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BIOTECHNOLOGY

# Biochemical characterization and antioxidant activity of walnut kernel (*Juglans regia* L.) of accessions from Middle and High Atlas in Morocco

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**ABSTRACT.** Biochemical composition and antioxidant activity were determined in kernel nuts for eleven Moroccan walnut (*Juglans regia* L.) accessions representing its main cropping area. Total oil, carbohydrates, crude protein, energy value, crude fiber and flavonoid have varied significantly between accessions, respectively within the range values of 54.50-65.48%, 8.17-19.25%, 11.5-25.58%, 648.91-713.83 Kcal, 4.17-6.75% and 12.59-62.11mg RE 100 $g^{-1}$ DM. Besides, mineral composition (mg 100 $g^{-1}$) of kernel have varied also significantly among accessions and particularly for phosphorus (338.1-675.87), copper (2.08-6.67), zinc (3.39-18.63), iron (1.17-2.64), chromium (0.16-0.20), nickel (1.26-1.45) and boron (0.07-1.49). However, dry matter (96.75-98.56%), moisture (1.44-3.24%), ash (1.67-2.53%), total phenols content (1017-3739 mg GAE 100 $g^{-1}$ DM), DPPH radical scavenging activity (75.02-85.96%), potassium (210.10-338.93 mg 100 $g^{-1}$), magnesium (79.15-374.54 mg 100 $g^{-1}$), sodium (1.17-12.63 mg 100 $g^{-1}$) and manganese (0.79-1.67 mg 100 $g^{-1}$) did not show significant variations between accessions. Furthermore, the results showed that Moroccan walnut constitutes an important source of nutrient elements, essentially fat, carbohydrates, protein, phosphorus and zinc, and natural antioxidants, phenolic compounds. Accordingly, consumption of all studied kernels accessions would be beneficial to health. This study showed considerable biochemical variation between the analyzed walnut accessions, which could help to select genotypes with desired traits according to their chemotypes.

**Keywords:** Variation; Proximate composition; Mineral composition; Bioactive compounds.

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## Introduction

*Juglans rejia* L. is an important fruit among the nut species (Zwarts, Savage, & McNeil, 1999). It's cultivated throughout the world for their timber and nutritional values (Sen, 1986; McGranahan & Leslie, 1990). In 2017 (Food Agriculture Organization of the United Nations [FAOSTAT], 2017) world production of walnuts was almost 3,829,626 tons and China, United States, Iran and Turkey were the principal producers, with respectively 50.27, 14.92, 9.12 and 5.48% of total global production, while Morocco, with an annual value of 12,736 tons, ranking last globally.

Walnut has a high calories level and rich nutrient composition. Walnut kernel contains about 60% of lipids and it is a good source of macronutrients and micronutrients and other bioactives (Souci, Fachmann, & Kraut, 2008; Bolling, Dolnikowski, Blumberg, & Chen, 2010; U. S. Department of Agriculture [USDA], 2018; Yerlikaya, Yucel, Erturk, & Korukluoğlu, 2012), and walnuts oil is rich in unsaturated fatty acids (Ozakan, 2009; Muradoglu, Oguz, Yildiz, & Yilmaz, 2010; Tapia et al., 2013). Many previous studies stated that walnut is an excellent source of phosphorous, potassium, magnesium, iron, zinc, sodium, calcium and natural antioxidants like polyphenols, folate, tannins (Li et al., 2006, Cosmulescu, Baciu, Achim, Botu, & Trandafir, 2009; Tapia et al., 2013). It can significantly contribute to daily meals and has functional importance in medical biochemistry and physiology. More, kernel walnut was revealed to be endowed with antimicrobial capacity against many different bacteria and fungi (Pereira et al., 2008).

In Morocco, the walnut fruit has a great place in diet and customs and it is consumed, fresh or toasted, alone or in other edible products. The consumption of walnut is increasing due to existence of high concentration of natural antioxidants that have been reported as being protective against certain types of cancer and may also decrease the risk of cardiovascular diseases (Miraliakbari & Shahidi, 2008; Yang, 2009; Carvalho et al., 2010). The walnut cropping in Morocco is concentrated in Medium and High Atlas mountains and represented mainly by local cultivars, but still yet conducted under traditional technical itinerary. In order to identify cultivars with researched traits according to their chemotypes, the present study aimed to analyze the biochemical content and antioxidant activity of kernel from several Moroccan walnut accessions representing the main cropping area. Such knowledge is indispensable to assess the daily dietary intake of beneficial constituents of kernel as well as to select the plant source for establishing new orchards.

## Material and methods

### Plant material

During September 2014, mature and healthy nuts were collected from 11 Moroccan walnut accessions (Table 1, Figure 1), representing its main cropping area in Morocco. From each accession, three trees were sampled on different sides. The walnut fruits were conserved in shell in dark bags. Dried nuts were shelled manually and the parameters were analyzed for each sample.

**Table 1.** Geographic and ecological characteristics of walnut accessions used in this study

| Accessions | Code | Geographic origin | Altitude (m) | Latitude N | Longitude W | Zone | Rainfall (mm) |
|---|---|---|---|---|---|---|---|
| Aghbala | AGH | 32 Km North East of Aghbala | 1673 | 32°32’ | 5°39’ | Middle Atlas | 450 |
| Naour | NAO | Naour | 1300 | 32°29’ | 5°58’ | Middle Atlas | 600 |
| Taghzirte | TAG | 12 Km East of Tagzirte | 650 | 32 26 | 6° 12’ | Middle Atlas | 700 |
| AitBougamez | ABZ | Ait Bougamez | 1996 | 31°38’ | 6° 28’ | High Atlas | 580 |
| AitMhamed | AMD | 20 Km South East of Azilal | 1728 | 31° 25’ | 2° 28’ | High Atlas | 450 |
| Demnate | DEM | Demnate | 932 | 31° 43’ | 6° 58’ | High Atlas | 350 |
| Imlil | IML | 17 km South of Asni | 1763 | 31° 8’ | 7° 55’ | High Atlas | 459 |
| Anougal | ANG | 40 km South of Amzmiz | 1569 | 31° 9’ | 8° 15’ | High Atlas | 681 |
| Beram | BER | Midelt | 1521 | 32° 40’ | 4° 44’ | High Atlas | 210 |
| Amouguer | AMG | 40 km West of Rich | 1569 | 32° 12’ | 5° 8’ | High Atlas | 250 |
| Tabrijjate | TBR | 70 km East of Imilchil | 1831 | 32° 16’ | 4° 56’ | High Atlas | 319 |

