Research Article

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Kernel oil content and oil composition in walnut (*Juglans regia* L.) accessions from north-eastern Italy

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Abstract

BACKGROUND: Walnut oil use is currently limited by its poor oxidative stability due to the high percentage of polyunsaturated fatty acids. Modifying the oil composition may be a goal in walnut breeding to increase interest in this crop. Exploring natural variability and identifying the main environmental factors affecting oil quality are necessary in crop selection. Therefore 190 wild accessions were collected and evaluated during 2013 and 2014 for oil content and its fatty acid profile and compared with five commercial cultivars as references.

RESULTS: High variation in kernel oil content and fatty acid composition was found in the native walnut. Kernel oil content ranged from 54.2 to 72.2% (w/w). The major fatty acids were linoleic (range 46.9–68.6%), oleic (10.0–25.1%), linolenic (6.9–17.6%), palmitic (3.9–11.4%) and stearic (1.1–5.2%) acids. Some accessions had oil with a fatty acid ratio very different from the reference commercial cultivars, especially the oleic acid/polyunsaturated fatty acid (PUFA) ratio. A significant linear relationship and positive correlation between the daily minimum temperature and oleic acid content was observed in the wild walnuts.

CONCLUSION: The wide variation in fatty acid content and composition allows superior accessions to be selected for diffusion among growers. A suitable strategy would be to make a selection against PUFA content rather than just for high oleic acid. In addition, the selected high oleic accessions, before being utilized *per se* or as donor parents in breeding programs, have to demonstrate they are not adversely affected by the environment.

Supporting information may be found in the online version of this article.

Keywords: walnut germplasm; oil content; fatty acid composition; PUFAs; oleic acid content; walnut breeding

INTRODUCTION

Common or Persian walnut (*Juglans regia* L.) is an important fruit tree that has been grown since ancient times in Europe and Asia. Nowadays, walnut is commercially cultivated throughout southern Europe, northern Africa, eastern Asia, the USA and western South America, with a total surface of about 1 Mha in 2014.[1] It is a valuable lipid food source; indeed the oil content, ranging in commercial walnut varieties from 620 to 740 g kg^{-1} kernel,[2] is the predominant component of this nut crop.[2–4] Walnut oil is naturally rich in polyunsaturated fatty acids (PUFAs), mainly linoleic (55–70%) and linolenic (10–18%) acids, and is consequently poor in monounsaturated fatty acids (MUFAs), represented by oleic acid (10–20%), and in saturated fatty acids (SFAs).[2,4–6] Walnuts are mainly consumed as fruit, and their nutritional qualities and use depend mainly on their oil fatty acid profile. Walnut oil is excellent if used fresh for edible purposes, because it is rich in the so-called ω-3 and ω-6 fatty acids that are essential for the human diet. On the contrary, given its high PUFA content, walnut oil is very susceptible to oxidation and rancidity and is consequently unsuitable for long storage, refining and deep frying or any other cooking applications because of its low smoking point. Its short shelf life limits many food and non-food applications. Changes in oil composition are currently a goal in many oilseed crop breeding programs. In addition, several novel products, mainly in the food industry, have been developed from oil crops through conventional breeding approaches. Some crops have thus been brought to a similar fatty acid composition through breeding. An example is the high oleic acid content in sunflower, soybean and canola, as well as the high stearic acid content in sunflower and soybean and low SFA content in soybean and canola.[7] Breeding to modify the quality of walnut oil has received little attention until recently, but changing the oil composition on the basis of nut end-use would contribute to the expansion of walnut oil utilization. The development of alternative walnut oil feedstocks with improved functionality while maintaining nutritional quality has therefore become a priority for

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the food industry. The specific goal in walnut breeding could be improving the oxidative stability through reduction of PUFAs and relative increase of MUFAs.[2,5,6] The objective of improved stability could be reached by raising the SFA content, but that can have adverse effects on human health due to the increase of low-density lipoprotein (LDL) in blood caused by high palmitic acid (C16:0) consumption.[8] Genotypes with high oleic acid content are naturally present in walnut. Contents higher than 400 g kg^{-1} and up to 300 g kg^{-1} have been reported for walnuts grown in Turkey[9] and New Zealand[10] respectively.

