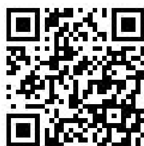

GRASAS Y ACEITES 67 (1)
January–March 2016, e116
ISSN-L: 0017-3495
doi: http://dx.doi.org/10.3989/gya.0256151

Genotype and year variability of the chemical composition of walnut oil of Moroccan seedlings from the high Atlas Mountains

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Submitted: 26 February 2015; Accepted: 15 September 2015

SUMMARY: Protein and oil content, fatty acid composition and tocopherol concentration were determined for two years in the kernel of ten candidate walnut selections from the high Atlas Mountains in Morocco. Considerable variation between genotypes was found for all parameters. The ranges of protein content (11.58–14.5% of kernel dry weight, DW), oil content (54.4–67.48% of kernel DW), oleic (12.47–22.01% of total oil), linoleic (55.03–60.01%), linolenic (9.3–15.87%), palmitic (6.84–9.12%), and stearic (1.7–2.92%) acid percentages, γ-tocopherol (188.1–230.7 $mg{\cdot}kg^{-1}$ of oil), δ-tocopherol (23.3–43.4 $mg{\cdot}kg^{-1}$), and α-tocopherol (8.9–16.57 $mg{\cdot}kg^{-1}$) contents agreed with previous results obtained from other commercial walnut cultivars. The effect of year was significant for all the chemical components, except for oil content and palmitic acid percentage. Some genotypes showed high oil contents and consistently high values of γ-tocopherol in both years of study. The introduction of these genotypes as new cultivars by vegetative propagation may result in a an increase in quality of the walnuts from the high Atlas Mountains of Morocco, and as a seed source for forest walnut propagation in the same region.

KEYWORDS: *Fatty acid profile; Genetic resources; Oil content; Protein content; Tocopherol content; Walnut*

RESUMEN: ***Variabilidad genotípica y anual de la composición química del aceite de nuez de plantones de nogal del Alto Atlas***. Se determinó durante dos años el contenido en proteína y aceite, la composición en ácidos grasos y la concentración en tocoferoles en la pepita de diez plantones de nogal seleccionados en el Alto Atlas de Marruecos, encontrándose una considerable variación entre genotipos para todos los parámetros. Los rangos del contenido en proteína (11.58–14.5 % del peso seco, PS), contenido en aceite (54.4–67.48 % PS), porcentaje de ácido oleico (12.47–22.01% del aceite total), linoleico (55.03–60.01 %), linolénico (9.3–15.87 %), palmítico (6.84–9.12 %), y esteárico (1.7–2.92 %), contenido en γ-tocoferol (188.1–230.7 $mg{\cdot}kg^{-1}$de aceite), δ-tocoferol (23.3–43.4 $mg{\cdot}kg^{-1}$) y α-tocoferol (8.9–16.57 $mg{\cdot}kg^{-1}$), coincidieron con resultados previos de otros cultivares comerciales de nogal. El efecto del año fue significativo para todos los componentes químicos, excepto para el contenido en aceite y el porcentaje de ácido palmítico. Algunos genotipos destacaron por un elevado contenido en aceite y valores consistentes de γ-tocoferol en ambos años del estudio. Estos genotipos muestran interés para su propagación vegetativa como nuevos cultivares para aumentar la calidad de las nueces del Alto Atlas, así como fuente de semillas para la propagación forestal del nogal en la misma región.

PALABRAS CLAVE: *Contenido en aceite; Contenido en proteína; Contenido en tocoferoles; Nogal; Perfil de ácidos grasos; Recursos genéticos*

Citation/Cómo citar este artículo: Kodad O, Estopañán G, Juan T, Socias i Company R, Sindic M. 2016. Genotype and year variability of the chemical composition of walnut oil of Moroccan seedlings from the high Atlas Mountains. *Grasas Aceites* **67** (1): e116. doi: http://dx.doi.org/10.3989/gya.0256151.

