietary fibre was the second most abundant component (20.03%), originating from the developing shell tissue and characterised by a very low soluble-to-insoluble fibre ratio.

In walnut kernels, fat was the predominant component (63.37%), followed by a substantial protein fraction (20.19%) and a comparatively low dietary fibre content (14.42%). These results are consistent with previous reports describing lipid levels of 60–71%[32,33] and protein fractions of 7–21% in walnut kernels.[34,35] Notably, the dietary fibre content observed here exceeds the values commonly reported in the literature (approximately 4–7%),[36] suggesting variability related to cultivar, climate, and soil.

3.2 Fatty acid profile

The fatty acid profiles of the walnut samples are detailed in SI1 – Table S2, and the main fatty acids are presented in Table 1. Polyunsaturated fatty acids (PUFAs) predominated in all samples containing kernels (Fig. 2), with *cis*-linoleic acid as the major constituent, ranging from 42 g per 100 g of fat in whole commercial walnuts to 60 g per 100 g of fat in green walnuts. Notably, due to the low fat content observed in green walnuts (0.8%), their absolute PUFA content was markedly lower compared to kernels, whole commercial and whole defective walnuts.

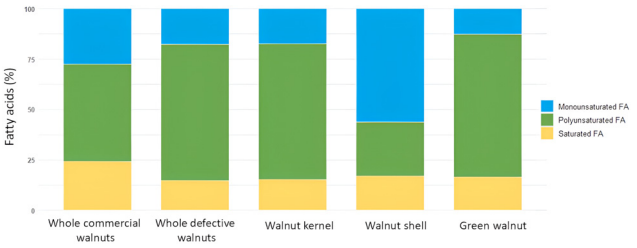


Fig. 2 Fatty acid composition of walnut samples. From left to right: whole commercial walnut, whole defective walnut, walnut kernel, walnut shell and green walnut. Data are expressed as percentages of total fat (g per 100 g of fat).

Walnut shells, which also contained very low fat levels (1.0%), exhibited a distinctive fatty acid profile, characterised by a higher proportion of monounsaturated fatty acids, particularly *cis*-oleic acid (54.9%), compared to PUFAs, in agreement with previous findings by Herrera *et al.* (2020).[37] Accordingly, lipophilic extracts from most walnut by-products are rich in *cis*-linoleic acid, whereas shells constitute a distinct subfraction characterised by higher *cis*-oleic acid levels, supporting their potential for valorisation in food and cosmetic applications.[38]

Within the PUFA fraction, α-linolenic acid (ALA), an omega-3 fatty acid, was the second most abundant component in the fat fraction, comprising approximately 10.0% of the total fatty acids in defective and green walnuts, but only 3.4% of the total fat in walnut shells. Unlike most other nuts, such as almonds, peanuts, and hazelnuts, whose PUFA profiles are dominated by omega-6 linoleic acid with only negligible amounts of ALA,[39] walnuts are a rich source of this fatty acid, contributing to a more nutritionally favourable *n*-6/*n*-3 ratio.[40] Consequently, lipid-rich walnut by-products could be used to increase dietary ALA intake, which is associated with enhanced cognitive performance, reduced inflammation, and protective

Table 1 Concentration of the main fatty acids quantified in walnut samples

	Whole commercial walnut	Whole defective walnut	Walnut kernel	Walnut shell	Green walnut
∑ Saturated fatty acids	24.25	14.76	15.06	17.01	16.29
∑ Monounsaturated fatty acids	27.38	17.69	17.32	56.24	12.59
∑ Polyunsaturated fatty acids	48.37	67.55	67.63	26.75	71.12
∑ Omega-3 fatty acids	5.98	11.73	10.74	3.35	11.00
∑ Omega-6 fatty acids	42.00	55.63	56.70	22.74	59.94
C(16:0) palmitic acid	13.70	9.24	9.00	10.60	10.83
C(18:1) *cis*-oleic acid	25.30	16.71	16.58	54.90	10.53
C(18:2) *cis*-linoleic acid	42.00	55.63	56.67	22.70	59.94
C(18:3) α-linolenic acid	5.62	11.52	10.50	3.35	11.00

Values are expressed in g per 100 g of fat.

effects against cardiovascular and neurological diseases, as well as certain types of cancer.[5,6]