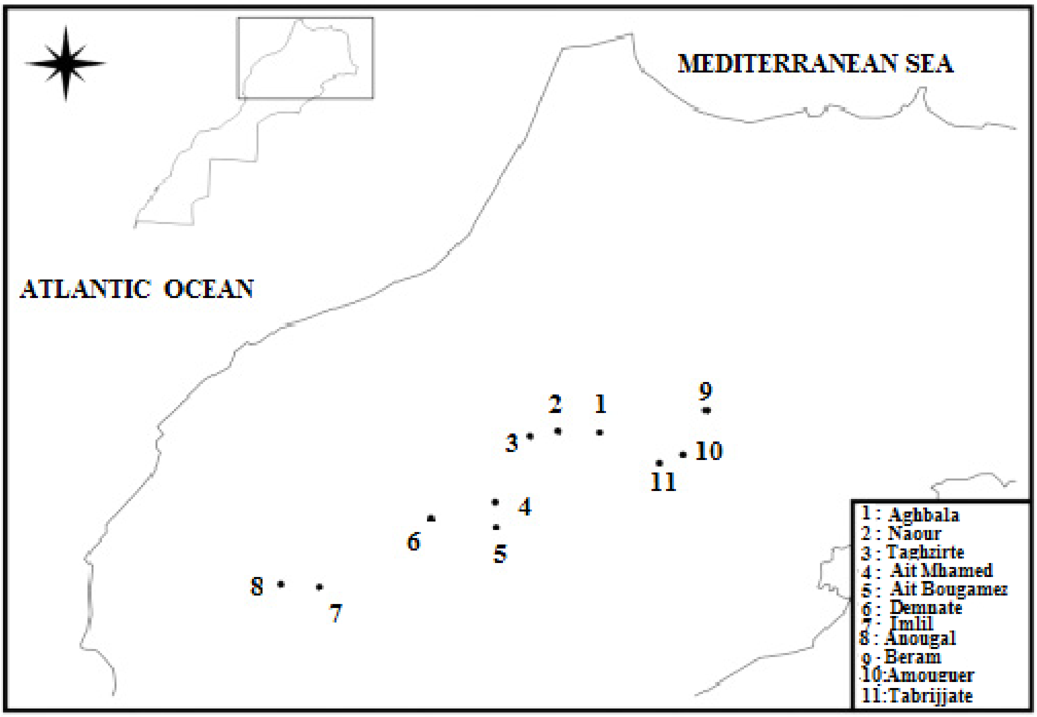


**Figure 1**. Map of Morocco Showing locations of the walnut accessions analyzed.

## Chemical analyses

From each accession, the following chemical characteristics were determined:

The Dry matter (DM) was determined by drying 5g of the samples in an oven at a temperature of 105°C for 24 hours to a constant weight and the moisture (M) was calculated on a dry weight and fresh weight basis (Mikdat, 2010). The Total ash (Ash) composition of 5 g of kernel was obtained by using a muffle furnace at 600 °C for 240 minutes and then weighed (Association of Official Analytical Chemists [AOAC], 1995).

Total oil (Oil) was determined by extraction from 5 g dried, ground kernels per replicate with N-hexane as solvent using a Soxhlet apparatus at 55-60°C for 8 hours (AOCS, 1998). The oil content was expressed as the difference in weight of the dried kernel samples before and after extraction. The crude protein (Prot) was obtained indirectly by determining the total N content obtained by Kjeldahl method (AOAC, 1995) and multiplying by a nitrogen-protein conversion factor of 6.25.

The carbohydrates content (Cbhydt) was estimated by calculating the differences based on the other components and using the following formula: Carbohydrate content (%) = 100% - (moisture (%) + protein (%) + oil (%) + ash (%) (Grosso, Nepote, & Guzman, 2000) and the energy value (EV) was expressed in kilocalories, using the following formula: Energy kcal = 4 x (protein g + carbohydrate g) + 9 x (lipid g) (Pereira et al., 2008). To determine the crude fiber, 5 g of the ground walnut samples were digested in 100 mL of 1.25% $H_2SO_4$. The solutions were boiled for 45 minutes and then were filtered and washed with hot distilled water. The filtrates were digested in 100 mL of 1.25% Sodium Hydroxide solutions. These solutions were heated for 60 minutes, filtered and washed with hot deionized water and over dried and measured. The final over-dried residues have been incinerated in a furnace at 550°C for 3 hours. The loss in weight represented the crude fiber amount (Aryapak & Ziarati, 2014).

The mineral composition was determined according to Ranganna (1995) method with some modifications. About 0.1 g of dried ash was moistened with distilled water (0.5-1 mL) and 1 mL of concentrated HCl was added and evaporated to dryness. Finally, 5 mL of 1N HCl was added for 10 min. and then filtered through Whatman No 42. The filtrates were collected in 50 mL Erlenmeyer flasks and analyzed by ICP-AES for estimating Phosphorus (P), Potassium (K), Magnesium (Mg), Sodium (Na), Copper (Cu), Zinc (Zn), Manganese (Mn), Iron (Fe), Chromium Cr, Nickel (Ni) and Boron (B) concentrations. The mineral contents of the samples were quantified against standard solutions of known concentrations which were analyzed concurrently.

Extract preparation, 5 g of ground kernel was extracted using 50 mL of 80% methanol and then centrifuged for 10 minutes and filtered. The concentration of the supernatant was carried out by a rotary evaporator (BUCHI B-490) (Jacki et al 2011). The Phenolic compounds concentration in the kernel methanol extracts was estimated by a colorimetric assay based on procedures described by Singleton and Rossi (1965) with some modifications. Briefly, 1 mL of sample was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 minutes, 1 mL of saturated sodium carbonate solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 minutes, after which the absorbance was read at 725 nm. Gallic acid was used for constructing the standard curve (0.01–4 mM). The results are expressed as mg of gallic acid equivalents 100 $g^{-1}$ of extract (GAEs).

The flavonoids content (Fld) of the all kernel methanol extracts was quantified using a modified colorimetric method of Yang (2009). Briefly, 1:10 diluted extracts (0.25 mL) was mixed with distilled water (1.25 mL) and subsequently with 5% sodium nitrite solution (0.07 mL) and allowed to react for 5 minutes Then 10% aluminium chloride solution (0.15 mL) was added and allowed to react further for 6 minutes before addition of one molar sodium hydroxide (0.5 mL). Finally, distilled water was added to all samples in 1 ml portions. The absorbance of the mixture was immediately measured at 510 nm. The flavonoids content was determined by using a rutin standard curve (0.02–20 mg $L^{-1}$) and stated as mg rutin equivalents (REs) per 100g of sample ± SE. More, scavenging activity of kernel extracts towards the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was monitored according to a method reported by Hatano, Kagawa, Yasuhara and Okuda, (1988). The sample extracts (0.3 mL) were mixed with 2.7 mL of methanolic solution containing DPPH radicals ($6x10^{-5}$mol $L^{-1}$). The mixture was shaken vigorously and left to stand in the dark until stable absorption values were obtained. The reduction of the DPPH radical was measured by monitoring continuously the decrease of absorption at 517 nm. DPPH scavenging effect was calculated as percentage of DPPH discoloration.

The percentage of DPPH radical scavenging activity (RSA) by each sample of kernel methanol extract was calculated using the equation:

$$RSA\ (\%) = [(A_0 - A_S)/A_0] \times 100.$$

where $A_0$ is the absorbance of control and $A_S$ is the absorbance of the sample.

### Statistical analyses

All the assays were carried out in triplicate and the results are expressed as mean values and standard error of the mean. Analysis of variance (One-Way ANOVA) was used to determine if significant differences were present among means. A LSD multiple range test was worked out to determine if significant ($p < 0.05$) differences occurred between individual accessions. Correlation between studied variables was established using Pearson correlation coefficient ($\alpha = 0.05$). All these analyses were performed by using SPSS program (2011). Principal components (PCA) and hierarchical cluster analyses were also carried out on the matrix of mean values of measured characters using respectively, the XLSTAT program (XLSTAT, 2014) and Statistica Software (Statistica StatSoft, 1997).

## Results

Results related to chemical composition are illustrated in Table 2. The analysis of variance exhibited significant differences between accessions for the most analyzed parameters: total oil, crude protein, carbohydrates content, energy value, crude fiber, flavonoids content and some minerals such as P, Cu, Zn, Fe, Cr, Ni and B.

The moisture content of the kernel ranged from 1.44% for Tabrijjate accession to 3.24% for Ait Bougamez accession with an average of 2.52. By averaging 2.08%, the total Ash varied from 1.67% for Ait Mhamed accession to 2.53% for Amouguer accession. Regarding the total oil content, with an average of 60.86%, it scaled from 54.50% for Aghbala accession to 65.48% for Ait Mhamed accession. The crude protein average determined in the current study was 15.60% and spanned from 8.17% for Tabrijjate accession to 19.25% for Amouguer accession. With an average of 18.93%, the carbohydrates content has changed within an interval of 11.15% for Ait Mhamed accession to 25.85% for Tabrijjate accession. Accordingly, walnut consumption conducted to a high input level of energetic value that varied between 713.83 kcal in Demnate accession to 648.41kcal in Aghbala accession with an average of 685.86 kcal. In addition, the crude fiber has varied widely between 4.17% for Amouguer accession and 6.75% for Naour accession, the recorded average value being 5.51%.