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1. INTRODUCTION

The Persian or English walnut (*Juglans regia* L.) is native of the mountain ranges of Central Asia (Leslie and McGranahan, 1998). It is a traditional fruit crop in North Africa and its first introduction into the Maghreb is attributed to the Romans (Germain, 1992). Walnut trees cover an area of 7600 ha in Morocco, and are considered a forestry fruit tree by local farmers and populations. Walnut trees are found in mountainous and remote areas between 800 and 1800 m above sea level and in different environments (Lansari *et al.*, 2001). The most important populations were identified in the high and medium Atlas Mountains. More than half of the trees are seedlings resulting from the prevailing way of propagation known by farmers, since grafting is unknown. As a consequence, the genetic variability of the local Moroccan walnut population is assumed to be very large (Lansari *et al.*, 2001). Morocco, as well as other countries with forestry resources, is paying great attention in protecting seedling stands as valuable tools for biodiversity conservation, and as a source of high quality plant material. Moreover, the selection of productive and drought tolerant genotypes with high kernel quality could be essential for the conservation of walnut trees in the mountain regions of Morocco once defined and propagated by grafting, offering extra economical benefits to the farmers and local populations. The studies on the genetic diversity of the local walnut populations of Morocco are scarce, with the Moroccan germplasm being characterized by smaller fruits, more shriveled kernels, higher aborted buds and lower fruiting potential than the French germplasm, although some genotypes were selected because of their high physical quality (Lansari *et al.*, 2001). Nevertheless, the chemical quality of the kernel of these local populations has not been approached.

Information on the composition of edible vegetable oils is required to ascertain their quality in terms of storage stability, flavor, taste, and nutrition. Such knowledge is informative for assessing the dietary intake of beneficial components of the oil (Crews *et al.*, 2005), as well as to choose the plant source for establishing a new orchard (Malvolti *et al.*, 2010). The high nutritive value of walnut kernels arises mainly from their high lipid content, ranging between 52 and 70%, which constitutes an important caloric source (Amaral *et al.*, 2003). Furthermore, the high lipid content in walnut kernels is relevant as a source of carbon and energy during germination and seedling growth (Chenevard *et al.*, 1994). The major constituents of walnut oil are triglycerides, in which the fatty acid (FA) profile shows that unsaturated FA (UFA) amount to about 70% of the walnut kernel oil. Monounsaturated FA (MUFA, mainly oleic acid) and polyunsaturated FA (PUFA, mainly linoleic and α-linolenic acids) are present in high amounts (Crews *et al.*, 2005; Martinez *et al.*, 2006). Their ratios are indices of the economic and nutritional value of the nut. Lower linoleic and linolenic acid contents may allow for a longer shelf life, whereas MUFAs may be more desirable because of their potential health benefits (Amaral *et al.*, 2003). The content of UFAs and the level of lipid oxidation are the most important quality parameters in walnuts for storage stability (Jensen *et al.*, 2001). High levels of UFAs result in oxidation products with an undesirable rancid taste.

Tocopherols are natural monophenols with antioxidant activities (Reische *et al.*, 1998) with several homologues depending on the position and number of methyl groups. Their main biochemical function is believed to be the protection of PUFAs against peroxidation and can stabilize FAs in oil (Savage *et al.*, 1999). The content of tocopherol homologues in nut oils is important owing to their antioxidant property and their positive nutritional effects on human metabolism. In walnuts, γ-tocopherol has been identified as the major vitamin E homologue, followed by α- and δ-tocopherol (Amaral *et al.*, 2005). High levels of γ-tocopherol may be associated with a reduction in blood cholesterol levels and in death from cardiovascular diseases. Several epidemiological and experimental studies suggest that the walnut is probably a potent chemo-preventive agent (Naito *et al.*, 2005), and that it has an antioxidant protective function of lipid matrix for both seed germination and human consumption (Verardo *et al.*, 2009).

The main objective of the present study was to determine the oil content, FA profile and tocopherol concentration of ten candidate walnut selections identified in the high Atlas Mountains of Morocco in order to select the genotypes of higher quality as seed sources for reforestation and as bud sources for grafting propagation as new cultivars.

