Emirates Journal of Food and Agriculture. 2017. 29(7): 557-561
doi: 10.9755/ejfa.2016-10-1390
http://www.ejfa.me/

SHORT COMMUNICATION

Soil and climate determine antioxidant capacity of walnuts

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ABSTRACT

The aim of this work was to study the influence of soil and climate factors in the antioxidant capacity of 10 different varieties of nuts, obtained from two experimental cultivars with different weather and soil conditions. Ten different varieties of walnut samples were obtained from two experimental cultivars, analyzing the antioxidant capacity by ORAC, DPPH• and ABTS+ methods. Eureka and Tehama walnuts showed the highest antioxidant capacity. Only Chico and Serr varieties (measured by ABTS+ method) and Franquette variety (measured by ORAC assay) increased their antioxidant capacity when cultured in Torre Blanca plots. The growing in El Chaparral plots increased the antioxidant capacity of every different variety. Minor temperature (especially when it reaches over -0 ºC) and humidity, as well as an increase on the annual rainfall are noteworthy factors that influence on the increase of antioxidant capacity of the wide majority of the analyzed walnuts.

Keywords: Antioxidant capacity; Climate; Nut; Soil; Walnut

INTRODUCTION

Walnut is specie adapted to temperate and continental climates. However, for nut production, the ideal habitat coincides with temperate climate similar to the Mediterranean. The early frost in autumn and winter temperatures too low can cause significant damage to the tree, while late frosts in spring reduce or cancel production, besides damaging new shoots. Excessive humidity favors the incidence of disease, supporting high summer temperatures. Walnut adapts to drought and different soil types, but it is typical to perform crop irrigation as well as use deep soils, fertile, not too alkaline. Moreover, in compact land it is poorly adapted (Salvadó et al., 2005).

Recent studies have found walnut to contain several groups of polyphenols, mainly identified as tannins (Christopoulos and Tsantili, 2012, Fukuda et al., 2003). Most of them contribute to the overall antioxidant capacity of walnut extracts with different *in vitro* and *in vivo* antioxidant estimation models (Fukuda et al., 2003, Fukuda et al., 2004). In addition, some monomeric phenols such as gallic acid and ellagic acid have been reported in walnut extracts, showing oxidation inhibition of plasma LDL *in vitro* (Anderson et al., 2001).

The interest in the study of bioactive compounds in foods and their contribution to antioxidant activity (Serafini et al., 2011) has increased the number of studies in which this activity is measured, among the consequent protective effect against various diseases (Kunyanga et al., 2012, Vadivel and Biesalski, 2011). Numerous studies have focused on analyzing the antioxidant activity of both seed nuts (Arcan and Yemenicioğlu, 2009, Samaranayaka et al., 2008). Some factors such as variety, soil and climatic conditions can influence the nutritional composition of walnuts and its resultant activity, giving an added value for their potential beneficial effects on health. Therefore, the aim of this work was to study the influence of soil and climate factors in the antioxidant capacity of 10 different varieties of nuts.

MATERIALS AND METHODS

Plant material

Ten different varieties of walnut samples were obtained from two experimental cultivars of the Instituto Murciano

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Received: 13 October 2016; **Revised:** 19 June 2017; **Accepted:** 25 June 2017; **Published Online:** 21 July 2017

de Investigación y Desarrollo Agrario y Alimentario (IMIDA) (Torre Pacheco, southeastern Spain). All varieties were grown in the same conditions. Finally, nuts were collected in October, 2013.

Edaphoclimatic description cultivar

El Chaparral cultivar, placed in Cehegín (Murcia) is at an altitude of 432 m, latitude 38º 6' 39,35" and length of land 1º 40' 59,06". The planting is 6 x 8 m. The soil has a clay loam texture based classification criteria of the USDA (Service 1975). After conducting an analysis of soil saturation extract, the land had the following grading: 34.38% sand, 27.84% silt and 37.78% of clay.

Torre Blanca cultivar, placed in Torre Pacheco (Murcia) is at an altitude of 31 m, latitude 37º 46' 25,89" and length of land 0º 53' 54,62". The planting is 6 x 8 m. The soil has a clay loam texture based classification criteria of the USDA (Service 1975). After conducting an analysis of soil saturation extract, the land had the following grading: 31.77% sand, 32.53% silt and 35.7% of clay.

By comparing the climate in the two cultivars, it was observed that El Chaparral has a number of annual hours below 0 °C significantly higher ($p \leq 0.05$) that is recorded in Torre Blanca cultivar (220 h vs. 6.92 h, respectively). The precipitations are higher in El Chaparral cultivar ($p \leq 0,05$) and the average temperature and humidity are higher in Torre Blanca cultivar ($p \leq 0,05$) (Table 1).