Average phenolic content was 2579 mg GAE 100 $g^{-1}$ DM for all accessions, with values ranging from 1017 mg GAE 100 $g^{-1}$ DM for Taghzirte accession to 3739mg GAE 100 $g^{-1}$ DM for Ait Mhamed accession, and concerning the total flavonoid content was higher in Aghbala accession (62.11mg RE 100 $g^{-1}$ DM) and lower in Amouguer accession (12.59 mg RE 100 $g^{-1}$) with a mean of 37.18mg RE 100 $g^{-1}$ DM. The scavenging activity on DPPH radical for the Moroccan walnut accessions reached a mean of 79.73%, with values changed between 75.02% for Tabrijjate accession and 85.96% for Amouguer accession.

The mineral content of Moroccan walnut estimated by ICP-AES seemed to be very important. The analyzed accessions contain high level of P with values ranging from 338.1 mg 100 $g^{-1}$ for Aghbala to 675.86 mg 100 $g^{-1}$ for Amouguer with an average of 569.59 mg 100 $g^{-1}$ DM; and K with a mean of 261.97 mg 100 $g^{-1}$ DM varied between 210.1 mg $kg^{-1}$ (Ait Mhamed) and 338.93 mg 100 $g^{-1}$ (Beram).The average of Mg content was determined as 125.17 mg 100 $g^{-1}$ and established between 79.15 mg 100 $g^{-1}$ (Tabrijjate) and 374.53 mg 100 $g^{-1}$ (Naour); and as for Na amount was determined between 1.17 mg 100 $g^{-1}$ for Ait Mhamed accession and 12.63 mg 100 $g^{-1}$ for Amouguer accession with an average of 4.06 mg 100 $g^{-1}$, which was 5.50 mg 100 $g^{-1}$ for Cu content with a variation from 2.09 mg 100 $g^{-1}$ in Aghbala to 6.67 mg 100 $g^{-1}$ in Beram. As regards Zn content that spanned from 3.39 mg 100 $g^{-1}$ for Ait Mhamed accession to 18.63 mg 100 $g^{-1}$ for Aghbala accession the recorded mean was 6.80 mg 100 $g^{-1}$. By contrast, the amount of the other minerals determined as mg per 100 g in kernel extracts was low and varying within the range of 0.79-1.67 for Mn, 1.17-2.64 for Fe, 0.16-0.20 for Cr, 1.26-1.45 for Ni and 0.07-1.49 for B.

Correlations among all biochemical parameters are summarized in Table 3. The significant and positive correlations were recorded by polyphenols content with total flavonoids content ($R=0.79^{**}$) and radical scavenging activity (R=0.70**), the energy value with oil content (R=0.97**) and carbohydrates (0.66*), P element with K (R=0.53**), Cu (R=0.64*) and Ni (R=0.76**). Moreover, we found strong positive correlations between B and Zn (R=0.87**) and between Cu and Ni (R=0.69**).

**Table 2.** Mean values and descriptive statistics of the biochemical parameters measured in the 11 walnut accessions.

| Propertie | Aghbala | Naour | Taghzirte | Ait Bougamez | Ait Mhamed | Demnate | Imlil | Anougal | Beram | Amouguer | Ibrijjate | Mean | F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DM (%) | 97.38± 1.34a | 96.98± 1.31a | 97.86± 0.96a | 96.75± 0.21a | 96.97± 031a | 97.41± 0.91a | 97.49± 1.25a | 98.03± 1.53a | 97.67± 0.64a | 97.11± 0.68a | 98.56± 0.31a | 97.47 | 0.91 |
| M (%) | 2.62± 1.34 a | 3.02± 1.31a | 2.14± 0.96a | 3.24± 0.21a | 3.03± 0.30a | 2.59± 0.92a | 2.51± 1.25a | 1.96± 1.53a | 2.32± 0.64a | 2.89± 0.68a | 1.44± 0.31a | 2.52 | 0.91 |
| Ash (%) | 1.84± 0.65a | 2± 0.20ab | 2.25± 0.17ab | 1.80± 0.10a | 1.67± 0.12a | 2.08± 0.21ab | 2.13± 0.12ab | 2.25± 0.61ab | 2.11± 0.28ab | 2.53± 0.18b | 2.26± 0.33ab | 2.08 | 1.71 |
| Oil (%) | 54.50± 0.00a | 59.93± 0.89abc | 65.23± 1.78bc | 58.49± 3.71ab | 65.48± 4.42c | 65.09± 1.68bc | 61.30± 6.57bc | 59.15± 4.78abc | 58.89± 2.29abc | 58.88± 1.87abc | 62.54± 4.77bc | 60.86 | 2.85* |
| Prot (%) | 15.17± 2.67bc | 15.17± 5.63bc | 15.71± 1.75 bc | 12.83± 3.64 ab | 18.67± 0.01 bc | 18.17± 1.88 bc | 14.00± 1.75 bc | 16.33± 4.04 bc | 18.08± 1.01 bc | 19.25± 3.03 c | 8.17± 3.64a | 15.60 | 3.27* |
| Cbhydt (%) | 24.44± 3.24cd | 19.88± 4.63bcd | 14.67± 3.00ab | 23.63± 0.65cd | 11.15± 5.22a | 13.83± 3.09ab | 19.47± 4.25abcd | 20.30± 7.22bcd | 18.58± 1.84abcd | 16.69± 4.36abc | 25.58± 6.97d | 18.93 | 3.15* |
| EV (kcal) | 648.91± 6.1a | 679.56± 7.6abc | 708.6± 11.8 bc | 672.28± 18.6ab | 708.6± 21.7bc | 713.8± 17.5c | 685.60± 37.7abc | 678.85± 30.1abc | 676.63± 10.3abc | 673.70± 9.2ab | 697.89± 23.8bc | 685.86 | 2.83* |
| Fiber (%) | 4.97± 1.45abc | 6.75± 0.73c | 6.49± 0.21c | 6.36± 0.36c | 6.49± 0.21c | 5.60± 0.17bcd | 4.35± 0.48ab | 4.85± 1.34abc | 4.65± 0.82abc | 4.17± 0.16a | 5.95± 0.54cd | 5.51 | 5.03** |
| RSA (%) | 80.28± 2.69ab | 79.43± 5.92ab | 78.64± 4.16ab | 78.16± 3.73ab | 82.27± 2.10ab | 79.98± 6.00ab | 81.31± 2.75ab | 78.64± 4.06ab | 77.37± 6.42a | 85.96± 1.59b | 75.02± 1.65a | 79.73 | 1.44 |
| PPT (mg GAE 100 $g^{-1}$) | 2659± 230ab | 1394± 277ab | 1017± 155a | 3487± 229b | 3739± 1202ab | 3389± 1451ab | 3236± 1044ab | 2307± 1458ab | 2587± 1125ab | 1154± 707ab | 3396± 2205ab | 2579 | 2.22 |
| Fld (mg RE 100 $g^{-1}$) | 62.11± 26b | 29.25± 14.35ab | 15.7± 12.13a | 50.11± 23.66ab | 49.40± 17.74ab | 59.47± 28.66b | 39.09± 8.40ab | 21.46± 15.31a | 28.05± 7.09ab | 12.59± 10.9a | 41.72± 30.76ab | 37.18 | 2.32* |
| P (mg 100 $g^{-1}$) | 338.1± 46.4a | 610.83± 3.4bc | 555.7± 14.8 bc | 576.2± 120.5 bc | 485.1± 55.3ab | 514.2± 75.2bc | 640.87± 43.7bc | 559.77± 141bc | 646.47± 2.29bc | 675.9± 46.4 c | 662.27± 117c | 569.59 | 3.83** |
| K (mg 100 $g^{-1}$) | 251.6± 79.7 ab | 249.2± 48.0ab | 249.1± 24.6ab | 269.07± 12.7ab | 210.1± 15.7a | 227.5± 38.9 a | 291.23± 29.1ab | 261.63± 57.7ab | 338.93± 88.3b | 266.8± 13.9 ab | 266.53± 50.7ab | 261.97 | 1.42 |
| Mg (mg 100 $g^{-1}$) | 101.25± 9.0a | 374.5± 54.2a | 99.84± 7.83a | 100.53± 17.6a | 94.65± 10.2a | 91.88± 12.5a | 96.62± 11.77a | 100.38± 20.6a | 117.57± 6.6 a | 120.5± 10.2a | 79.15± 62.98a | 125.17 | 0.76 |
| Na (mg 100 $g^{-1}$) | 4.15± 2.54a | 2.46± 0.80a | 2.63± 0.86a | 1.78± 0.56a | 1.17± 0.84a | 2.95± 2.06a | 2.86± 1.14a | 6.80± 2.05ab | 3.57± 2.50a | 12.6± 11.5b | 3.48± 1.18a | 4.06 | 2.14 |
| Cu (mg 100 $g^{-1}$) | 2.08± 1.56a | 3.06± 0.81a | 4.82± 0.26b | 5.59± 0.37bc | 6.24± 0.27c | 5.96± 0.45c | 6.50± 0.06c | 6.43± 0.07c | 6.67± 0.29c | 6.65± 0.08c | 6.56± 0.22c | 5.50 | 21.7*** |
| Zn (mg 100 $g^{-1}$) | 18.63± 6.6b | 6.73± 0.92a | 4.78± 1.20a | 6.41± 1.03a | 3.39± 0.68a | 6.03± 2.92a | 4.97± 2.82a | 8.52± 2.70a | 4.31± 1.15a | 6.94± 4.09a | 4.10± 1.43a | 6.80 | 6.28*** |
| Mn (mg 100 $g^{-1}$) | 1.61± 0.95a | 1.05± 0.05a | 1.04± 0.34a | 1.55± 1.19a | 0.79± 0.16a | 1.65± 0.35a | 0.96± 0.54a | 1.34± 0.48a | 1.67± 0.54a | 1.05± 0.59a | 0.99± 0.18a | 1.25 | 0.90 |
| Fe (mg 100 $g^{-1}$) | 1.88± 0.06a | 1.85± 0.54a | 1.78± 0.10a | 1.66± 0.63a | 1.85± 0.27a | 1.45± 0.32a | 1.71± 0.40a | 2.64± 0.54b | 1.17± 0.45a | 1.30± 0.28a | 1.68± 0.18a | 1.72 | 2.90* |
| Cr (mg 100 $g^{-1}$) | 0.18± 0.01a | 0.19± 0.0ab | 0.19± 0.00b | 0.19± 0.00b | 0.20± 0.00b | 0.20± 0.00b | 0.19± 0.00b | 0.18± 0.02a | 0.19± 0.00b | 0.16± 0.00b | 0.19± 0.00b | 0.19 | 2.54* |
| Ni (mg 100 $g^{-1}$) | 1.26± 0.04a | 1.42± 0.02b | 1.41± 0.05b | 1.43± 0.06b | 1.39± 0.03b | 1.43± 0.02b | 1.41± 0.02b | 1.45± 0.01b | 1.42± 0.03b | 1.40± 0.05b | 1.44± 0.01b | 1.42 | 5.23** |
| B (mg 100 $g^{-1}$) | 1.49± 0.41d | 0.50± 0.17bc | 0.46± 0.11abc | 0.70± 0.28c | 0.29± 0.22ab | 0.25± 0.05ab | 0.35± 0.27abc | 0.18± 0.14ab | 0.26± 0.20ab | 0.56± 0.02bc | 0.07± 0.03a | 0.46 | 10.0*** |