Genetic resources are the basis of crop improvement, so the characterization of wild accessions with the identification of genetic variability for the target traits is the first step in new cultivar development. However, no information is reported about environmental effects on these genotypes. Significant variation in linoleic, linolenic and oleic acid contents exists naturally due to plant genotype and environmental factors that occur during fruit growth.[10] Some variations recorded in seed fatty acid profile within cultivars were associated with location[10] and irrigation management. Environmental factors such as latitude, temperature, drought, radiation and maturing stage/harvest date may also affect oil composition in most oil crops. An increase in the oleic/linoleic acid ratio with increasing temperature during grain filling has been widely reported in several oil crops such as sunflower[11–13] and soybean.[14] Among environmental factors, temperature is known to be the main one affecting oil composition in oil crop genotypes.[7] Many authors have worked on oil quality as affected by temperature in sunflower,[15–17] where minimum temperature seems to alter the oleic/linoleic acid ratio, but no studies can found in the literature on the effect of temperature on fatty acid composition in walnut. The aim of this work was (1) to characterize wild walnut accessions distributed throughout the Friuli Venezia Giulia region for oil content and fatty acid composition in comparison with popular commercial varieties and (2) to evaluate the effect of different daily temperature regimes during nut development on oil fatty acid profile.

EXPERIMENTAL

Walnut sample collection

A total of 58 and 166 nut samples were collected from wild walnut trees in 2013 and 2014 respectively throughout the Friuli Venezia Giulia region in the north-eastern Italian Alps (Fig. 1(a)). The harvest time was during the last two weeks of September in both years, when husk was beginning to split for all genotypes. Thirty-five of these accessions were common to both years. Most trees were more than 30 years old. They grew between 1 and 1073 m above sea level and were genetically characterized in a previous paper.[18] The sampled trees had never undergone either pruning or other horticultural practice (fertilization and irrigation), nor pest inspection. Nuts of five commercial cultivars (Lara, Franquette, Hartley, Howard and Sorrento) were sampled from commercial orchards, except Sorrento which was supplied by the National Council for Agricultural Research and Economics, CREA-FRUT of Rome, and included as references. All genotypes were characterized for oil content and oil fatty acid composition.

Meteorological data

Meteorological data were provided by OSMER (Friuli Venezia Giulia Meteorological Service), and, as they suggested, the Friuli Venezia Giulia region was divided into six climatic zones, namely Carnia Alps, Pre-Alpine belt, Friuli plain, foothills, Tarvisio Alps and Canal del Ferro valley[19] (Fig. 1(b)). Minimum, average and maximum daily temperatures of both years of experimentation, average mean temperature and rainfall of the last 25 years and altitude above sea level for each location were obtained from OSMER (Table 1).

Sample preparation

Forty nuts were sampled before nut fall from each accession and taken to the laboratory for analyses. Samples stored in a dry room were inspected regularly; when the husk was open, fruits were dehusked and the nuts washed in water with 10 g L^{-1} sodium hypochlorite, dried in a heater at 30 °C for 3 days and stored at 4 °C in a cold store at 60% relative humidity. At the time of analysis, nuts were manually cracked and shelled and the kernels chopped in an MKM 6000 coffee mill (Bosch, Stuttgart, Germany).

Moisture content

Moisture content was measured in duplicate. It was determined on 5 g samples dried in an oven at 105 ± 2 °C until constant weight was reached.

Oil content determination

Oil content was determined by nuclear magnetic resonance (NMR) on whole kernels. NMR is a non-destructive method used in oil crop breeding.[20] A preliminary trial was conducted to compare the Soxhlet oil extraction method with NMR, the results of which are reported as 'Supporting information'.