Source of the walnuts and samples preparation

These commercial walnuts varieties were: Algaida, Amigo, Chico, Eureka, Franquette, Payne, Pedro, Serr, Sunland and Tehama. All varieties were grown under the same agricultural practices. 2 kg of walnuts were obtained for each variety. Walnuts, free of mesocarp, were obtained at optimum ripeness, discarding those that had defects like cracks or visual symptoms of microbiological contamination. From each batch, 50 randomly selected fruits were shelled to remove kernels. Finally the walnut kernels were vacuum-packed in plastic bags and stored at -80 °C until analysis.

Table 1: Climatological characteristics of El Chaparral and Torre Blanca cultivars

Statistical value	Cultivars	
	El Chaparral	Torre Blanca
Hours below 0°C	220.06*	6.92
Relative humidity (%)	61.53*	76.35
Annual rainfall (mm)	344.07*	271.07
Average annual temperature (°C)	15.38*	17.05
Maximum temperature reached (°C)	28.14	27.57

(*) Significant differences ($p \leq 0,05$) between cultivars

DPPH• assay

For the analysis of antioxidant capacity by DPPH$^{\bullet}$, the method described by Bondet et al. (1997) was performed. The antioxidant capacity was expressed as µM Trolox equivalents/g of walnuts.

ABTS+ assay

The TEAC assay described by Miller and Rice-Evans (1997) was used to assess the radical scavenging capacity of the walnuts extracts against the stable ABTS^{+} radical Results were expressed as mmol of Trolox determined in g of walnuts.

ORAC assay

The oxygen radical absorbance capacity was determined as described by Dávalos et al. (2004) with slight modifications (Dávalos et al., 2004). The antioxidant activity was expressed as µM Trolox equivalents/g of walnuts.

Statistical analysis

For statistical analysis the statistical package IBM SPSS Statistics (version 21.0) and analysis of variance (ANOVA) was used. Previously, the assumption of normality was tested. In variables with significant differences ($p<0.05$) the Tukey HSD test (Honest Significant Difference) was applied to determine the existence of differences between means, establishing a confidence level of 95%. To study the relationship between qualitative variables, the correlation coefficients Pearson and Kendall were calculated contrast to both linear and nonlinear association.

RESULTS AND DISCUSSION

When defining antioxidant capacity of foods, is advisable to use different assays, offering different information due to the diverse chemical reactions carried out in the different methods (Frankel and Meyer, 2000). The DPPH$^{\bullet}$ andABTS^{+} are non-biological radicals extensively used to test the antioxidant capacity of plant samples. Other widespread method for the evaluation of the antioxidant capacity of vegetal is ORAC method, based on the ability of peroxyl radical scavenging (Bondet et al., 1997). Therefore the use of various methods can provide a more complete evaluation of the antioxidant capacity of the studied nuts, whose results are shown in Table 2.

DPPH$^{\bullet}$

DPPH$^{\bullet}$ is one of the few stable and commercially available organic nitrogen radicals and has an UV−vis absorption maximum at 515 nm. Upon reduction, the solution color fades and the reaction progress is conveniently monitored by a spectrophotometer (Huang et al., 2005). All individual cultured varieties of El Chaparral plots showed higher

Table 2: Antioxidant capacity of the individual varieties of walnuts depending on the farm (uM Trolox Equivalents (TE)/g of walnut±SD

Variety	DPPH$^{\bullet}$		ABTS+		ORAC	
	El Chaparral	Torre Blanca	El Chaparral	Torre Blanca	El Chaparral	Torre Blanca
Algaida	169.95±8,74*	141.59±7.67	166.25±7.71	166.87±7.38	219.26±15.92	196.52±10.28
Amigo	143.31±6.87*	117.85±7.97	156.95±3.34*	117.86±7.96	176.20±11.48*	153.97±6.51
Chico	192.15±20.44*	128.40±15.06	133.97±5.81	161.94±6.45*	182.77±11.26*	146.76±6.57
Eureka	261.02±9.37*	175.77±9.48	256.60±7.10*	182.19±10.63	285.36±16.89	256.46±14.11
Franquette	146.22±4.98*	103.52±1.90	146.40±7.06	119.48±12.00	157.70±5.70	196.46±6.50*
Payne	184.96±12.55*	135.87±5.58	152.51±1.34	163.70±16.72	191.79±8.35	188.95±5.66
Pedro	162.21±9.06*	106.21±11.58	169.75±3.30*	108.83±9.48	211.54±11.55	193.90±16.62
Serr	169.69±3.98*	104.41±4.59	105.02±4.75	101.57±2.42	221.08±21.01*	153.77±4.50
Sunland	185.06±0.86*	140.80±5.21	158.18±3.93	167.56±0.45*	248.17±13.80*	210.17±10.47
Tehama	262.76±10.67*	154.33±6.89	217.79±5.61*	169.77±5.62	268.43±15.35	262.43±7.98

(*) Significant differences ($p \leq 0,05$) between cultivars

values than Torre Blanca plots ($p \leq 0,05$) when analyzed by DPPH$^{\bullet}$ assay. Mayor differences were found after the analysis of Tehama variety, showing differences of 42% between the two plots (262,76 ± 10,67 uM TE and 154,33 ± 6,89 uM TE respectively). Contrarious, minor differences were obtained from Algaida variety, which presented a difference of 17% (169,95 ± 8,74 uM TE and 141,59 ± 7,67 uM TE respectively).