Significance level, *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. Means marked with different letters, within each column, are significantly different ($p < 0.05$). Bold values are minimum and maximum

While the significant and negative correlations were indicted by the carbohydrates content with oil content (R=-0.69**) and protein content (R=-0.71**), the Zn with Cu, Cr and Ni respectively R=-0.72**, R=-0.78** and R=-0.81**. Furthermore, the B has negative correlation with Ni (R=-0.88**), Cu (R=-0.78**) and P (R=-0.67*), and the Fe with Cr (R=-0.71**).

The two first axes of principal components analysis (PCA) retained explain 64.53% of the total variation with each component explaining respectively 46.76% and 17.76. The highest contribution to PC1 corresponded negatively to total oil, energy value and some minerals like Cu, Cr and Ni, whereas corresponded positively to carbohydrates content, in addition to minerals such as P, Zn and B. The PC2 indicated that crude protein and Cu had the greatest negative contribution, while crude Fiber, flavonoid content and Cr had the highest positive contribution to this component. Figure 2 illustrated accessions on the plot of the first two axis space. The accessions may constitute two groups. The first one formed by the accessions belonging to Middle and High Atlas Mountains namely Naour (NAO), Taghzirte (TAG), Amouguer (AMG), Beram (BER), Anougal (ANG), Imlil (IML), Tabrijjate (TAB), Ait M'hamed (AMD), AitBougamaz (ABZ) and Demante (DEM) which are characterized by a high amount of oil, energy value, fiber, proteins, Cu and Cr. The second one is composed of one accession of Middle Atlas Mountain, Aghbala (AGH) with a greatest level of flavonoids, carbohydrates, Zn and remarkable content of B.

**Table 3.** Correlation coefficients among biochemical parameters measured in the walnut accessions.

| | DM | H | Ash | Oil | Prot | Cbhydt | EV | Fber | RSA | PPT | Flvd | P | K | Mg | Na | Cu | Zn | Mn | Fe | Cr | Ni |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | -1.00** | | | | | | | | | | | | | | | | | | | | |
| Ash | 0.52 | -.51 | | | | | | | | | | | | | | | | | | | |
| Oil | 0.17 | -.16 | 0.03 | | | | | | | | | | | | | | | | | | |
| Prot | -.47 | 0.47 | 0.01 | 0.08 | | | | | | | | | | | | | | | | | |
| Cbhydt | 0.29 | -.29 | 0.02 | -.69** | -.71** | | | | | | | | | | | | | | | | |
| EV | 0.23 | -.23 | 0.07 | 0.97** | 0.06 | 0.66* | | | | | | | | | | | | | | | |
| Fiber | -.18 | 0.18 | -.50 | 0.47 | -.27 | -.13 | 0.44 | | | | | | | | | | | | | | |
| RSA | -.59* | 0.59* | 0.12 | -.05 | 0.68* | -.51 | -.11 | -.37 | | | | | | | | | | | | | |
| PPT | -.02 | 0.02 | -.57* | 0.11 | -.33 | 0.20 | 0.13 | 0.06 | 0.70** | | | | | | | | | | | | |
| Flvd | -.22 | 0.22 | -.73** | -.03 | -.20 | 0.17 | -.02 | 0.20 | -.15 | 0.79** | | | | | | | | | | | |
| P | 0.21 | -.21 | 0.63* | 0.17 | -.18 | 0.04 | 0.19 | -.18 | -.07 | -.19 | -.64* | | | | | | | | | | |
| K | 0.25 | -.25 | 0.33 | -.45 | -.12 | 0.40 | -.43 | -.55* | -.28 | -.05 | -0.35 | 0.53* | | | | | | | | | |
| Mg | -.36 | 0.37 | -.08 | -.15 | 0.05 | 0.04 | -.17 | 0.36 | 0.04 | -.46 | -.22 | 0.16 | -.06 | | | | | | | | |
| Na | 0.07 | -.07 | 0.74** | -.37 | 0.33 | 0.02 | -.35 | -.66* | 0.55 | -.51* | -.55* | 0.30 | 0.16 | -.09 | | | | | | | |
| Cu | 0.28 | -.28 | 0.40 | 0.41 | 0.08 | -.28 | 0.44 | -.33 | 0.03 | 0.29 | -.29 | 0.64* | 0.29 | -.51 | 0.24 | | | | | | |
| Zn | -0.10 | 0.11 | -.19 | -.73** | 0.02 | 0.45 | -.73** | -.26 | 0.13 | -.10 | 0.35 | -.72** | -.11 | 0.01 | 0.20 | -.72** | | | | | |
| Mn | -.06 | 0.06 | -.16 | -.48 | 0.15 | 0.28 | -.40 | -.24 | -.28 | 0.22 | 0.36 | -.33 | 0.33 | -.16 | -.03 | -.19 | 0.45 | | | | |
| Fe | 0.23 | -.23 | -.14 | -.08 | -.18 | 0.17 | -.08 | 0.17 | -.17 | -.02 | -.04 | -0.34 | -.36 | 0.06 | -.08 | -.21 | 0.29 | -.17 | | | |
| Cr | -.20 | 0.2 | -.04 | 0.64* | -.01 | -.41 | 0.62* | 0.3 | 0.02 | 0.26 | 0.06 | 0.48 | 0.02 | -.08 | -.30 | 0.48 | -.78** | -.26 | -.71** | | |
| Ni | 0.24 | -.24 | 0.34 | 0.48 | -.14 | -.15 | 0.52 | 0.17 | -.29 | 0.03 | -.45 | 0.76** | 0.17 | 0.06 | 0.01 | 0.69** | -.81** | -.18 | 0.02 | 0.42 | |
| B | -.41 | 0.41 | -.35 | -.67* | 0.05 | 0.34 | -.71** | -.09 | 0.26 | -.12 | 0.35 | -.67* | -.10 | 0.06 | 0.06 | -.78** | 0.87** | 0.35 | 0.01 | -.47 | -.88** |