Fatty acid determination

The walnuts were extracted with petroleum ether (b.p. 40–60 °C) using a Soxhlet apparatus (see 'Supporting information'). Petroleum ether was removed by evaporation under nitrogen gas flow and the oil was dried over anhydrous sodium sulfate. Afterwards, an aliquot of oil was dissolved in 1 mL of *n*-hexane. Fatty acids were converted into fatty acid methyl esters (FAMEs) by transesterification with methanolic potassium hydroxide solution (2 mol L^{-1}) and then vortexed for 30 s. Composition was determined by gas chromatography with flame ionization detection (GC-FID), with every fatty acid expressed as a percentage of the total detected in the oil. The gas chromatograph was fitted with a 60 m HP-88 capillary column (Agilent Technologies, Santa Clara, CA, USA). Helium was used as carrier gas (2 mL min^{-1} constant flow). The injector, detector and oven temperatures were 230, 250 and 200 °C respectively. Samples (5 μL) were injected in split mode (split ratio 1:100). A 5 mm i.d. precision inlet split liner was used. Different FAMEs were identified by comparison with known standards.

Statistical analysis

Descriptive metrics such as mean, standard deviation and range of data distribution were calculated.

Pearson correlation coefficients were computed to measure the strength of linear association among investigated traits.

Non-parametric bootstrap analysis was applied to select the best temperature predictor for fatty acid composition.[15] Statistical analysis was performed using R Version 2.15.0 (R Development Core Team, Vienna, Austria), utilizing the Shapiro–Wilk normality test to analyze the normality condition. A total of 10 000 random samplings with replacement were performed. The sample size of each bootstrap iteration was the same as the original one. Several temperature parameters were evaluated by comparing the distribution of the 10 000 R^2 values.

