By-Products of Walnut \((Juglans regia)\) as a Source of Bioactive Compounds for the Formulation of Nutraceuticals and Functional Foods †

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Abstract: The scarcity of natural resources and higher incidence of diseases related to inappropriate eating habits have prompted the search of food and nutraceutical products with functional properties that are also respectful of the environment. Agro-industrial by-products are a profitable source for obtaining bioactive compounds, with various biological properties, including antioxidant, anti-inflammatory, and anticancer properties, which contribute to immunity and reduce the negative effects of infections, inflammation, and oxidative stress. In the case of the walnut \((Juglans regia)\) oil industry, various by-products are generated, among which we can mention pomace, green shell, shell, skin and leaves. Therefore, there is an opportunity for the recovery of waste, the recovery of target molecules and the formulation of new products, whether they are nutraceuticals, pharmaceuticals, or food additives, contributing to the circular economy and consumer health. Walnut is commonly characterized by its high content of lipids (58–65%), mainly polyunsaturated fatty acids, tocopherols and phytosterols. In addition, the current literature states that its by-products are rich in phenolic compounds, mainly phenolic acids and flavonoids. In accordance, the antioxidant potential of different extracts of nuts, shells and leaves has bee studied using different methods such as the reduction potency assay, scavenging effect of DPPH (2,2-diphenyl-1-picrylhydrazyl) and inhibition of lipid oxidation by \(\beta\)-carotene linoleate system, with EC \(_{50}\) values less than 1 mg/mL. The results obtained showed that all walnut extracts have a strong antioxidant capacity against ROS species. For this reason, this work focuses on the bibliographic review of the bioactive compounds present in the by-products of the walnut industry, as well as mentioning their biological properties and possible applications in the food sector.

Keywords: walnut; by-products; polyphenols; circular economy; biological activity

1. Introduction

The walnut is the fruit of the walnut tree \((Juglans regia; Juglandaceae family)\). There are two species, the Persian or English walnut and the black walnut. It is harvested all over the world, but China, the United States and Europe stand out as major producers. It is well-known for its broad nutritional attributes, sensory properties, and health benefits. Whole walnuts are usually consumed, but they are also employed in various food preparations such as cakes, cookies, energy bars, salads or ice cream. In some countries such as Slovenia, green walnuts are used in the preparation of a liqueur. Regarding the contribution of
nutrients, they contain lipids, mainly unsaturated fatty acids, phytosterols and tocopherols, as well as proteins, vitamins, minerals and a significant amount of antioxidant compounds such as phenolic compounds [1]. These nutrients are bioactive components since they provide beneficial properties for the health of the consumer. Current research has shown that walnut consumption can provide natural antioxidants and it has a protective role against diseases influenced by oxidative stress such as cancer and cardiovascular diseases [2,3]. On the other hand, it is known that non-edible parts such as leaves, shell, skin, green shell, and bark have been used in traditional medicine for the treatment of different ailments. For example, in some countries, an infusion of walnut leaves is used for its antioxidant and antimicrobial properties. In addition, an extract of the green peel has been used to treat skin diseases and inflammation [4]. The industrialization of the fruit causes a large amount of plant residues. In this sense, it is estimated that 70% of the fruit is transformed into residue, mainly peel, bagasse, green peel, skin, and leaves (Figure 1), which contain a significant number of bioactive compounds valuable for their use and exploitation. These residues are commonly disposed of in landfills, burned, or used for composting. However, a most efficient use of this waste would be a circular economy strategy, that would reduce environmental impact and at the same time boost economic sector. In this sense, the agricultural residues of the walnut have been widely investigated in the search for natural products. There is evidence that all parts of the walnut tree can be used as a source of compounds that express an important antioxidant, antimicrobial, antidiabetic, immunomodulatory, hepatoprotective, and anti-inflammatory potential [3,5,6]. Therefore, this review aims to discuss the recent scientific literature on the importance of the walnut, including the different parts of its fruit, as well as to mention its biological properties and possible applications in the food industry.

Figure 1. Valorization of walnut by-products following circular economy approaches, extracting compounds with biological properties for bio-based products in different industrial sectors.

