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

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Abstract: The scarcity of natural resources and a higher incidence of diseases related to inappropriate eating habits have focused research on the search for food and nutraceutical products with functional properties that are 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 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. The nut 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, for example total phenolic concentrations in the range 74 to 410 mg/g of GAE/FW are reported. In accordance, it is reported that the antioxidant potential of different extracts of nuts, shells and leaves was 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 β-carotene linoleate system, with EC50 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 (*Juglans regia*) industry, as well as mentioning their biological properties and possible applications in the food industry

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

1. Introduction

The Walnut is the fruit of the walnut tree (*Juglans*) 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. It is known throughout the world for its broad nutritional attributes, sensory properties, and health benefits. It is mainly consumed as whole walnuts or in various food preparations such as cakes, cookies, energy bars, salads, ice cream and in some countries such as Slovenia green walnuts are used in the preparation of a liqueur. Regarding the contribution of nutrients, the contribution of 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 (Gunduc and El 2003). 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 have a protective role against diseases influenced by oxidative stress such as cancer and cardiovascular diseases (Yang, Liu, and Halim 2009; Catanzaro et al. 2018). 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 the infusion of walnut leaves is used for Its antioxidant and antimicrobial properties, in addition, the extract of the green peel was used for skin diseases and inflammation (Jahanban-Esfahlan et al. 2019a). The industrialization of the fruit causes a large amount of plant residues, 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 the use and exploitation. These residues are commonly disposed of in landfills, burned or used for composting. However, the efficient use of this waste would be a circular economy strategy that would conserve the environment and at the same time boost the 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 potential, anti-inflammation (Jahanban-Esfahlan et al. 2019b; Medic et al. 2021; Catanzaro et al. 2018). Therefore, this review aims to discuss the recent scientific literature on the importance of the nut, including the different parts of its fruit, as well as to mention its biological properties and possible applications in the food industry

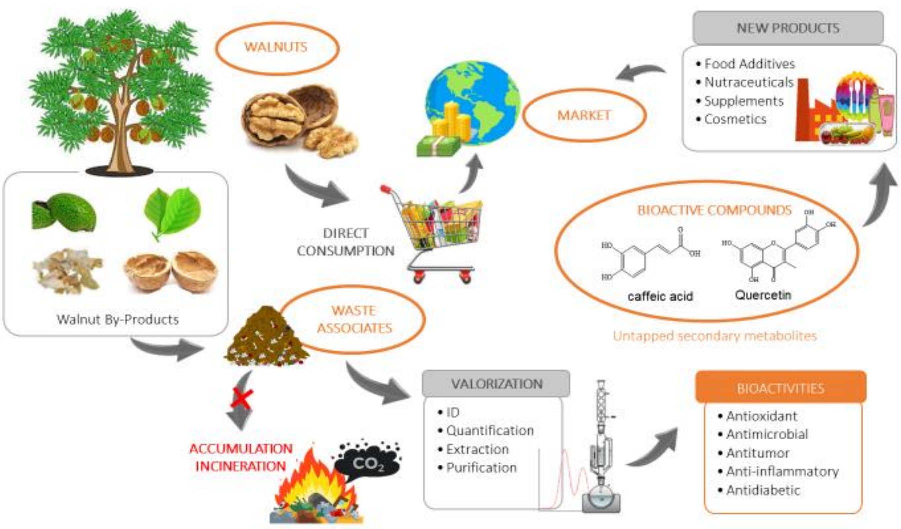

Figure 1.

2. By-Products Derived from Walnut

The walnut is made up of four different parts Figure 1. The green outer layer 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, it is a hard layer which surrounds the seed, 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 skin. In the following section we will address the main bioactive compounds present in each by-product, as well as the possible industrial uses

Walnut Husk

It is a characteristic by-product of walnut cultivation, it is generated in the same 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 and in traditional medicine 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 (Jahanban-Esfahlan et al. 2019a), so their recovery would be interesting. 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, water/methanol (Fernández-Agulló et al. 2013). TPC recovery is reported for extracts derived from walnut shells in the range of 32.61 to 166.7 mg GAE/100 g 1 DW (Oliveira et al. 2008; Fernández-Agulló et al. 2013; Sfahlan et al. 2009) and total flavonoids from 22.91 to 423.97 mg CE/100 g 1 DW (Cosmulescu et al. 2014), with Soxhlet extraction being the conventional technique that allowed greater recovery of TPC and TFC. However, ultrasound-assisted extraction is reported to be a conventional technique that allows efficient recovery of phenolic compounds (Tabaraki and Rastgoo 2014). Additionally, the DPPH radical scavenging activity of walnut shells has been evaluated in different studies, reporting values for the 50% effective concentration (IC50) mg/mL in the range of 0.30–0.80 mg/mL (Oliveira et al. 2008; Fernández-Agulló et al. 2013; Sfahlan et al. 2009). Other authors determined Antioxidant activity measured by FRAP 0.45 ± 0.04 mmol Fe^{2+}/g DS (Tabaraki and Rastgoo 2014). Among the main phenolic compounds present in the walnut shell, hydrolysable tannins have been identified, mainly ellagic acid, reporting 98.3 ± 5.56 mg GAEs/L extract and tannic acid 120.4 ± 4.19 mg GAEs/L extract (Stampar et al. 2006; Akbari et al. 2012). In addition, the presence of 27 naphthoquinones and their derivatives is reported, with juglone being the most important 1404 ± 96.8 mg GAEs/L extract (Stampar et al. 2006). In addition, the presence of Gallic acid and Protocatechuic acid is reported, reporting 122 ± 10.0 and 23.0 ± 4.78 mg/100 g DW, respectively (Jakopic et al. 2008). 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 (Cosmulescu et al. 2014). 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 (Beiki, Najafpour, and Hosseini 2018). As for the food industry, it is described as a natural antioxidant that could replace the use of synthetic additives (Salejda et al. 2016). It is reported that the green shell of the walnut can be used as a functional additive in the meat industry as a low-cost source of valuable phytochemicals (Salejda et al. 2016).