2. MATERIALS AND METHODS

2.1. Plant material

This study was carried out with 10 walnut genotypes from the Asni region in the high Atlas Mountains (center of Morocco), with wealthy genetic resources. These genotypes were selected after four years of observations based on morphological and homological traits, such as the general status of the plant (vigor, ramification, foliar density and appearance), lateral fruiting, and physical quality of the kernel, as appreciated by the local population. These genotypes were unique seedlings; therefore each genotype is a single tree. The best genotypes were marked and fruits were collected in winter during two consecutive years (2009–2010). The nuts were collected in lots of about 1 kg when the fruit mesocarp had fully dried and split and peduncle abscission was complete. Then the mesocarp was removed and the nuts were dried and stored at ambient temperature for three weeks. From each lot, representing each genotype, two independent samples of 30 fruits were randomly taken for analysis. After cracking, the kernels were tromped in liquid nitrogen and then ground using an electrical grinder (IKA, Staufen, Germany) to obtain a fine flour.

2.2. Oil and protein determination

Oil was extracted from 5 g of ground walnut kernel using a fat extractor Soxtec during 5 h and using hexane as a solvent (AOCS, 1973). The oil content was expressed as the difference in weight of the dried kernel samples before and after extraction. The protein content was obtained indirectly by determining the total N content obtained by the Kjeldahl method (AOAC, 1995) and multiplying by a nitrogen-protein conversion factor (Kc=6.25; % protein=Kc* % total N).

2.3. Fatty acid determination

FAs were extracted using hexane (AOCS, 1973) and analyzed by Gas Chromatography (Series 6890, column CP 9205 WAX MS, Agilent, Santa Clara, CA, USA) as the methyl ester derivatives (FAMEs) after trans-esterification using a methanolic solution of Boron trifluoride (BF3, 10%). These methyl esters were separated using a flame ionization detector (FID). The carrier gas was helium at a flow rate of 1 $mL·min^{-1}$. The temperatures of the inlet and detector were held at 220 and 275 °C, respectively. The initial column temperature was 100 °C for 3 min. The oven temperature was increased from 100 to 150 at 20 $°C·min^{-1}$ ramp rate for 1 min, from 150 to 200 at 15 $°C·min^{-1}$ ramp rate for 3 min, and from 200 to 240 at 3 $°C·min^{-1}$ ramp rate. The temperature was kept at 240 °C for 4 min. The injection volume was 0.5 µL. The identification of individual fatty acid was achieved by comparison with the relative retention times of previous fatty acid standard injections and was expressed as percent of total fatty acids.

2.4. Tocopherol concentration

Tocopherol concentrations were determined in each duplicate sample as previously described (López-Ortiz *et al.*, 2008). Samples of 0.30 g of walnut oil were dissolved in 2 mL of 1-propanol by shaking at air temperature for 3 min. The resulting mixture was then filtered through a 0.45 µm nylon syringe membrane (Phenomenex, Torrance, CA, USA) before measurement. The final extract was kept in a dark vial in a refrigerator at 2°–4 °C until chromatographic analysis. Tocopherol homologue determinations were performed using a Kontron HPLC 360 equipped with a double piston pump (Kontron, Eching, Germany). Tocopherol homologues were detected using a Kontron 440 photodiode array detector and a Sfu 25 fluorescence detector. The chromatographic conditions finally selected for the simultaneous determination of all tocopherol homologues were as follows: 20 µL of sample were injected into the chromatography column Phenomenex Luna C18 (2) (0.5×4.6 mm, 5 µm particle size, 100Å pore size) while the mobile phase consisted of an acetonitrile:methanol (30:70) mixture at 30 °C at a flow rate of 1.2 $mL·min^{-1}$ and maintained at 30 °C. Tocopherol homologues were detected at 295 nm. Peak areas were measured, and retention times were compared with standards of the three homologues (Sigma-Aldrich, Madrid, Spain), with a purity of 95% for α-tocopherol, 96% for γ-tocopherol, and 90% for δ-tocopherol. The values were confirmed by their characteristic spectra using the photodiode array detector, which also confirmed their purity. To quantify all the homologues, calibration curves were drawn. Standard linearity was verified in each case by analysis of six standards in triplicate each containing 20–200 $mg·kg^{-1}$ oil for α-tocopherol, 0.1–8 $mg·kg^{-1}$ oil for γ-tocopherol and 0.05–5 $mg·kg^{-1}$ oil for δ-tocopherol. With this method, the recovery of the three tocopherol homologues was approximately 98%. Under these experimental conditions the limits of detection calculated from the residual error of the calibration curves were: 5.5 $mg·kg^{-1}$ oil for α-tocopherol, 0.2 $mg·kg^{-1}$ oil for γ-tocopherol and 0.1 $mg·kg^{-1}$ oil for δ-tocopherol. Tocopherol compositions were the mean values of three replicates (n=3) from each duplicate sample and were expressed as $mg·kg^{-1}$ oil.