All these DPPH$^{\bullet}$ values were similar to those previously showed for walnut (*Juglans regia* L.) kernels (Christopoulos and Tsantili, 2012), higher than for Brazil nut (*Bertholletia excels*) kernel and whole nut (John and Shahidi 2010), and also higher than baru nuts (*Dipteryx alata* Vog) with and without peels (Lemos et al., 2012).

ABTS^{+}

The free radical scavenging ability of plant samples is also studied using a moderately stable nitrogen-centred radical species: ABTS^{+} radical (Oboh and Ademosun, 2012). With respect to ABTS^{+} results from the individual walnuts throws different results between the two plots. Mayor differences ($p \leq 0.05$) were obtained from the varieties Amigo, Chico, Eureka, Pedro, Sunland and Tehama. While Amigo, Eureka, Pedro and Tehama varieties showed higher antioxidant capacity when cultured in El Chaparral plots (156,95 ± 3,34 uM TE, 256,60 ± 7,10 uM TE, 169,75 ± 3,30 uM TE and 217,79 ± 5,61 uM TE respectively), antioxidant capacity of Chico and Sunland was higher when cultured in Torre Blanca plot (161,94 ± 6,45 uM TE and 167,56 ± 0,45 uM TE respectively).

Other publications have shown the high antioxidant activity against ABTS^{+} radical of nuts and nuts extracts. Slightly higher values than our results were reported for tree nut oils (Miraliakbari and Shahidi, 2008). Nonetheless, we obtained higher ABTS^{+} results than fresh or dry hazelnuts, walnuts and pistachios assayed with their seed coats (Arcan and Yemenicioğlu, 2009); and considerably higher than walnut (*Juglans regia*), almond, apricot, raisin, fig, hazelnut (Kamiloglu et al., 2014), and the extracts of cashew shoots (*Anacardium occidentale*) (Razali et al., 2008).

ORAC$_{FL}$

ORAC$_{FL}$ assay provides a direct measure of hydrophilic chain-breaking antioxidant capacity against peroxyl radical (Ou et al., 2001). Regarding the common antioxidant capacity of walnuts (obtained as a mean of all varieties), ORAC assay results in higher antioxidant capacity ($p \leq 0,05$) of the walnuts analyzed, showing significant differences when compared to DPPH$^{\bullet}$ and ABTS^{+} assays in both cultivars (Fig. 1). Variety which showed minor antioxidant capacity by ORAC$_{FL}$ method was Franquette (157,70 ± 5,70 uM TE) from El Chaparral plot, and Chico variety for Torre Blanca plot (146,76 ± 6,57 uM TE). From the different varieties, Tehama was the one which showed mayor antioxidant capacity by ORAC$_{FL}$ method in both El Chaparral (268,43 ± 15,35 uM TE) and Torre Blanca (262,43 ± 7,98 uM TE) plots. The values obtained for the walnuts are minor than other observed for cashews in a recent study, in which reported 711,4 ± ug of TE/100g for raw cashews (Samani, 2017).

However, ORAC values were similar ($p > 0.05$) between El Chaparral plot (216,23 ± 13,12 uM TE) and Torre Blanca plots (195,94 ± 8,92 uM TE). In case of DPPH$^{\bullet}$ and ABTS^{+} assay, values were different regarding the two plots. El Chaparral plots showed statistically significant ($p \leq 0.05$) higher antioxidant capacity when measured by DPPH$^{\bullet}$ assay (187,73 ± 8,75 uM TE) and ABTS^{+} assay (166,34 ± 4,98 uM TE) than Torre Blanca plots (DPPH$^{\bullet}$: 130,88 ± 7,59 uM TE; ABTS^{+}: 145,98 ± 7,91 uM TE).

