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Original Research Article

Comparative study of the nutritional and bioactive compounds content of four walnut (*Juglans regia* L.) cultivars

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ABSTRACT

The proximate and mineral composition, fatty acid profile, total polyphenol, melatonin and serotonin contents were assessed in four walnut (*Juglans regia* L.) cultivars (cv. Serr, Hartley, Chandler and Howard). The aim was to decide which cultivar is the most suitable from a nutritive, but also commercial, point of view. Proteins and fats accounted for more than 70% of the walnut kernel weight. Due to their content in bioactive compounds such as melatonin, serotonin and total phenols, their high content in magnesium (up to 443 mg 100 g^{-1} FW) and other minerals, their high contents in polyunsaturated fatty acids (up to 78.0% of total fatty acids) and their favorable *n*−6/*n*−3 ratio, among other healthful properties, consumption of all the studied cultivars would be potentially beneficial to health. According to its fatty acid profile, Howard would be the most healthful cultivar, but also it would be theoretically the most sensitive to rancidity, and thus the cultivar with the shortest shelf life. Nonetheless, this cultivar showed the highest content in the antioxidants melatonin and total polyphenols.

1. Introduction

Walnut trees (*Juglans regia*) are cultivated in southern Europe, northern Africa, eastern Asia, the USA and western South America. Walnuts, commonly consumed as part of the Mediterranean diet and in many communities throughout the world, are an excellent source of many nutrients. They have generated great interest in recent years and have been proposed as a promising natural functional food. The beneficial effects of walnut consumption for prevention, management and treatment of diseases related to diet are proven and well-documented, and include protection from cardiovascular disease (Banel and Hu, 2009; Ros, 2009) and diabetes (Kendall et al., 2011), reduction of adiposity and low-grade systemic inflammation (Casas-Agustench et al., 2009; Tulipani et al., 2011) and the improvement of blood lipid profile (Mukuddem-Petersen et al., 2005).

The health-promoting effect of walnuts can be said to be related to their chemical composition. Besides having a low glycemic index, nuts are rich sources of polyphenols, polyunsaturated fatty acids (PUFA) [mainly the essential fatty acids linoleic (*n*−6) and α-linolenic (*n*−3)], a high *n*−6 to *n*−3 ratio, proteins unusually rich in essential amino acids (and thus with a high nutritive value), biogenic amines (melatonin and serotonin), minerals (magnesium, potassium, calcium, etc.), fiber, etc. (Anderson et al., 2001; Amaral et al., 2003, 2005; Reiter et al., 2005; Yang et al., 2009). All these compounds may contribute in a synergistic way (Liu, 2003; Yang et al., 2009), so that a complex and dynamic interaction between an array of essential nutrients and phytochemicals seems to be the mechanism responsible for the health benefits associated with walnut consumption (Chen and Blumberg, 2008).

To date, walnut research has mostly focused on its nutritional composition and lipid profile and, as far as we know, there are no studies in the literature that evaluate the nutritional value of Howard walnut cultivar. Differences in composition among walnut cultivars produced in Portugal (Amaral et al., 2003; Pereira et al., 2008), Italy (Ruggeri et al., 1998), Serbia (Rabrenovic et al., 2008) and New Zealand (Savage, 2001) were observed, but data remain

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limited regarding the influence of cultivar (genetics), harvest season, geographical origin, environmental conditions (temperatures, rainfall and light), soil composition, maturity level, methods of cultivation, processing and storage on walnut composition. It is known that all these factors highly influence the composition of nuts, and of fruits in general. It should be borne in mind that consumers cannot always choose the variety of walnut, and often mixtures of several varieties are sold.

Polyphenols (Anderson et al., 2001; Chen and Blumberg, 2008; Yang et al., 2009), melatonin (Reiter et al., 2005) and serotonin (Feldman and Lee, 1985) are considered to be important bioactive compounds, although there are very few information concerning the melatonin and serotonin contents in walnuts. A more detailed research focusing on the nutritional quality and these health-promoting components in walnuts is of interest.

The main objective of this study was to characterize the most appropriate walnut variety from a nutritive and commercial point of view. For that, we compared the nutritional value (protein, fat, minerals and fatty acid composition), total polyphenols and bioactive compound content (melatonin and serotonin) of the four walnut cultivars (Serr, Hartley, Chandler and Howard) best adapted to soil and climate of Extremadura, a region of Spain that leads the Spanish production of walnuts.