Significance level: *: $p < 0.05$; **: $p < 0.01$

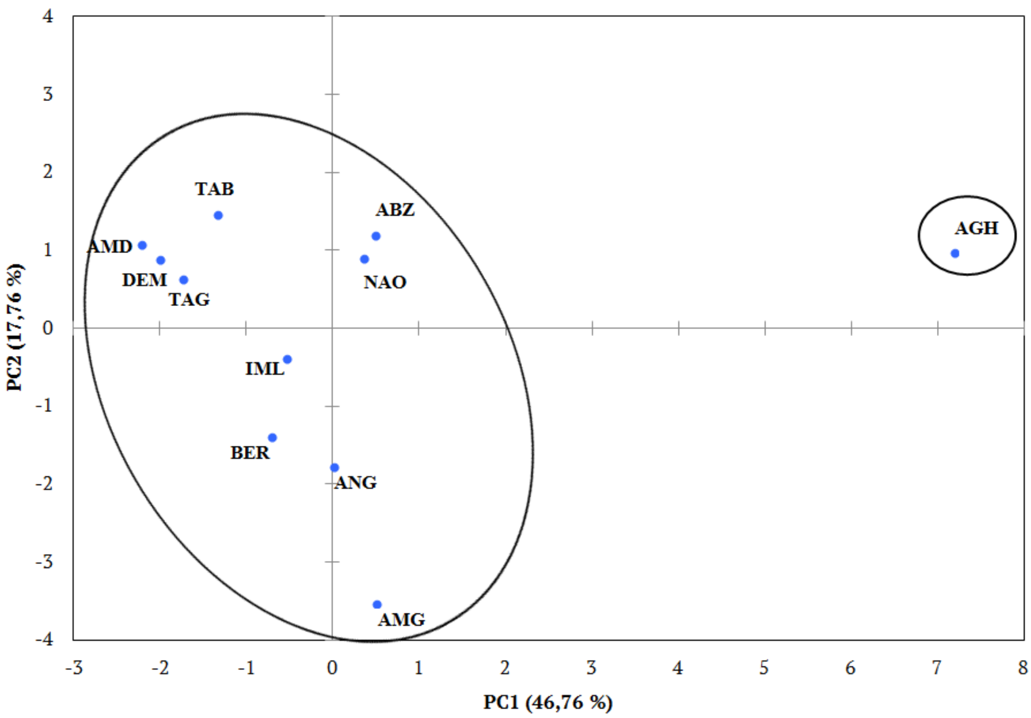


**Figure 2.** Plot on the first two principal components of 11 walnut accessions.

Likewise, Hierarchical cluster analysis led to identify two major groups (Figure 3). One is made of Aghbala accession originating from Middle Atlas Mountain. The other group bifurcated in two subgroups: one formed of Naour accession arising from Middle Atlas Mountain and the other one containing the rest of accessions that were partitioned also in two subgroups. A subgroup composed of Taghzirte, belonging to Middle Atlas Mountain, and Anougal, Ait Bougamez, Ait Mhamed and Demante emanating from High Atlas Mountain. The other subgroup is formed of Imlil, Tabrijjate, Amouguer and Beram coming from High Atlas Mountain.

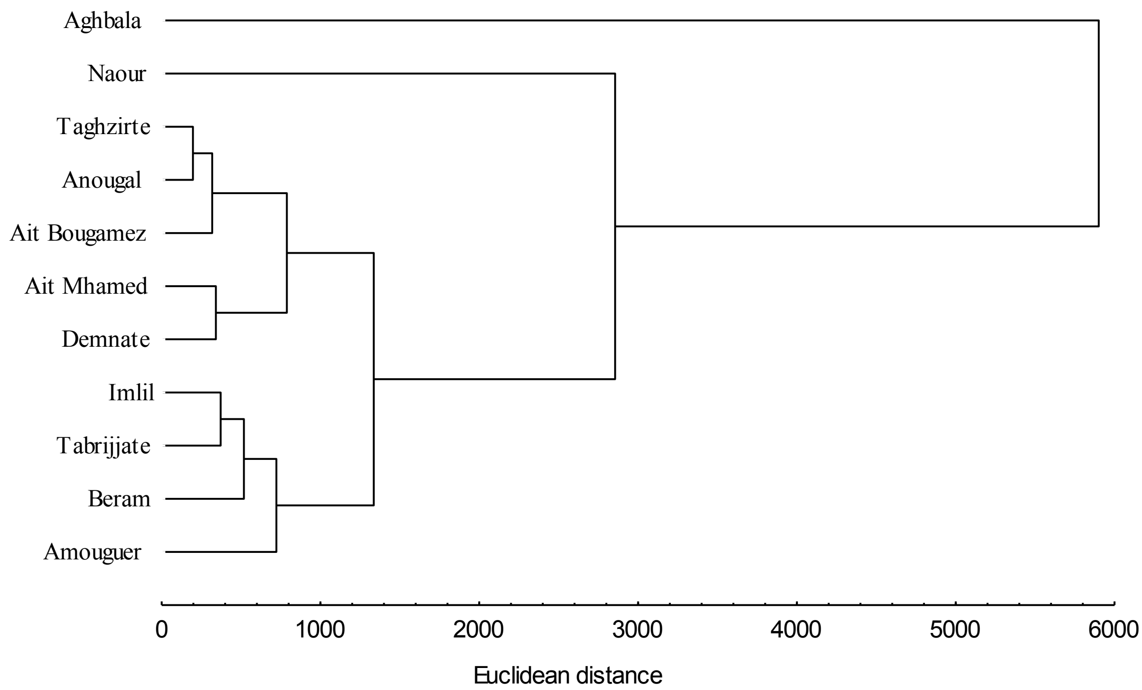


**Figure 3.** Dendrogram of 11 accessions of Moroccan walnut based on biochemical parameters

## Discussion

For the purpose of this study, 22 biochemical parameters were chosen to characterize Moroccan walnut germplasm. This study revealed that analysis of biochemical parameters showed significant differences between Moroccan walnut accessions. These results are strengthening by previous work on the same accessions by using morphological and pomological traits (Kabiri, Bouda, Elhansali, & Haddioui, 2018).