2.5. Statistical analysis

All statistical analyses were performed with the SAS program (SAS, 2000). The analysis of variance with the PROC GLM procedure was applied to

distinguish the effect of the genotype and the year. The additive linear model for the statistical analysis was:

$$P_{ij}=\mu+g_i+y_j+(g\times y)_{ij}+\varepsilon_{ij}$$

Where P_{ij} is the phenotypic value of the i genotype in the j year, μ is overall mean, g_i is the genotype effect, y_i is the year effect, $(g\times y)_{ij}$ is the genotype and year interaction and ε_{ijk} is the residual effect. The mean separation was done with the Duncan test at $P\leq 0.05$. The LSMEANS option of the GLM procedure was used to calculate least-square means for genotypes in each year to observe rank changes of genotypes from year to year. The coefficients of correlation between the studied variables were obtained and the Pearson's correlation coefficients were calculated following the PROC CORR procedure.

3. RESULTS AND DISCUSSION

3.1. Oil and protein content

Oil and protein contents were highly variable among genotypes (Table 1). Protein content was significantly different between years (Table 2), with a higher mean value in 2009 (Table 1). The effect of year was not significant for oil content (Table 2), although it had been significant in three other walnut cultivars (Martinez *et al*., 2006). Oil content ranged between 54.4 and 67.48%, and protein between 11.58 and 14.5% in 2009, (Table 1), whereas in 2010 these ranges were between 55.29 and 66.25% for oil, and between 11.99% and 14% for protein (Table 1). Protein content agreed with previous reports (Amaral *et al*., 2003; Savage *et al*., 1999), as well as fat content, although the lowest fat value was lower than any previous report (Crews *et al*., 2005; Amaral *et al*., 2003; Martinez *et al*., 2006; Bada *et al*., 2010). The significance of the year×genotype interaction (Table 2) indicated that oil content variability was significant between years for some genotypes, as a result of the specific response by these genotypes to the environmental conditions of the year.

3.2. Major fatty acid profile

These oils showed a high content for PUFAs (linoleic and linolenic acids), intermediate for MUFAs (oleic acid) and low for saturated FAs (palmitic and stearic acids) as in other walnut oils (Amaral *et al*.,

TABLE 1. Oil and protein content and fatty acid composition of the kernels of 10 Moroccan walnut seedlings over two consecutive years