Palmitic acid was the most prevalent saturated fatty acid across all samples, ranging from 9.0% of the total fat in kernels to 13.7% in whole commercial walnuts. Some studies reported slightly lower percentages of palmitic acid in walnut kernels, indicating that fatty acid composition can vary according to cultivar and climatic factors.[41] Overall, the distinct fatty acid composition of walnut by-products supports their potential for health-promoting applications[42,43] and their use as functional ingredients in food, feed, and cosmetic formulations.[38,44]

3.3 Phenolic profile

The concentrations of phenolic compounds quantified in the walnut samples are presented in Table 2. Ellagic acid, gallic acid, and catechin were identified as the predominant phenolic compounds in all samples. Nevertheless, phenolic composition can vary substantially depending on the walnut fraction, cultivar, growing conditions, ripening stage, and post-harvest treatments.[45,46]

Walnut shells exhibited the highest phenolic content of all samples analyzed. Ellagic acid was the most abundant phenolic compound overall and showed significant variation among fractions ($p < 0.001$) (Fig. 3).

Walnut shells contained the highest levels of ellagic acid (71.3 ± 0.76 mg per 100 g), whereas green walnuts presented the lowest levels (11.2 ± 2.77 mg per 100 g). The recovery of ellagic acid is highly dependent on the extraction method, with advanced techniques such as ultrasound-assisted, enzyme-assisted, or pressurized liquid extraction resulting in significantly enhanced yields compared to conventional methods.[26] For example, ellagic acid concentrations in walnut shells have been reported to range from 70 mg per 100 g when using traditional liquid extraction to over 1000 mg per 100 g when pressurized liquid extraction is applied.[26] Despite this variability, ellagic acid, which is derived from the hydrolysis of ellagitannins, is widely recognized as the major phenolic constituent of walnuts,[47–49] and its recovery from shells represents a viable valorisation strategy.[50] Ellagic acid is of considerable biological interest as it serves as a precursor to urolithins,

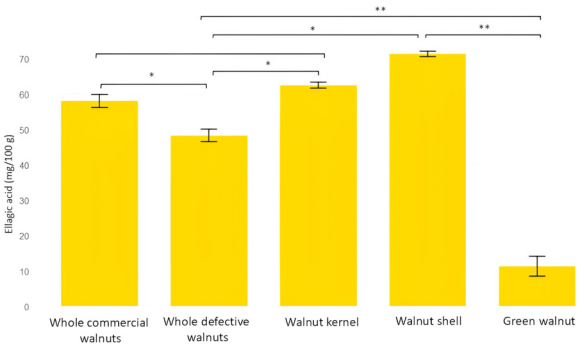


Fig. 3 Concentration of ellagic acid in walnut samples. From left to right: whole commercial walnut, whole defective walnut, walnut kernel, walnut shell, and green walnut. Data are expressed as mean ± standard deviation (n = 3 replicates per group) in mg per 100 g of sample. ** $p < 0.01$ and * $p < 0.05$ indicate significant differences.

metabolites noted for their anti-inflammatory properties,[51] and is associated with improvements in athletic performance, cognitive function, insulin resistance, body composition, and cardiovascular health.[52] Furthermore, ellagic acid is valued in cosmetic applications for its skin-brightening and photoprotective properties.[53,54]

Gallic acid was most abundant in green walnuts (6.5 ± 0.30 mg per 100 g), followed by walnut shells (5.7 ± 0.03 mg per 100 g), with the lowest concentration found in the kernel (2.6 ± 0.02 mg per 100 g). Masek *et al.* (2019)[55] similarly reported gallic acid as a major phenolic compound in green walnut husks (>6 mg g^{-1}). Other studies have found significantly higher levels in walnut kernels and shells than those observed here, likely reflecting differences in cultivar, growing region, and extraction procedures.[26,56,57] Gallic acid is known for its antioxidant capacity, beneficial effects on glycaemic regulation, and antimutagenic potential.[58]

Catechin was another abundant phenolic compound, being highest in walnut shells (7.19 ± 0.12 mg per 100 g). This flavonoid has been associated with neuroprotective effects, cardiovascular benefits, and the prevention of several types of neoplasms.[59] Comparable concentrations were reported by Sheng *et al.* (2021),[60] who noted that the seed coat (pellicle) surrounding the kernel contains markedly higher levels of several