DISCUSSION

Individual variations of the different walnuts were also accomplished. In summary, the varieties which showed

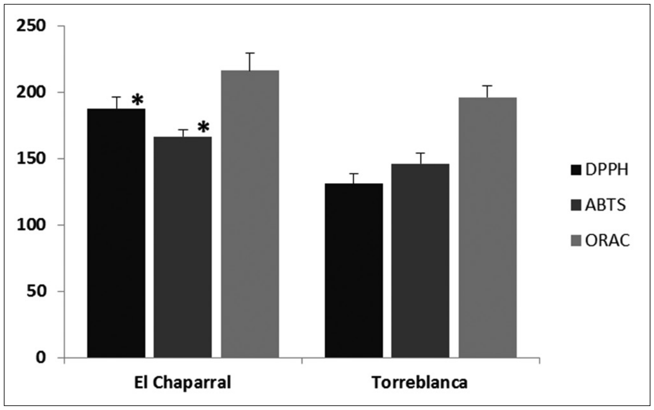

Fig. 1. Antioxidant capacity of walnuts depending on the farm (uM Trolox Equivalents (TE) / g of walnut). (*) significant differences (p ≤ 0,05) between cultivars.

higher antioxidant capacity measured by the three methods were Eureka and Tehama. Meanwhile, Amigo, Franquette and Serr exhibit the minor values for the three methods. Regardless the few difference on the average antioxidant capacity measured by ORAC, individual values of the different varieties lead to many noteworthy differences. ORAC values from El Chaparral plots cultivar were statistically higher ($p \leq 0.05$) in case of Amigo, Chico, Serr and Sunland varieties (176,20 ± 11,48 uM TE, 182,77 ± 11,26 uM TE, 221,08 ± 21,01 uM TE, 248,17 ± 13,80 uM TE respectively). However, only Franquette variety was found to exert higher antioxidant capacity ($p \leq 0.05$) when cultured in Torre Blanca plots (196,46 ± 6,50 uM TE).

All these ORAC data were higher than previously reported for Brazil nut (*Bertholletia excels*) kernel and whole nut (John and Shahidi, 2010), and higher than over 100 different kinds of foods, including nuts and fruits, vegetables, dried fruits, spices and cereals from the United States (Wu et al., 2004); although lower than roasted peanut, hazelnut and almonds skins (Monagas et al., 2009). Moreover, it has been demonstrated that the consumption of walnuts or walnut meal increase the ORAC activity in plasma in 21 healthy men and postmenopausal women (McKay et al., 2010), and in healthy individuals (Haddad et al., 2014).

Judging by the results, the different varieties of tested walnuts can effectively scavenge different types of ROS under *in vitro* conditions. The range of results reported (Fig. 1) indicates that multiple mechanisms may be responsible for their antioxidant capacity, related to their unsaturated fatty acids and other bioactive compounds: high-quality vegetable protein, fiber, minerals, tocopherols, phytosterols, and phenolic compounds (Ros, 2010). Although all the antioxidant methods have different nature and origin, all varieties of walnuts followed a similar trend in all the methods in general. In summary, the combination of phytochemicals and synergistic mechanisms in the matrix is highly responsible for the potent antioxidant activities of fruits and vegetables (Gironés-Vilaplana et al., 2014), including walnuts.

Soil and climatic conditions of the different regions differ widely, being a crucial factor in the antioxidant capacity of the walnuts analyzed. The growing in El Chaparral plots has shown to increase the antioxidant capacity of the different varieties. In fact, Eureka and Tehama grown in this plot were the most powerful antioxidant varieties when measured by the three methods. Only Chico and Serr varieties measured by ABTS+ method, and Franquette variety measured by ORAC assay, have been found to increase their antioxidant capacity when grown in Torre Blanca plots.

Other authors have described the increase on the phenolic content and the consequent increase on the antioxidant capacity on Batavia lettuce (Marin et al., 2015); however, it did not increase in other lettuce varieties. Same authors described the increment on phenolic and antioxidant capacity with descend on the temperature of the cultivar (Marin et al., 2015). Therefore, the minor temperature (especially when it reaches over -0 ºC) and humidity, as well as an increase on the annual rainfall are noteworthy factors that influence on the increase of antioxidant capacity of the wide majority of the analyzed walnuts.

CONCLUSION

Judging by data from our study, it can be concluded that a minor temperature and humidity -due to an increase on the annual rainfall- seems to influence the increase of the antioxidant capacity of the wide majority of the different varieties of walnut analyzed in the present study. The high antioxidant capacity of some varieties of walnuts shows their value as agents with potential applications in diet-associated diseases related to oxidative stress.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of Catholic University San Antonio of Murcia. The Authors also declares that there is no conflict of interest. Special thanks to Mr. Connor Mc. Mullen for the English-native correction of the manuscript.

Author contribution

F. F. carried out the analytical processes and the treatment of the data. A. M. C. supervised the analytical processes. J. M. and A. G. V. wrote and discussed the present paper. D. V. helped with the revision of the manuscript. B. C.,

J. M. and P. Z. designed, supervised, and discussed this research work.

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