Genotype	Protein content (% of kernel DW)	Oil content (% of kernel DW)	Palmitic acid (% of total oil content)	Stearic acid (% of total oil content)	Oleic acid (% of total oil content)	Linoleic acid (% of total oil content)	Linolenic acid (% of total oil content)
Crop year 2009							
Asni-1	13.56±0.15	54.04±0.41	8.03±0.08	2.42±0.05	12.47±0.25	58.47±0.33	14.40±0.02
Asni-2	14.43±0.22	65.72±2.28	8.53±0.13	2.91±0.13	15.04±0.52	59.28±0.29	15.49±0.12
Asni-3	14.50±0.04	58.33±3.03	8.20±0.1	2.48±0.16	16.84±0.26	55.96±0.46	14.6±0.04
Asni-4	14.43±0.04	65.73±0.26	7.93±0.32	2.83±0.08	14.30±1.4	57.42±0.1	13.20±0.04
Asni-5	11.58±0.03	62.51±0.36	8.19±0.45	2.71±0.09	15.33±0.41	58.02±0.96	13.86±0.12
Asni-6	12.06±0.08	67.48±0.75	8.06±0.12	2.45±0.03	16.97±0.23	57.83±1.77	13.48±0.09
Asni-7	14.04±0.15	60.89±0.41	6.84±0.1	2.72±0.13	15.18±0.53	59.87±0.06	14.49±0.13
Asni-8	14.43±0.29	64.09±0.49	7.72±0.09	2.68±0.05	13.53±1.16	60.01±0.90	15.87±0.08
Asni-9	12.69±0.07	57.56±0.73	8.24±0.26	2.51±0.12	16.81±0.36	56.27±0.42	12.55±0.05
Asni-10	14.14±0.13	57.09±0.74	8.23±0.23	2.70±0.19	17.45±0.54	56.85±0.48	12.38±0.16
Means	13.53±1.06	61.82±4.44	7.97±0.71	2.63±0.21	15.16±1.38	58.13±1.19	14.22±1.59
Crop year 2010							
Asni-1	12.31±0.03	65.16±2.25	8.28±.2	1.70±0.11	14.40±0.26	57.68±0.45	13.47±0.09
Asni-2	12.41±0.56	63.15±5.19	7.03±0.11	2.13±0.25	16.43±0.33	57.17±0.97	12.79±0.07
Asni-3	12.78±0.32	65.45±1.79	8.44±0.25	2.30±0.2	14.95±0.21	58.64±0.26	14.07±0.07
Asni-4	13.17±0.16	64.71±2.29	7.77±0.21	1.80±0.09	15.98±0.86	57.29±0.06	13.97±0.02
Asni-5	14.01±0.03	66.25±2.46	9.12±0.22	2.89±0.04	16.14±0.34	57.96±0.45	13.94±0.04
Asni-6	13.64±0.04	64.59±3.55	7.43±0.04	2.60±0.02	17.50±0.42	58.01±0.75	14.02±0.04
Asni-7	12.82±0.09	57.58±0.19	8.40±0.18	2.40±0.08	19.27±0.24	56.99±0.14	12.43±0.09
Asni-8	12.07±0.56	57.33±2.15	8.00±0.11	2.50±0.14	22.01±1.19	55.03±1.12	9.30±0.1
Asni-9	13.87±0.33	55.29±0.34	7.37±0.17	2.17±0.07	17.62±1.12	56.18±0.55	12.73±0.03
Asni-10	11.99±0.22	56.86±0.57	8.40±0.13	2.30±0.2	17.39±0.76	57.06±0.42	12.50±0.08
Mean	13.01±2.15	61.79±5.08	7.98±0.47	2.28±0.36	17.14±2.92	57.22±2.11	12.97±1.42

Table 2. Analysis of variance for oil content (%), protein content (%) and fatty acid composition of Moroccan walnut seedlings

Source of variation	df	Mean square[a]	*F*-test	*p*-value
Protein content				
Genotype	9	3.01***	25.30	<.0001
Year	1	6.97***	58.46	<.0001
Year×Genotype	9	2.16***	18.11	<.0001
Error	40	0.12		
Oil content				
Genotype	9	104.99***	26.11	<.0001
Year	1	1.28 ns	0.32	0.5755
Year×Genotype	9	10.60*	2.64	0.0169
Error	40	4.02		
Palmitic acid				
Genotype	9	0.66***	14.99	<.0001
Year	1	0.01 ns	0.24	0.6258
Year×Genotype	9	1.16***	26.29	<.0001
Error	40	0.04		
Stearic acid				
Genotype	9	0.23***	11.03	<.0001
Year	1	1.96***	92.31	<.0001
Year×Genotype	9	0.23***	10.80	<.0001
Error	40	0.02		
Oleic acid				
Genotype	9	14.82***	47.49	<.0001
Year	1	8.48***	27.18	<.0001
Year×Genotype	9	18.19***	58.28	<.0001
Error	40	0.31		
Linoleic acid				
Genotype	9	3.99**	3.70	0.0019
Year	1	7.02*	6.51	0.0147
Year×Genotype	9	10.10***	9.36	<.0001
Error	40	1.08		
Linolenic acid				
Genotype	9	7.45***	18.56	<.0001
Year	1	33.40***	83.23	<.0001
Year×Genotype	9	5.55***	13.83	<.0001
Error	40	0.40		