Table 2 Concentration of phenolic compounds identified in walnut samples

	Whole commercial walnut	Whole defective walnut	Walnut kernel	Walnut shell	Green walnut	p-Value
Ellagic acid	58.07 ± 1.81b	48.27 ± 0.76c	62.40 ± 0.87b	71.33 ± 0.76a	11.27 ± 2.77d	<0.001
Gallic acid	4.75 ± 0.05b	5.64 ± 0.35b	2.63 ± 0.06c	5.71 ± 0.03ab	6.49 ± 0.30a	<0.001
Catechin	5.55 ± 0.06b	5.09 ± 0.03b	2.65 ± 0.02c	7.19 ± 0.12a	1.65 ± 0.03d	<0.001
Epicatechin	0.14 ± 0.02b	0.07 ± 0.01b	0.04 ± 0.02b	0.23 ± 0.06b	0.54 ± 0.02a	<0.001
Chlorogenic acid	0.11 ± 0.01b	0.09 ± 0.03b	<LOD	0.20 ± 0.03a	0.19 ± 0.03a	0.004
Hesperidin	0.03 ± 0.01a	0.02 ± 0.01a	0.02 ± 0.01a	<LOQ	<LOD	0.288
Naringenin	0.21 ± 0.01a	0.21 ± 0.01a	0.02 ± 0.01c	0.13 ± 0.01b	<LOD	<0.001
Quercetin	<LOQ	<LOQ	<LOD	<LOQ	<LOD	—

Values are expressed as the mean of the replicates ± standard deviation, in mg per 100 g of sample. LOD: limit of detection; LOQ: limit of quantification. Differences between groups were assessed using one-way permutation ANOVA, followed by pairwise t-tests with Bonferroni correction. Lowercase letters indicate statistically significant differences: a > b > c > d.

phenolic compounds, including gallic acid and catechin, than the kernel itself.

Although not specifying individual compounds, research has shown that phenolic-rich walnut by-products can extend shelf life and inhibit lipid oxidation in packaged sardines, owing to their antioxidant and antimicrobial effects.[61] The pellicle is consistently identified as the richest source of phenolic compounds in walnuts, containing more than 30 distinct phenolics,[62] and exhibiting ellagic acid concentrations up to 20-fold higher than the kernel.[63,64]

3.4 Tocopherols and carotenoids

The concentrations of the main carotenoids and tocopherols quantified in the walnut samples are summarized in Table 3. These lipophilic bioactive compounds are associated with various health benefits, including reduced oxidative stress, lower inflammatory markers, and improved lipid profiles.[65,66] While these effects are largely attributed to the antioxidant properties of carotenoids, members of the carotene subgroup exhibit provitamin A activity, as they can be converted into retinol after ingestion. Among these, β-carotene is considered the most bioactive form due to its high conversion efficiency.[67]

Green walnuts exhibited the highest total carotenoid content (803.7 ± 48.6 mg kg^{-1}), comprising lutein (4.6 ± 0.1 mg kg^{-1}), α-carotene (169.4 ± 9.1 mg kg^{-1}), and predominantly β-carotene (629.7 ± 39.5 mg kg^{-1}). In contrast, β-carotene levels in the remaining samples were only slightly above 10 mg kg^{-1}, with no significant differences observed between them (Fig. 4).

Although β-carotene has been identified as a dominant bioactive compound in green walnut husks,[68] quantitative data for this matrix remain scarce. For comparison, walnut leaves are reported to contain approximately 300 mg kg^{-1} β-carotene[69] and have been investigated as a natural source of antioxidant and antimicrobial compounds with potential applications in food and cosmetic formulations.[70,71]

Regarding tocopherols, γ-tocopherol was the predominant form in all samples, followed by δ-tocopherol, whereas α-tocopherol, the most biologically active form of vitamin E,[72] was not detected (Table 2). Walnut kernels exhibited the highest concentration of γ-tocopherol (226.9 ± 8.1 mg kg^{-1}), which was not significantly different from whole walnuts (193.6 ± 11.2 mg kg^{-1}) (Fig. 5) or defective walnuts (150.8 ± 10.9 mg kg^{-1}). Green walnuts had significantly lower concentrations of γ-tocopherol (51.2 ± 2.2 mg kg^{-1}) than the kernel-containing samples, yet still showed significantly more than walnut shells, which presented the lowest levels of both γ- and α-tocopherol (25.7 ± 0.4 mg kg^{-1} and 21.6 ± 0.1 mg kg^{-1}, respectively).