As expected, the walnut kernels had low moisture content (1.44 to 3.24%) which is similar to that reported by Muradoglu et al. (2010) (1.1-2.7%), Yerlikaya et al. (2012) (1.91-4.48) and Polat, Okatan, and Guçlu (2015), (2.14-3.98%) for Turkish walnut, but inferior of those found in Portugal cultivars by Pereira et al. (2008), (3.85-4.50%), in Romanian cultivars by Leahu, Damian, Oroian and Hretcanu (2013) (3.85-4.58%) and in Spanish cultivars by Tapia et al. (2013) (1.29-4.67%). In fact, low moisture content is important factor to maintain quality and augment shelf life of kernels as this parameter decreases the likelihood of microbial growth, premature germination of seeds and late biochemical changes associated with these processes.

For the total ash, the accessions studied present 1.67 to 2.53%. This result is comparable with that obtained in Turkish cultivars (1.99-2.53; 1.5-2.8; 1.44-2.14%) respectively by Özcan, İman, and Arslan (2010), Muradoglu et al. (2010) and Polat et al. (2015) and in Romania cultivars (1.7-2.2%) by Leahu et al. (2013), but it is higher than that presented by genotypes of walnut in Spain (1.12-1.29%) by Tapia et al. (2013) and lower than that recorded in Portugal walnut (3.31-4.26%) by Pereira et al. (2008). These differences could be attributed to the cultivar, harvesting year and accompanying conditions such as climate, geographical origin and the methods of cultivation. Indeed, different values of temperature, rainfall and light can influence chemical composition of fruits.

Fat was the predominant constituent in all accessions and total oil content recorded (54.50-65.48%) is in agreement with that found in Romanian walnut (53.81-66.1%) by Leahu et al. (2013), Spanish walnut (58.3-65.2%) by Tapia et al. (2013) and Turkish walnut (58.44-67.14%) by Polat et al. (2015), but it is lower than that reported by Pereira et al. (2008) (68.83-72.14%) for Portugal walnut.

Protein contents of kernel showed a wide variation depending on accessions, ranging from 8.17 to 19.25%. These values are within the range of consulted literature concerning Turkish walnut (10.58-18.19%; 12.8- 22.3%; Yerlikaya et al. (2012) and Muradoglu et al. (2010) respectively), Romanian walnut (13.81 - 17.22%; Leahu et al. (2013)) and Iranian cultivars (13.50- 20.20%; Aryapak & Ziarati (2014)). Then, walnuts are a good source of vegetable protein; they may be used in vegetarian meals. Accordingly, walnuts have been associated with low cardiovascular crisis compared to protein of animal origin (Yang, 2009; Carvalho et al., 2010).

The studied kernels accessions were revealed very energetic (648.01 - 713.83 kcal) while they were very rich in natural sugars varying from 11.5 to 25.85%. A direct correlation was reported between energetic value and carbohydrates amount in walnut kernel (Gharibzahedi, Ghasemlou, Razavi, Jafarii, & Faraji, 2011; Leahu et al. 2013) which strengthening our findings. The result of carbohydrates content is similar to that reported by Leahu et al. (2013) (12.1-24.38%) in Romanian walnut and higher than the values obtained in Portugal cultivars (3.75-7.16%) (Pereira et al., 2008). These differences might be because of differences in harvesting year and environmental conditions, with different temperature, rainfall and light, which could affect the chemical composition of fruits (Parcerisa et al., 1995). So, fat was the predominant component in all kernel accessions, followed by carbohydrates, protein, fiber, moisture and ash and this decreasing order of proximate composition is in line with that established by Food Composition Database (USDA, 2018) and Food Composition and Nutrition Tables (Souci et al., 2008).

The determination of phenolic content in walnut has gained increasing interest with the recognition that these compounds are bioactive and particularly have antioxidant properties (Anderson et al., 2001; Fukuda, Ito, & Yoshida, 2003) that were evidenced in the present work by a strong association between total phenolic content and antioxidant activity (R=0.70**). Phenolic content reached appreciable levels in the Moroccan walnut cultivars and ranging within values from 1,017 to 3,739 mg GAE 100 $g^{-1}$ DW. These findings are somewhat higher than that reported by Leahu et al. (2013) (1,728-2,352 mg GAE 100 $g^{-1}$ DW) for Romanian walnut. Their contents depend on many environmental conditions, as well as genotype of different accessions (Mpofu, Sapirstein, & Beta, 2006; Solar, Colarič, Usenik, & Stampar, 2006; Tapia et al., 2013)

The antioxidant activity of walnut kernel was assessed by scavenging activity on DPPH radicals which varied from 75.02 to 85.96%. The obtained results are in agreement with the ones published by Akbari, Heidari and Jamei (2014) (53.83-94.09%) for some walnut cultivars in Iran. The antioxidant activity of walnut kernel is mainly attributed to the presence of high content of polyphenols, which are considered among the more powerful natural antioxidants (Pereira et al., 2008; Gharibzahedi et al., 2011; Akbari, Jamei, Heidari, & Jahanban-Esfahlan, 2012). Therefore, this study indicates that the fruit of Moroccan walnut can be considered as a good source of the natural compounds with antioxidant activities that can be used in the therapeutic as well as condiments in functional foods.