[a]ns, *, **, ***: not significant or significant at P<0.05, 0.01, 0.001.

2003; Savage *et al.*, 1999). The ranges of variability for the main FAs, as shown in Table 1, were different for the two years, but similar to those already reported in other walnut oils (Amaral *et al.*, 2003; Martinez *et al.*, 2006; Savage *et al.*, 1999; Bada *et al.*, 2010). The highest values of the walnut oil were found for PUFAs: the FAs were reported as the most effective in reducing low-density-lipoprotein cholesterol in humans, although high linoleic and linolenic acid contents may result in a poor oxidative stability and shorter shelf-life of the oils (Abbey *et al.*, 1994). However, the results of Savage *et al.* (1999) showed that the most unstable walnut oils, extracted from 'Esterhazy' and G139, did not have higher levels of linoleic and linolenic acids than the more stable oils from 'Vina', G120, and 'Stanley'. In addition, MUFAs were as effective as PUFAs in the reduction of low-density-lipoprotein cholesterol in humans (Mensik and Katan, 1989), and the most important MUFA in walnuts is oleic acid (Amaral *et al.*, 2003). Therefore, the composition of the oil from these local Moroccan walnuts points out that these seedlings may be of great benefit to health and nutrition in the human diet due to their fatty acid profile.

The effect of genotype, year, and the interaction year×genotype were significant for all acids, except for the year effect for palmitic acid (Table 2). The year effect had been significant for all fatty acids, except for stearic and linoleic acids, in an Argentinian study (Martinez *et al.*, 2006). The variability in the FA composition has been well studied in walnuts (Amaral *et al.*, 2003; Martinez *et al.*, 2006; Bada *et al.*, 2010), concluding that this composition depended primarily on the genotype, with significant effects of the year, the environment, the geographical origin, and agricultural practices. In our study, the highest contents of linoleic and linolenic acids were obtained in 2009 and, conversely, that of oleic acid in 2010 (Table 1). These seedlings were not irrigated, and water stress may significantly reduce the PUFAs, especially linoleic and linolenic acids (Amaral *et al.*, 2003). The climatic conditions during the period of fruit growth and kernel filling (May to October) were more humid and cooler in 2010 than in 2009 (data not shown). A general negative correlation of PUFAs and growing temperature had been established: PUFAs increased in the membrane as well as in the seed storage lipids with decreasing temperatures (Rennie and Tanner, 1989). Nitrogen fertilization reduced the content of oleic and linolenic acids, and increased that of linoleic acid (Verardo *et al.*, 2013), but our seedlings were not fertilized. The negative correlation between the contents of oleic and linoleic acids ($r=-0.58$ in 2009 and $r=-0.77$ in 2010) and of oleic and linolenic acids ($r=-0.68$ in 2009 and $r=-0.8$ in 2010) may explain the high value of linoleic acid in 2010. Similar correlations had been previously reported (Malvolti *et al.*, 2010; Martinez and Maestri, 2008).