The observed distribution of tocopherols is consistent with their lipophilic nature, as these compounds are efficiently solubilized in the lipid-rich walnut kernel, whereas their presence in the lipid-poor shell is minimal. Previous studies on nut oils have similarly reported walnut oil as particularly rich in γ-tocopherol

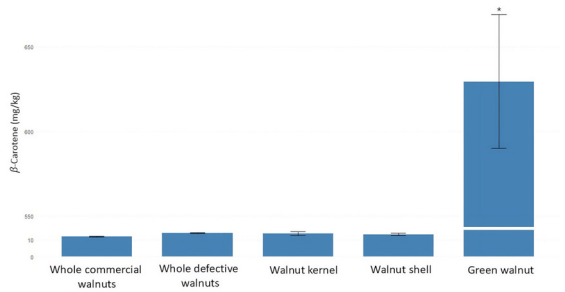


Fig. 4 Concentration of β-carotene in walnut samples. From left to right: whole commercial walnut, whole defective walnut, walnut kernel, walnut shell and green walnut. Data are presented as mean ± standard deviation (n = 3 replicates per group) in mg kg^{-1} of sample. ** $p < 0.01$ and * $p < 0.05$ indicate significant differences.

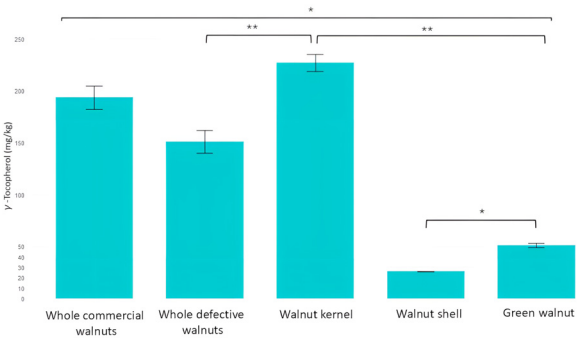


Fig. 5 Concentration of γ-tocopherol in walnut samples. From left to right: whole commercial walnut, whole defective walnut, walnut kernel, walnut shell and green walnut. Data are presented as mean ± standard deviation (n = 3 replicates per group) in mg kg^{-1} of sample. ** $p < 0.01$ and * $p < 0.05$ indicate significant differences.

Table 3 Concentration of carotenoids and tocopherols identified in walnut samples

	Whole commercial walnut	Whole defective walnut	Walnut kernel	Walnut shell	Green walnut	p-Value
Lutein	3.3 ± 0.01b	2.7 ± 0.24b	1.9 ± 0.34b	3.3 ± 0.21b	4.6 ± 0.10a	<0.001
α-Carotene	<LOD	<LOD	<LOD	8.8 ± 1.32b	169.4 ± 9.07a	<0.001
β-Carotene	12.0 ± 0.29b	14.0 ± 0.15b	13.6 ± 1.19b	13.3 ± 0.70b	629.7 ± 39.45a	<0.001
γ-Tocopherol	193.6 ± 11.20ab	150.8 ± 10.91b	226.9 ± 8.10a	25.7 ± 0.39d	51.2 ± 2.16c	<0.001
δ-Tocopherol	33.6 ± 0.15b	35.3 ± 1.39ab	43.8 ± 0.44a	21.6 ± 0.02c	35.6 ± 1.83ab	<0.001

Values are expressed as the mean of the replicates ± standard deviation, in mg kg^{-1} of sample. LOD: limit of detection; LOQ: limit of quantification. Differences between groups were assessed using one-way permutation ANOVA, followed by pairwise t-tests with Bonferroni correction. Lowercase letters indicate statistically significant differences: a > b > c > d.

(330 mg kg^{-1}), followed by δ-tocopherol (35 mg kg^{-1}), consistent with the trends observed here.[41,73] Although not the most biologically active form of vitamin E, γ-tocopherol demonstrates notable anti-inflammatory and antioxidant properties.[74]

3.5 Antioxidant compound profile overview

The Principal Component Analysis (PCA) biplot (Fig. 6) and alluvial diagram (SI1 – Fig. S1) summarize the antioxidant compound profiles of the walnut samples, illustrating the distribution and relationships among phenolic, tocopherol, and carotenoid compounds. In the PCA biplot, the first two principal components accounted for 84.63% of the total variance, indicating a clear separation of samples based on their bioactive compound composition.