2. By-Products Derived from Walnut

The walnut is made up of four different parts (Figure 1). The green outer layer is known as the hull or shell; when the fruit ripens, it cracks. It must be separated manually from the middle shell, which we know as the walnut. This is a hard layer that surrounds the seed, and, in the same way, it must be broken mechanically to release the seed or nucleus, which is the edible part. In addition, the seed is covered by a thin brown layer called the skin. In the following section, we have compiled several bioactive compounds identified in these by-products (Table 1).
Table 1. Compounds and functional ingredients recovered from walnut residues with various extraction methods and their applications.

| Biomass Source | By-Product | Compound(s) | Extraction Method | Biological Property | Application | Refs. |
|----------------|------------|-------------|-------------------|---------------------|-------------|-------|
| Nut Skin       |            | TPC         | Soxhlet           | DPPH                | Natural antioxidants | [7]   |
|                |            | TPC         | Ultra sonic bath  |                      | Food additives | [8]   |
|                |            | TPC         | Soxhlet           | DPPH                | Natural antioxidants | [7]   |
| Shell          |            | TPC         | HAE               | ORAC                | Food additives | [10] |
|                |            | TPC         | Ultrasonic bath   | DPPH                | Food additives | [9]   |
| Nut Bagasse    |            | TPC         | Cholesterol bath  | DPPH                | Food additives | [11] |
| Nut Green husk | Essential oil | TPC         | HAE               | Antimicrobial activity | Gram positive and Gram-negative bacteria, and fungi | [13] |
|                | Leaves     | TPC         | HAE               | DPPH                | Natural antioxidants | [15] |
|                | Leaves     | TPC         | HAE               | DPPH                | Natural antioxidants | [14] |
|                | Leaves     | TPC         | HAE               | -                   | Food additives | [16] |

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|                |            | TPC         | Ultrasonic bath   | DPPH                | Food additives | [9]   |
| Nut Bagasse    |            | TPC         | Cholesterol bath  | DPPH                | Food additives | [11] |
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|                | Leaves     | TPC         | HAE               | DPPH                | Natural antioxidants | [14] |
|                | Leaves     | TPC         | HAE               | -                   | Food additives | [16] |
Table 1. Cont.

| Biomass Source | By-Product | Compound(s)                        | Extraction Method | Biological Property | Application | Refs. |
|----------------|------------|------------------------------------|-------------------|---------------------|-------------|-------|
| Tocopherols    |            | 282.20 mg/100 g DW                |                   |                     |             |       |
| TPC/HPLC:      |            | 3-O-caffeoylquinic acid, pro-cyanidins and taxifolin derivatives |                   |                     |             |       |
|                |            | HAE                                | H$_2$O, 100 °C, 5 min | DPPH                | 0.151–0.202 mg/mL | Food additives | [17]  |
|                |            |                                    |                   | TBARS               | 189.92–269.27 g/mL |             |       |
|                |            |                                    |                   | HeLa                | 294.87 g/mL |             |       |
|                |            |                                    |                   | MCF-7               | 209.28–242.14 g/mL |             |       |
|                |            |                                    |                   | ORAC                | 2.17 ± 0.22 µmol Trolox/mg | Food additives | [18]  |
| TPC            | 270 mg GAE/g DW |                                    | H$_2$O/EtOH, 40 °C, 10 min | ORAC                | 2.17 ± 0.22 µmol Trolox/mg | Food additives | [18]  |

Notes & abbreviations: TPC, total phenolic compounds; DPPH, 2,2-difenil-1-picrilhidrazilo; HPLC, high performance liquid chromatography; TFC, total flavonoid compounds; HAE, heat assisted extraction; MAE, microwave assisted extraction; TBARS, Thiobarbituric acid reactive substances; ORAC, Oxygen Radical Antioxidant Capacity; GAE, gallic acid equivalents; DW, dry weight; EtOH, ethanol; MeOH, methanol; H$_2$O, water; EtOAc, ethyl acetate; Pet, petroleum; Ace, acetone; Hex, hexane; N. A., nitric acid; S. Ac., sulfuric acid; Chl, chloroform; Eg, ethylene glycol.

2.1. Walnut Husk

Walnut husk is a characteristic by-product of walnut cultivation; it is generated in the harvest, when the ripe fruit breaks to give rise to the fruit. In rural areas, it is used as a source of energy for heating. In traditional medicine, it is used for the treatment of skin diseases and pain relief. It has been reported that this by-product has significant amounts of phenolic compounds and flavonoids [4], so its recovery would be useful. For the preparation of the extract, it is necessary to choose the most appropriate solvent and extraction technique. In this sense, it is reported that better TPC and TFC results are achieved, as well as better antioxidant activities in extracts treated with mixtures of water/ethanol or water/methanol [13]. TPC recovery for extracts derived from walnut shells has been reported to oscillate between 32.61 to 166.7 mg GAE/100 g DW [12–14], and total flavonoids from 22.91 to 423.97 mg CE/100 g DW [19]. Generally, Soxhlet extraction is the conventional technique that allows greater recovery of TPC and TFC. However, ultrasound-assisted extraction has also shown promising results [20]. Among the main phenolic compounds present in the walnut shell, hydrolysable tannins have been identified, mainly ellagic acid, with 98.3 ± 5.56 mg GAEs/L extract reported, and tannic acid, with 120.4 ± 4.19 mg GAEs/L extract [7,21]. In addition, the presence of 27 naphthoquinones and their derivatives has been reported, with juglone being the most important with 1404 ± 96.8 mg GAEs/L extract [21]. In addition, the presence of gallic acid and proto-catechuic acid has been reported, with 122 ± 10.0 and 23.0 ± 4.78 mg/100 g DW, respectively [22]. Regarding the compounds belonging to the group of flavonoids, (+)-catechin 530.80 ± 15.39 mg GAEs/L extract and La (-)-epicatechin 350.33 ± 11.91 mg GAEs/L extract stand out [19]. Additionally, the DPPH radical scavenging activity of walnut shells has been evaluated in different studies, reporting values for the half effective concentration (IC$_{50}$) in the range of 0.30–0.80 mg/mL [12–14]. Other authors determined antioxidant activity by FRAP, showing a result of 0.45 ± 0.04 mmol Fe$^{2+}$/g DS [20].