The significant year×genotype interaction for all acids (Table 2) indicated a different genotype behavior in relation to the climatic conditions, as already pointed out for other cultivars and climatic conditions (Amaral *et al.*, 2003; Martinez *et al.*, 2006). The genotypes Asni-6 and Asni-10 showed consistently high oleic acid contents over the two years (Table 1). In the walnut, the FA metabolism is controlled by a large number of diverse genes

(Dandekar *et al.*, 2005) that may react differently to stress, since a plant is a biological entity and does not behave mechanically every year. Therefore, the evaluation of the FAs over several years in the same environment is an important criterion for identification of stable genotypes to establish commercial orchards in order to produce walnuts without fluctuation in these important quality parameters.

3.3. Tocopherol concentration

The tocopherol profile showed that γ-tocopherol was the major homologue, followed by α- and δ-tocopherol (Table 3), as in other genotypes (Amaral *et al.*, 2005; Bada *et al.*, 2010). The three tocopherol homologues showed high variability among the genotypes (Table 4), as already observed in nine cultivars, where tocopherol content was also affected by environmental factors (Amaral *et al.*, 2005), although few significant differences were observed for the individual tocopherol homologues in the other three cultivars, where the crop year was the main variability source (Martinez *et al.*, 2006). The mean value over the two years ranged between 188.1 and 230.7 mg·kg^{-1} oil for γ-tocopherol (Table 3). This range agreed with previous results (Amaral *et al.*, 2005), but the maximum values of the Moroccan seedlings were lower than those previously reported (Savage *et al.*, 1999; Bada *et al.*, 2010). The concentration of δ-tocopherol ranged from 23.3 to 43.4 mg·kg^{-1} oil (Table 3), higher than in a previous report (Amaral *et al.*, 2005), but lower than in others (Savage *et al.*, 1999; Bada *et al.*, 2010). The range in variability for α-tocopherol was between 8.9 and 16.57 mg·kg^{-1} oil (Table 3), similar to that of a previous report (Amaral *et al.*, 2005), but lower than in others (Savage *et al.*, 1999; Bada *et al.*, 2010). These results confirm that γ- and δ-tocopherols are the most variable homologues in walnuts.

In both years γ-tocopherol showed positive and significant correlations with oil content (r=0.66 in 2009 and r=0.71 in 2010), with linoleic acid (r=0.81 in 2009 and r=0.62 in 2010), and with linolenic acid (r=0.79 in 2009 and r=0.57 in 2010), and a negative correlation with oleic acid (r=−0.58 in 2009 and r=−0.68 in 2010), agreeing with previous studies (Malvolti *et al.*, 2010). As a consequence, genotypes with high oil contents also show high concentrations of γ-tocopherol and of linoleic and linolenic acids. Thus, the amount of oil content could be a reliable index for the amount of PUFAs in walnut oil, and consequently a reduction in time and cost of evaluating walnut oil at large scale.

The tocopherol concentration in walnuts is affected by climatic conditions (Martinez *et al.*, 2006; Amaral *et al.*, 2005) and geographical origin (Amaral *et al.*, 2005). The effect of year was statistically significant for all tocopherol homologues (Table 4). The mean value of all tocopherol homologues was higher in 2009 than in 2010 (Table 3). Tocopherol levels increase in response to a variety of abiotic stresses, considered as evidence of its protective role (Munné-Bosch and Alegre, 2002). The climatic conditions of the year, mainly temperature, affect the concentration of the different tocopherol homologhes in several nut crops (Maranz and Wiesman, 2004), indicating that these components depend on the temperature and the occurrence of drought during fruit ripening. Since heat and drought stresses were higher in the 2009 fruit growing season than in 2010 (data not shown), they could increase the concentration of all tocopherol homologues in these non-irrigated genotypes. The year×genotype interaction for all tocopherol homologues (Table 4) indicated a different genotype behavior in relation to the climatic conditions, as already pointed out (Martinez *et al.*, 2006). The genotypes Asni-3, Asni-4 and Asni-5 showed high and consistent values for γ-tocopherol and oil contents in both years. Taking into account the information on the protective function of γ-tocopherol (Verardo *et al.*,

Table 3. Tocopherol concentration in the kernel oil of Moroccan walnut seedlings over two consecutive years