Green walnuts (red) are clearly separated from the other groups along Dim1, primarily due to their higher levels of α- and β-carotene, lutein, and epicatechin. Whole commercial (purple) and defective walnuts (orange) are clustered closely together, reflecting similar bioactive compound composition characterised by elevated concentrations of catechin, naringenin, hesperidin, ellagic acid, and tocopherols. Walnut shells (green) are also clearly separated and associated with a diverse phenolic profile and relatively low levels of δ-tocopherol. Finally, walnut kernels (blue) are distinguished by their high contents of γ- and δ-tocopherols.

The PCA biplot suggests tissue-specific patterns of secondary metabolite accumulation in walnuts. The positive correlations observed between catechin, naringenin, and ellagic acid likely reflect co-localization in the husk and pellicle, as well as shared biosynthetic pathways. The accumulation of pigments such as α- and β-carotene and lutein in green walnut husks may support photoprotection and antioxidant defence in exposed tissues.[75] In contrast, walnut kernels are enriched in tocopherols, consistent with their role as a storage tissue that protects lipids from oxidative damage. Negative correlations, such as those between tocopherols and phenolic acids or carotenoids and flavonoids, may further reflect metabolic compartmentalization and functional specialization in walnut tissues.

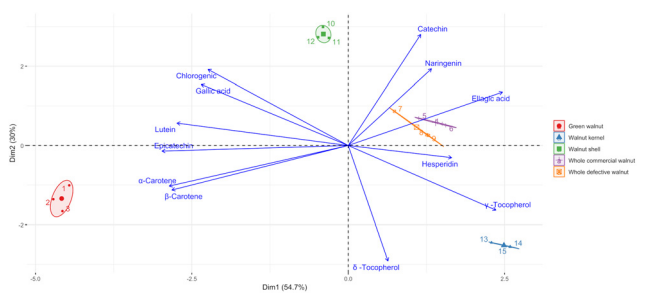


Fig. 6 PCA-biplot of antioxidant compounds in walnut samples. Symbols and colours represent the five sample groups (green walnut, walnut kernel, walnut shell, whole commercial walnut, and whole defective walnut). Arrows indicate the relative contributions of each compound. The first two principal dimensions explain 84.63% of the total variance (Dim1: 54.67%; Dim2: 29.96%).

4. Conclusions

This study highlights the nutritional and technological potential of green walnuts and by-products of walnut processing, including defective walnuts and walnut shells. Green walnuts showed the highest carotenoid content, primarily β-carotene, which was mainly localized in the outer tissues. Given the high moisture content of green walnuts, the application of drying processes could enhance the concentration and practical utilisation of these valuable bioactive constituents. Walnut shells were identified as a rich source of insoluble fibre and phenolic compounds, particularly ellagic acid, with scope for improved nutritional and functional value through appropriate processing. Despite their low lipid content, walnut shells exhibited a distinctive fatty acid profile dominated by oleic acid. Defective walnuts possessed a higher energy density than other by-products due to their protein and lipid composition. Although lower compared to the shell fraction, their phenolic content was still considerable, largely concentrated in the pellicle and shell. In contrast, tocopherols accumulated predominantly in the kernel.

In summary, these underutilised walnut fractions represent promising resources within a circular economy framework. Their potential applications extend beyond animal feed to include use as functional ingredients in food and cosmetic formulations, as well as in the development of bio-based materials, such as components of food packaging.

Future research should focus on (i) improving walnut shell functionality through treatments such as fermentation; (ii) optimizing drying processes for green walnuts and further characterisation of their bioactive-rich outer layers; and (iii) isolating the walnut cuticle as a concentrated source of bioactive compounds such as ellagitannins.

Author contributions

C. A.-R.: investigation, methodology, formal analysis, data curation, and writing – original draft. F. M. C.-M.: investigation, methodology, formal analysis, data curation, and writing – original draft. M. C.: formal analysis and writing – review & editing. M. P.: conceptualization, supervision, and writing – review & editing. A. V.-Q.: project administration, funding acquisition, supervision, and writing – review & editing.

Conflicts of interest

No potential conflict of interest was reported by the authors.

Data availability

The data supporting this article have been included as part of the supplementary information (SI). See the Raw Data.xlsx file.

Supplementary information is available. See DOI: https://doi.org/10.1039/d6fo00494f.

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