High level of the major minerals phosphorus, potassium and magnesium were found in Moroccan walnut accessions. By contrast, low levels of sodium were detected. According to the literature, walnut kernels contain high amounts of potassium (390-700 mg 100 $g^{-1}$), phosphorus (310-510 mg 100 $g^{-1}$) and magnesium (90-140mg 100 $g^{-1}$), and lower sodium (1-15mg 100 $g^{-1}$). The Moroccan walnut was revealed very rich in phosphorus (338.10-675.87mg 100 $g^{-1}$) in comparison with several works (Gharibzahedi et al., 2011: 289-365 mg 100 $g^{-1}$, Özcan et al. (2010): 222.62-260.42 mg 100 $g^{-1}$, Muradoglu et al. (2010): 299.1-434.7 mg 100 $g^{-1}$) and also with USDA National Nutrient Database for Standard Reference (346 mg 100 $g^{-1}$) released in 2018. In human body, phosphorus is needed to build strong-healthy bones, make energy, move muscles, filter waste and repair tissue and cells. The recommended daily intake for a human adult is about 700 mg and the walnut kernel consumption can cover the daily required value. The chemical composition and the pH of the soil are well reviewed to influence the chemical composition of fruits (Pereira et al., 2008; Muradoglu et al., 2010; Tapia et al., 2013). It's noteworthy that the bedrock of Moroccan soil is very rich in phosphorus and potassium elements which provide continuously the soil with these elements (Van Straaten, 2011). Accordingly, the soils of Morocco are not P and K deficient. The inherent P and K concentrations in soils are sufficiently high for P and K demanding crops, as well as in areas where continuous cropping has led to P and K deficiencies (Van Straaten, 2011). The potassium, that functions as an electrolyte, reached appreciable levels (up to 339 mg 100 $g^{-1}$) in Moroccan walnuts and it is accompanied by high quantities of magnesium (up to 374.5 mg 100 $g^{-1}$) which are higher than that established by USDA (2018). Magnesium plays a role in over 300 enzymatic reactions within the body, including the metabolism of food, synthesis of fatty acids and proteins, and the transmission of nerve impulses; recommended daily allowance is about 310 mg, and walnut kernel intake can respond to the daily demanded amount. Sodium is one of the most important electrolytes needed for the regulation of blood. The highest sodium content (12.63 mg 100 $g^{-1}$ DW) was determined for Amouguer accession as compared to other accession and which could be considered as an abundant source of sodium. As regards micronutrient contents, zinc (6.80 mg 100 $g^{-1}$) and copper (5.50 mg 100 $g^{-1}$) were generally higher than those reported by many studies in different walnut cultivars (Gharibzahedi et al., 2011; Muradoglu et al., 2010; Cosmulesco et al, 2009; Özcan, 2009; Tapia et al., 2013) and also by USDA (2018). Zinc is vital for a healthy immune system, correctly synthesizing DNA, promoting healthy growth during childhood, and healing wounds. Only a small intake of zinc (8 mg) is necessary to reap the benefits, and walnut kernel consumption can meet the recommended daily allowance. Whilst, Copper is an essential nutrient for the body, together with iron, it enables the body to form red blood cells and alone it helps maintain healthy bones, blood vessels, nerves, and immune function, and it contributes to iron absorption. The recommended daily allowance for Cu is around 0.9 mg for adolescents and adults, and then consumption of all studied accessions can satisfy this amount. By contrast, iron (1.72 mg 100 $g^{-1}$) and manganese (1.25 mg 100 $g^{-1}$) were somewhat less represented in Moroccan walnut in comparison with that of other countries as Spain (2.1 and 2.2 mg 100 $g^{-1}$ respectively) (Tapia et al., 2013). Iron is a vital mineral to the proper function of haemoglobin, promotes healthy pregnancy and increased energy, daily required intake depends on a person's age and sex, but for an human male adult is about 8 mg. Manganese is considered an essential nutrient for our body and is required for the normal functioning of brain, nervous system and many of body’s enzyme systems. So, the Moroccan walnuts are deficient in iron and manganese elemental nutrients. Accordingly, any future breeding programs should consider iron and manganese as among selection criteria. Appreciable level of nickel was determined in studied accessions and five out of 11 accessions having higher values than average (1.42 mg 100 $g^{-1}$). In human body, Nickel is used for increasing iron absorption, preventing anaemia, and treating weak bones. Values under 1 mg 100 $g^{-1}$ were obtained for chromium (0.19) and boron (0.46) and are complying with mineral composition in walnut kernels (Souci et al., 2008; Cosmulescu et al., 2009; Ozakan, 2009; USDA, (2018). Chromium is an essential trace mineral that can improve insulin sensitivity and enhance protein, carbohydrate, and lipid metabolism. The adequate intake of chromium for men is about 0.030 mg per day, and then consumption of all studied walnut accessions can cover the required amount. Among the multiple roles that boron plays in our organism are reduction of inflammation and oxidative stress. So, the descending order of nutritive elements contents (mg 100 $g^{-1}$) in Moroccan walnut studied cultivars was P> K> Mg> Zn> Cu> Na> Fe> Ni> Mn> B> Cr, whereas it was P>K>Mg>Ca>Fe>Mn>Zn>Cu>Na for Iranian walnuts (Gharibzahedi et al., 2011), K>P>Mg>B>Mn>Na>Fe>Zn>Cr>Cu>Ni for Turkish walnut (Özcan, 2009) and K>Mg>Ca>Mn>Fe>Zn> Cu>Na>Cr>Al>Se for Romanian walnuts (Cosmulesco et al., 2009). The order depending on contents of mineral elements in Moroccan walnuts was congruent with that released by USDA (2018) except for phosphorus and zinc which respectively ranked second and seventh in USDA listed mineral elements, while they ranked top and fourth in Moroccan accessions. Levels of these elements in the cultivated soil have a great influence on chemical composition in the fruits (Yerlikaya et al., 2012; Aryapak & Ziarati, 2014).

According to hierarchical analysis based on biochemical parameters, the Moroccan walnut accessions were structured into four distinct groups independently of the mountain range type. Corroborating it, the results obtained by Kabiri et al. (2018) by analyzing the same accessions using pomological and morphological traits. The first group included Aghbala accession, seems to diverge significantly from the others, has the highest level of flavonoids, Zn and B. The second group contains Naour accession with greatest level of fiber and Mg. The third group includes Taghzirte, Anougal, Ait Bougammez, Ait Mhamed and Demnate accessions generally characterized by high level of oil, energy value, phenolic compounds, Fe, Cr and Ni. The fourth group contained four accessions namely Imlil, Tabrijjate, Beram and Amouguer with high levels of protein, carbohydrate, ash, RSA, P, K, Cu, Na and low content of moisture.

## Conclusion

The present investigation shows that Moroccan walnut is characterized by increased biological and nutritional values. This finding should be exploited to select the best walnut genotypes with the high level of biochemical parameters in order to increase the production, as this tree takes a great place in Moroccan diet, customs and economic activities for the farmers in mountain. Because of economic value of the oil, these kernels could be used as potential sources of oils and be found between strategic foods in the future. Moreover, Moroccan walnuts were revealed as a good source of vegetable protein and may be used in vegetarian diets. Besides, this study, indicates that Moroccan walnuts can be considered as a good source of the naturals compounds with antioxidant activity that can be used in the therapeutic as well as condiments in functional foods. High variability was found between studied accessions for proximate-mineral composition and antioxidant activity. This variability could be attributed chiefly to genetic factors in conjunction with harvesting year, geographic origin, environmental conditions, soil elemental composition, maturity level and the methods of cultivation. The comparison of mineral composition of kernels walnuts revealed a significant richness of mineral elements, essentially phosphorus, zinc and copper, and a deficiency in iron and manganese. Taking into account the relevance of high kernel level of biochemical parameters studied, the walnut accessions from High Atlas Mountain should be considered as the first accessions to be useful as seed sources.

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## References

Akbari, V., Heidari, R., & Jamei, R. (2014). Fatty acid compositions and nutritional value of six walnut (*Juglans regia* L.) cultivars grown in Iran. *Advanced Herbal Medicine*, *1*(1), 36-41.

Akbari, V., Jamei, R., Heidari, R., & Jahanban-Esfahlan, A. (2012). Antiradical activity of different parts of Walnut (*Juglans regia* L.) fruit as a function of genotype. *Food chemistry. 135*(4), 2404-2010. doi: 10.1016/j.foodchem.2012.07.030

Anderson, K. J., Teuber, S. S., Gobeille, A., Cremin, P., Waterhouse, A. L., & Steinberg, F. M. (2001). Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. *The Journal of Nutrition, 131*(11), 2837–2842. doi: 10.1093/jn/131.11.2837

Aryapak, S., & Ziarati, P. (2014). Nutritive value of Persian walnut (*Juglans regia* L.) Orchards. *American-Eurasian Journal of Agricultural & Environmental Sciences*, *14*(11), 1228-1235. doi: 10.5829/idosi.aejaes.2014.14.11.12438

Association of Official Analytical Chemists [AOAC]. (1995). *Official Methods of Analysis* (16th ed.). Arlington, VA: AOAC

Association of Official Analytical Chemists [AOCS]. (1998). *Official methods and recommended practices of the American Oil Chemists'Society* (15th ed.). Washington, DC. AOCS, USA

Bolling, B. W., Dolnikowski, G., Blumberg, J. B., & Chen, C. Y. O. (2010). Polyphenol content and antioxidant activity of California almonds depend on cultivar and harvest year. *Food Chemistry, 122*(3), 819-825. doi: 10.1016/j.foodchem.2010.03.068

Carvalho, M., Ferreira, P. J., Mendes, V. S., Silva, R., Pereira, J. A., Jerónimo, C., & Silva, B. M. (2010). Human cancer cell antiproliferative and antioxidant activities of *Juglans regia* L. *Food and Chemical Toxicology, 48*(1), 441–447. doi: 10.1016/j.fct.2009.10.043

Cosmulescu, S., Baciu, A., Achim, G., Botu, M., & Trandafir, I. (2009). Mineral composition of fruits in different walnut (*Juglans regia* L.) cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 37*(2), 156-160. doi: 10.15835/nbha3723169

Food Agriculture Organization of the United Nations [FAOSTAT]. (2017). *World production of walnuts*. Rome, IT: FAOSTAT. Retrieved from http://www.fao.org/faostat/en/#data/QC

Fukuda, T., Ito, H., & Yoshida, T. (2003). Antioxidative polyphenols from walnuts (*Juglans regia* L.). *Phytochemistry, 63*, 795-801. doi: 10.1016/S0031-9422(03)00333-9

Gharibzahedi, S. M. T., Ghasemlou, M., Razavi, S. H., Jafarii, S. M., & Faraji, K. (2011). Moisture-dependent physical properties and biochemical composition of red lentil seeds. *International Agrophysics*, *25*(4), 343-347.