	δ-Tocopherol (mg·kg^{-1} oil)		**γ-Tocopherol (mg·kg^{-1} oil)**		**α-Tocopherol (mg·kg^{-1} oil)**	
Genotype	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**
Asni-1	32.12±2.03	23.30±1.64	238.49±6.93	207.49±5.32	21.39±1.42	8.70±0.87
Asni-2	52.12±0.24	35.12±0.13	244.07±4.84	205.79±3.42	14.16±1.74	13.55±1.24
Asni-3	37.11±0.92	49.74±0.63	228.09±1.94	234.59±2.41	11.10±1.23	10.49±2.41
Asni-4	19.34±1.32	31.63±1.08	218.75±5.68	218.50±7.32	14.35±0.91	19.09±1.08
Asni-5	43.33±0.98	25.51±3.25	264.34±14.32	262.53±11.2	15.52±1.77	12.05±1.52
Asni-6	29.27±1.24	26.60±1.34	234.93±7.36	184.36±3.78	11.45±1.15	15.13±0.52
Asni-7	24.41±2.42	26.47±1.75	240.56±12.35	162.20±20.54	18.83±0.47	13.07±0.33
Asni-8	41.20±3.54	29.30±1.22	273.91±2.75	174.32±4.32	19.96±1.33	17.15±2.01
Asni-9	54.09±4.35	31.98±3.57	212.87±11.23	182.75±13.47	15.10±2.12	13.18±0.89
Asni-10	28.34±1.68	23.76±2.66	212.16±7.35	201.48±9.24	17.55±1.21	16.60±0.94
Means	37.01±5.95	31.07±12.08	239.56±21.42	203.62±15.08	15.06±3.08	13.60±5.95

Table 4. Analyses of variance for the three main tocopherol homologues of Moroccan walnut seedlings

Source of variation	df	Mean square[a]	*F*-test	*p*-value
α-Tocopherol				
Genotype	9	93.00***	98.95	<.0001
Year	1	173.60***	184.70	<.0001
Year×Genotype	9	44.33***	47.16	<.0001
Error	40	0.94		
γ-Tocopherol				
Genotype	9	937.35***	7.97	<.0001
Year	1	31805.81***	270.33	<.0001
Year×Genotype	9	762.07***	6.48	<.0001
Error	40	117.66		
δ-Tocopherol				
Genotype	9	343.54***	113.33	<.0001
Year	1	8010.85***	2642.65	<.0001
Year×Genotype	9	227.32***	74.99	<.0001
Error	40	3.03		

[a]***: significant at P<0.001.

2009) and the relevance of high lipid contents as a source of carbon and energy during germination and seedling growth (Chenevard *et al*., 1994), these genotypes could be considered as a seed source for walnut propagation in the high Atlas Mountains in Morocco to recover from forest degradation, since the choice of the seed source is considered crucial for the success of future plantings in the silvicultural management (Hemery, 2008; Callaham, 1994).

4. CONCLUSIONS

The present results are the first report on the chemical composition of the walnuts from the high Atlas Mountains in Morocco. The oil from their kernels presents medium to high oil content and concentration of PUFAs and of tocopherol, mainly of linoleic and linolenic acids, and of γ- and δ-tocopherols. This composition is similar to that of different commercial walnut cultivars. Thus, this oil may be considered as a nutraceutical oil and a good source of vitamin E for the human diet. These findings confirm the genotype and the year effects on the composition of the walnut oil, but the genotype characteristics are determinant in the year-to-year stability of this composition. Some genotypes (Asni-3, Asni-4 and Asni-5) showed high oil contents and consistently high values of γ-tocopherol. The introduction of these genotypes as new cultivars by vegetative propagation may result in a quality increase in the walnuts from the high Atlas Mountains of Morocco. In addition, these genotypes could be used as a seed source for walnut propagation in the same region, since the choice of the seed source is considered crucial for the success of future plantings in the silvicultural management.

ACKNOWLEDGMENTS

This research was funded by the grants of "Healthy Food for life" program Marie Curie IRSES, and the Research Group A12 of Aragón.

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