2. Materials and methods

2.1. Materials

Four walnut (*J. regia* L.) cultivars ('Serr', 'Chandler', 'Howard' and 'Hartley') were collected from the local producer Nogaltec S. L. All samples come from different orchards of the same farm located in a southwest region of Spain (Montijo, Extremadura, 38°5′ N and 6°4′ W). All cultivars were harvested in 2009 (wet spring, summer hot and dry) from mid-September to mid-October, from 10 year-old walnut crops. After having been shaken from the tree, the fallen nuts were aligned in a row with a sweeper and then collected mechanically from the soil. After harvesting, leaves, twigs and stones were discarded. Green skin was removed mechanically and walnuts were washed and dried. Finally, the nuts were manually cracked and shelled, packaged in plastic bags and immediately transported to the laboratory.

For each walnut cultivar, 10 kg of peeled nuts were received. At the time of reception, each sample (10 kg of kernels of each cultivar) was subsampled into 500 g batches, vacuum packed in plastic bags, and stored at 4 °C in the dark until analysis. Each subsample was representative of each cultivar, and independent analyses were made from each subsample.

The walnuts were finely chopped and ground in an appliance mill (model A327R1, Moulinex, Madrid, Spain). For fatty acid or dry-degreased analysis, we used crude oil or walnut flour residue, respectively, from the Soxhlet.

2.2. Methods

2.2.1. Proximate analysis

Analyses of moisture, total fat, protein and ash contents were carried out according to AOAC Official Methods (AOAC, 2000). The carbohydrate content was estimated by difference of the other compounds using the following formula: carbohydrate content = 100% − (% moisture + % fat + % protein + % ash). The analysis was performed in quadruplicate and results are expressed as percentage.

2.2.2. Mineral composition

Zinc, copper, manganese, iron, magnesium and calcium were determined by atomic absorption spectrometry; sodium and potassium were determined by atomic emission spectroscopy following the method developed in our research institute. Both analyses were performed in an atomic spectrophotometer (Varian, model AA240FS, Varian Inc., Palo Alto, California, USA). Mineralization of 1 g defatted and dehydrated samples was carried out, by performing a wet acid digestion at high-pressure in a microwave digester set at 180 °C for 15 min (Milestone Ethos Plus, Milestone Helping Chemists, Bergamo, Italy). 8 mL of nitric acid 65% and 2 mL of hydrogen peroxide 31% were used as oxidizing medium.

2.2.3. Fatty acid composition

Fatty acid methyl esters (FAME) from the oil samples obtained in Soxhlet were obtained by alkaline treatment with 2.0 M KOH in methanol at room temperature. Gas chromatography was carried out for FAME separation and quantification following the European Commission regulation CEE 2568/91 21. The analysis was performed in an Agilent 7890A Gas Chromatograph (Agilent Technologies, Palo Alto, California, USA) equipped with a Flame Ionization detector (FID). Fatty acids were separated in a 60 m *cis/trans* FAME column (Agilent Technologies, 60 m × 0.320 mm ID × 0.25 μm film thickness) and the identification was carried out by comparing the retention time of the unknown compounds with those from the standards. For the chromatographic separation, chromatographic column was initially warmed at 165 °C for 35 min and after that; the temperature was increased up until 220° at 5 °C/min (Calvo et al., 2011). Under these conditions all the studied FAME were adequately separated in 62 min. Fatty acids were quantified by means of the external standard calibration method. The results are expressed in relative percentage of each fatty acid.

2.2.4. Bioactive compound analysis

Melatonin and serotonin. The melatonin and serotonin content in the cultivars was analyzed by HPLC–MS, by adapting the method proposed by Burkhardt et al. (2001), as described in González-Gómez et al. (2009). During the extraction procedure, light was avoided, since melatonin and serotonin are highly light sensitive. The results are expressed in ng g^{-1} fresh weight.

Table 1
Proximate composition (g 100 g^{-1}) of the four walnut cultivars.

	Cultivar								
	Serr		Hartley		Chandler		Howard		*p*-Value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Moisture	3.77[b]	0.13	4.67[a]	0.13	3.65[b]	0.02	3.36[c]	0.03	0.001
Fat	63.7[b]	0.8	58.3[d]	0.2	65.2[a]	0.8	61.3[c]	0.5	0.001
Protein	17.4[a]	0.2	16.5[b]	0.2	15.4[c]	0.3	15.1[d]	0.1	0.001
Ash	1.24[ab]	0.01	1.12[c]	0.02	1.17[bc]	0.02	1.29[a]	0.05	0.001
Carbohydrates[a]	13.9[b]	0.8	19.4[a]	0.3	14.6[b]	0.8	18.9[a]	0.5	0.001

[a] Carbohydrates contents were estimated by difference. (a)–(d) Different letters in the same row indicate significant statistical differences (Tukey's test, $p < 0.05$). SD, standard deviation ($n = 4$). All values are given on a fresh weight basis.