Grosso, N. R., Nepote, V., & Guzman, C. A. (2000). Chemical composition of some wild peanut species (*Arachis* L.) seeds. *Journal of Agricultural and Food Chemistry, 48*(3), 806-809. doi: 10.1021/jf9901744

Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. *Chemical and Pharmaceutical Bulletin, 36*(6), 2090–2097. doi: 10.1248/cpb.36.2090

Kabiri, G., Bouda, S., Elhansali, M., & Haddioui, A. (2018). Morphological and pomological variability analysis of walnut (*Juglans regia* L.) genetic resources from the middle and high atlas of Morocco. *Atlas Journal of Biology*, 575-582. doi: 10.5147/ajb.v0i0.179

Leahu, A., Damian, C., Oroian, M., & Hreţcanu, C.E. (2013). Estimation of biochemical properties of walnuts from the region of Suceava – Romania. *Food and Environment Safety*, *12*(2), 169-175.

Li, L., Tsao, R., Yang, R., Liu, C., Zhu, H., & Young, J. C. (2006). Polyphenolic profiles and antioxidant activities of heartnut (*Juglans ailanthifolia* Var. *cordiformis*) and Persian walnut (*Juglans regia* L.). *Journal of Agricultural and Food Chemistry*, *54*(21), 8033–8040. Doi: 10.1021/jf0612171

McGranahan, G., & Leslie, C. (1990). Walnuts (*Juglans*). In J.N. Moore & J. R. Ballington, (Eds.), Genetic Resources of Temperate Fruit and Nut Crops (Vol. 2, p. 907-951) Wageningen, NL: ISHS.

Mikdat, S. (2010). Determination of walnut genotypes with high fruit bearing and quality in Dicle, Hani, Egil and Kocaköy Townships. *Journal of Agricultural Faculty of Gaziosmanpasa University*, *27*(1), 85-93.

Miraliakbari, H., & Shahidi, F. (2008) Antioxidant activity of minor components of tree nut oils. *Food Chemistry*, *111*(2), 421–427. doi: 10.1016/j.foodchem.2008.04.008

Mpofu, A., Sapirstein, H. D., & Beta, T. (2006). Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. *Journal of Agricultural and Food Chemistry*, *54*(4), 1265–1270. doi: 10.1021/jf052683d

Muradoglu, F., Oguz, H. I., Yildiz, K., & Yilmaz, H. (2010). Some chemical composition of walnut (*Juglans regia* L.) selections from Eastern Turkey. *African Journal of Agricultural Research*, *5*(17), 2379-2385

Özcan, M. M. (2009). Some nutritional characteristics of fruit and oil of walnut (*Juglans regia* L.) growing in Turkey. *Iranian Journal of Chemistry and Chemical Engineering*, *28*(1), 57–62

Özcan, M. M., İman, C., & Arslan, D. (2010). Physico-chemical properties, fatty acid and mineral content of some walnuts (*Juglans regia* L.) types. *Agricultural Sciences*, *1*(2), 62-67. doi: 10.4236/as.2010.12009

Parcerisa, J., Rafecas, M., Castellote, A. I., Codony, R., Farràn, A., Garcia, J., ... & Boatella, J. (1995). Influence of variety and geographical origin on the lipid fraction of hazelnuts (*Corylus avellana* L.) from Spain: (III) oil stability, tocopherol content and some mineral contents (Mn, Fe, Cu). *Food Chemistry*, *53*(1), 71–74. doi: 10.1016/0308-8146(95)95789-9

Pereira, J. A., Oliveira, I., Sousa, A., Ferreira, I. C. F. R., Bento, A., & Estevinho L. (2008). Bioactive properties and chemical composition of six walnut (*Juglans regia* L.) cultivars. *Food and Chemical Toxicology*, *46*(6), 2103- 2111.

Polat, M., Okatan, V., & Güçlü, S.F. (2015). Determination of some physical and chemical properties of walnut (*Juglans regia* L.) Genotypes grown in the central district of Bitlis/Turkey. *Scientific Papers. Series B, Horticulture*, *59*, 81-6.

Ranganna, S. (1955). Handbook of analysis and quality control for fruit and vegetable products (2nd ed.). New Delhi, IN: Tata McGraw Hill.

Sen, S. M. (1986). *Ceviz Yetistiriciligi.* Samsun, TU: Eser Matbaas.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16(3)*,144–158.

Solar, A., Colarič, M., Usenik, V., & Stampar, F. (2006). Seasonal variations of selected flavonoids, phenolic acids and quinones in annual shoots of common walnut (*Juglans regia* L.). *Plant Science, 170*(3), 453-461. doi: 10.1016/j.plantsci.2005.09.012

Souci, S. W., Fachmann, W., & Kraut, H. (2008). Food composition and nutrition tables. Die zusammensetzung der lebensmittel, nahrwert-tabellen. La composition des aliments - Tableux des valeurs nutritives. Stuttgart, DE: MedPharm Gmbh Scientific

Statistica StatSoft Inc. (1997). *Statistica release 5.1* [Software]. Tulsa, OK: StatSoft Inc. Retrieved from http://www.statsoft.com

Statistical Package for the Social Sciences [SPSS]. (2011). *IBM SPSS Statistics for Windows, version 20.0* [Software]. Armonk, NY: IBM Corp.

Tapia, M. I., Sánchez-Morgado, J. R., García-Parra, J., Ramırez, R., Hernandez, T., & González-Gómez, D. (2013). Comparative study of the nutritional and bioactive compounds content of four walnut (*Juglans regia* L.) cultivars. *Journal of Food Composition and Analysis, 31*(2), 232-237. doi: 10.1016/j.fca.2013.06.004

United States Department of Agriculture [USDA]. (2018). *National Nutrient Database for Standard Reference - Release 1* (Basic Report, 12154) Retrieved from http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl

Van Straaten, P. (2011). The geological basis of farming in Africa. In A. Bationo, B. Waswa, J. M. Okeyo, F. Maina & J. M. Kihara (Eds.), *Innovations as key to the green revolution in Africa* (p. 31-47). Berlin, DE: Springer Science+Business Media. doi: 10.1007/978-90-481-2543-2_3,

XLSTAT. (2014). *Version 2014.5.03* [Software]. Retrieved from https://www.xlstat.com

Yang, J. (2009). Brazil nuts and associated health benefits: A review. *LWT - Food Science* and *Technology*, *42*(10), 1573–1580. doi: 10.1016/j.lwt.2009.05.019

Yerlikaya, C., Yucel, S., Erturk, Ü., & Korukluoğlu, M. (2012). Proximate composition, minerals and fatty acid composition of *Juglans Regia* L. genotypes and cultivars grown in Turkey. *Brazilian Archives of Biology and Technology, 55*(5), 677-683. doi: 10.1590/S1516-89132012000500006

Zwarts, L., Savage, G. P., & McNeil, D. L. (1999). Fatty acid content of New Zealand-grown walnuts (*Juglans regia* L.). *International Journal of Food Sciences and Nutrition, 50*(3), 189–19. doi: 10.1080/096374899101229