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** 4615–6059 mg GAE/100 g 1 DW **TFC** 810–1495 mg CE/100 g 1 DW	**Soxhlet** MeOH, 60 °C, 30 min	**DPPH** 0.09–0.20 mg/mL	Natural antioxidants	[1]

	TPC/HPLC syringic acid, juglone, and ellagic acid (1003.24, 317.90, and 128.98 mg/100 g)	**Ultrasonic bath** MeOH/BHT 60 °C, 30 min	-	Food aditives	[2]
	TPC 939–1968mg GAE/100 g 1 DW **TFC** 301–811 mg CE/100 g 1 DW	**Soxhlet** MeOH, 60 °C 30 min	**DPPH** 0.27–0.48 mg/mL	Natural antioxidants	[1]
Shell	**TPC** **HAE:** 20.6 mg GAE/g DW **Ultrasonic bath:** 25.8 mg GAE/g DW, **UAE:** 51.2 mg GAE/g DW	**HAE** EtOH/H_2O, 25 °C, 15 min **Ultrasonic bath** EtOH/H_2O, 25 °C, 15 min, 500 W **UAE** EtOH/H_2O, 25 °C, 15 min, 1500 W	**-**	Food aditives	[3]
	TPC 14.81 mg GAE g/DW	**HAE** n-hexane, 25 °C, 90 min	**ORAC** 3423.44 μmol Trolox g^{-1}	Food aditives	[4]
Bagasse	**TPC** 7.7 mg GAE g^{-1} DW	**Sonication** CH3OH/H_2O, 60 min	**DPPH** 83.46–93.08%	Food aditives	[5]
Green husk	**TPC** 32.61–74.08 mg (GAE)/g DW	**HAE** H_2O, 100 °C 45 min	**DPPH** 0.35–0.59 mg/mL **Antimicrobial activity**: Gram positive and Gram-negative bacteria, and fungi <1 mg/mL	Food aditives	[6]
	TPC 84.46 mg GAE/g DW	**HAE** EtOH/H_2O MeOH/H_2O	**DPPH** 0.33–0.70 mg/mL	Natural antioxidants	[7]
	TPC 43.9–166.7 mg GAE/g DW	**Soxhlet** MeOH, 80 °C, 30 min	**DPPH** 0.30–0.80 mg/mL	Natural antioxidants	[8]
Leaves	**Essential oil** α and β pinene	**hydrodis-tillation**	**DPPH** 34.5–56.4 μg/mL **Antimicrobial activity**: Gram positive and Gram-negative bacteria	Food aditives	[9]
	TPC	**HAE**	-	Food aditives	[10]

	HAE: 3.9 a 13.7 mg GAE/g DW **MAE:** 6.4 a 14.7 mg GAE/g DW	EtOH, 60 °C, 112 min. **MAE**: EtOH, 107.5 °C, 30 min			
	Tocopherols: 282.20 mg/100 g DW **TPC/HPLC**: 3-*O*-caffeoylquinic acid, Pro-cyanidins and taxi-folin derivatives	**HAE** H_2O, 100 °C, 5 min	**DPPH** 0.151–0.202 mg/mL **TBARS** 189.92–269.27 g/mL **HeLa** 294.87 g/mL **MCF-7** 209.28–242.14 g/mL	Food aditives	[11]
	TPC 270 mg GAE/g DW	**HAE** H_2O/EtOH, 40 °C, 10 min	**ORAC** 2.17 ± 0.22 µmol Trolox/mg	Food aditives	[12]

Notes & abbreviations: EtOH, ethanol; MeOH, methanol; H_2O, water; EtOAc, ethyl acetate; Pet, petroleum; Ace, acetone; Hex, hexane; N. A., nitric acid; S. Ac., sulfuric acid; Chl, chloroform; Eg, ethylene glycol.

References

1. Author 1, A.B.; Author 2, C.D. Title of the article. *Abbreviated Journal Name* **Year**, *Volume*, page range.
2. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, A., Editor 2, B., Eds.; Publisher: Publisher Location, Country, 2007; Volume 3, pp. 154–196.
3. Author 1, A.; Author 2, B. *Book Title*, 3rd ed.; Publisher: Publisher Location, Country, 2008; pp. 154–196.
4. Author 1, A.B.; Author 2, C. Title of Unpublished Work. *Abbreviated Journal Name* year, *phrase indicating stage of publication (submitted; accepted; in press)*.
5. Author 1, A.B. (University, City, State, Country); Author 2, C. (Institute, City, State, Country). Personal communication, 2012.
6. Author 1, A.B.; Author 2, C.D.; Author 3, E.F. Title of Presentation. In Proceedings of the Name of the Conference, Location of Conference, Country, Date of Conference (Day Month Year).
7. Author 1, A.B. Title of Thesis. Level of Thesis, Degree-Granting University, Location of University, Date of Completion.
8. Title of Site. Available online: URL (accessed on Day Month Year).