Currently, the green walnut shell has different applications in the industry. For example, it is used for the elimination of dangerous materials and heavy metal ions in industrial effluents, as well as for the elimination of synthetic dyes or other dangerous compounds. In addition, considering the presence of juglone, a natural dye, this by-product can be used as a hair dye. It is also a profitable, valuable, environmentally friendly compound [23]. For the food industry, it is described as a natural antioxidant that could replace the use of synthetic additives [24] and can be also used as a functional additive in the meat industry as a low-cost source of valuable source of phytochemicals [24].
2.2. Skin

The walnut skin is the thin brown layer that protects the seed from oxidation and microbial contamination. It represents 5 to 8% of the fruit and it is eliminated in the processing of the walnut, because it can cause a slight astringency and bitterness. Regarding phytochemical composition, it has been described to be the main source of polyphenols from the nut [5,8]. In this sense, several studies reported concentrations of the three most abundant phenolic compounds (juglone, syringic acid and ellagic acid) up to 20 times higher in the skin, compared to the seed [8]. In addition, it is reported that the concentration of phenolic compounds increases gradually from the fruiting stage to the mature stage, reporting TPC and TFC values of 52.05–279.3 mg GAE/g DW and 8.95 mg RE/g FW, respectively [5,25]. As can be seen, the presence of non-flavonoid-type phenolic compounds stands out [25]. Reports stated that most of the compounds present in the walnut skin belong to hydrolyzable tannins, such as digalloyl-glucose, ellagic acid galloyl pentose or galloyl methylgalloyl deoxyhexoside isomer. The high content of phenolic compounds gives this by-product important biological properties, such as antioxidant, antimicrobial, and anticancer capacity [3,5,7].

2.3. Shell

It is an inert, hard and biodegradable material, which constitutes about 70% of the total weight of the fruit. Thus, it is the most abundant by-product of the walnut. A study reported a chemical composition of 3.4% ash, 50.3% lignin, 22.4% hemicellulose and 23.9% cellulose [4]. Despite its high potential as a source of chemicals, it has been traditionally used as a source of energy for heating, an abrasive agent to clean and polish metals, plastics, wood, and as a filter medium to separate oil, hazardous materials, and heavy metals. Currently, due to this biofiltration potential, it is used to treat water extracted from oil fields and wastewater. In recent years, research has focused on the production of antioxidants and antimicrobials from walnut shells [4], which have been linked to the presence of phenolic compounds. In this sense, the recovery of these compounds through three extraction methods (ultrasonic bath, ultrasonic probe and standard agitation method) has been studied, recovering TPC of 51.2 mg of gallic acid equivalents/g of dry weight (GAE /g DW), respectively [9]. In agreement, another study reported TPC values of 49.10–63.60 mg gallic acid equivalents/g dry weight (GAE/g DW) [26]. Further analysis identified lignans (0.30 mg/g), stilbenes (0.02 mg/g) and flavonoids (0.69 mg/g). Antioxidant properties of walnut shell have been corroborated by DPPH assay, reporting values of 3.14–7.17 µg/mL [9].

2.4. Leaves

Leaves are generated in large quantities during harvest. They have been used in traditional medicine for the treatment of hemorrhoids, venous insufficiency and for their anthelmintic, depurative, antidiarrheal properties [15]. In this by-product, juglone is reported as the main phenolic compound, among other flavonoids [5]. The appropriate mass solvents for the recovery of phenolic compounds, water, ethanol and methanol have been reported as the most efficient [5]. In this sense, a study achieved the highest recovery of TPC and TFC in a methanolic extract, reporting values of 120.28 ± 2.32, 59.44 ± 0.87 mg/g DE, respectively. They also report on a higher presence of TPC in young leaves compared to mature leaves [27]. Another study identified ten phenolic compounds, namely 3- and 5-caffeoylquinic acids, 3- and 4-p-coumaroylquinic acids, p-coumaric acid, quercetin 3-galactoside, quercetin 3-pentoside derivative, quercetin 3-arabinoside, quercetin 3-xiloside and quercetin 3-rhamnoside in walnut leaves [15].
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