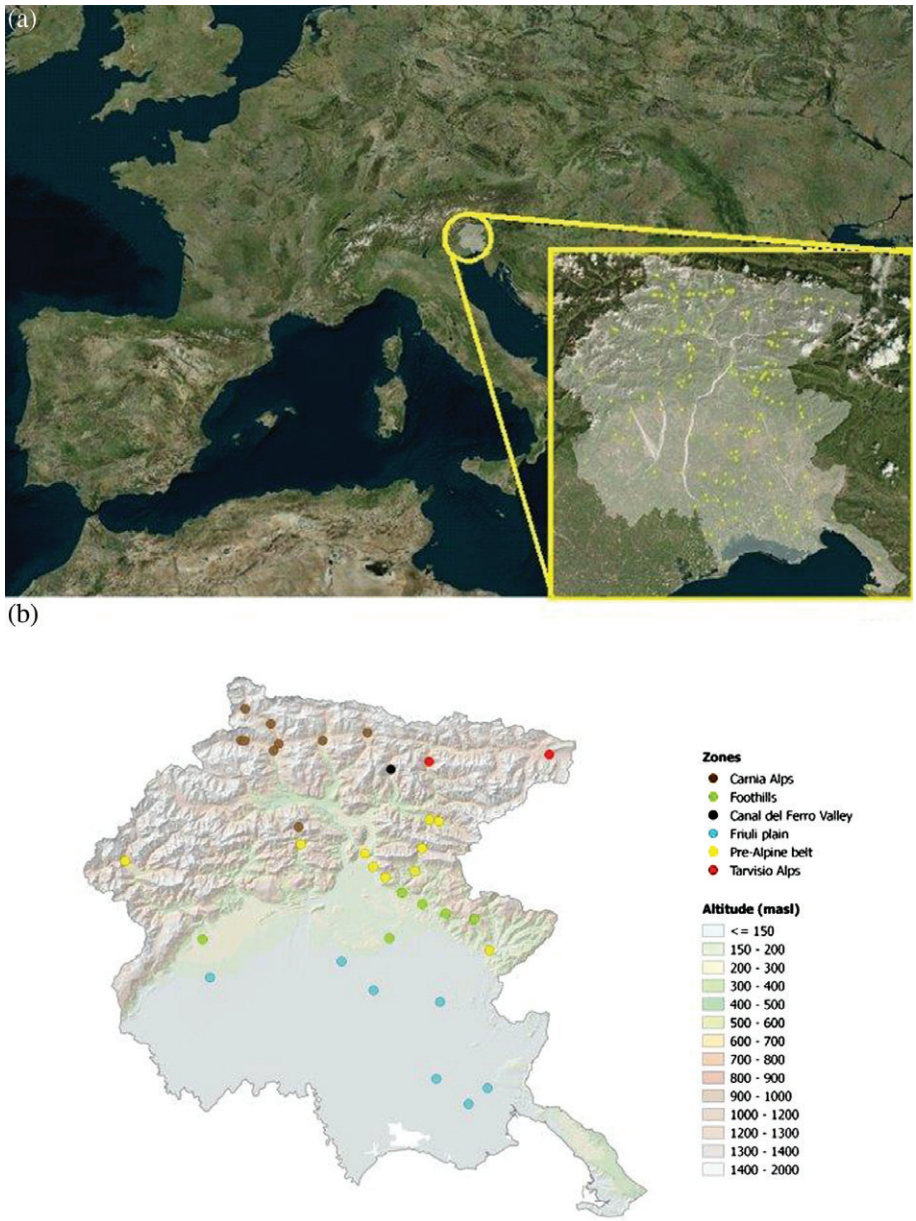

Figure 1. Geographic location of (a) walnut collection sites and (b) climatic zones.

RESULTS AND DISCUSSION

Moisture content

Although the kernel moisture could vary as a function of season, harvest time and environmental conditions, both wild accessions and commercial cultivars had the same moisture content of about 3.5% (w/w) (data not shown) after drying, suitable for long storage.[21] This value was in agreement with those reported by Amaral *et al.*[3] and Pereira *et al.*[4] and lower than those reported in other studies.[22,23]

Oil content

Oil content is reported in Table 2. Commercial varieties generally showed a higher mean oil content than wild genotypes, but the difference was quite small (~4% w/w) (Table 2). The oil content of the commercial varieties was comparable to the literature data for the same cultivars.[2,4,23] However, there were some differences between commercial varieties. Howard was the cultivar with the highest oil content, whereas Hartley showed the lowest one. Sorrento recorded a lower oil content than that reported by Martínez *et al.*[2] (72–74%) and higher than that reported by Malvolti *et al.*[24] (61%). Kernel oil content of the wild accessions ranged from 56.2 to 71.6% in 2013 and from 54.2 to 72.2% in 2014. The lowest oil content was found in the genotype W160 (Pre-Alpine belt) in both years, whereas the highest was recorded for W122 and W123 (Friuli plain, close to the sea coast) in 2013 and 2014 respectively; both the latter accessions were located in the same environmental area (Table 3).

Kernel oil concentration was slightly higher on average than those reported for other walnut collections in New Zealand[10,25] and Portugal.[3] Conversely, Malvolti *et al.*[24] obtained a lower kernel oil content, as the average of 190 walnut genotypes grown throughout the Italian peninsula, than those obtained in the present study. The range was similar to those reported by Ozkan

Table 1. Altitude, annual rainfall and mean temperature during seed development at locations where nut samples were collected in both years

Location	Elevation[a] (m a.s.l.)	Annual mean temperature[b] (1991–2014) (°C)	Annual rainfall[c] (1991–2014) (mm)	2013 Mean temperature during seed development[d] (°C)	2014 Mean temperature during seed development[d] (°C)	Accessions collected (*n*)
Carnia Alps	693	4.0–11.0	1681	19.5	14.7	9
Pre-Alpine belt	468	4.4–12.7	2347	14.2	17.6	10
Friuli plain	50	13.0–13.2	1450	23.4	22.0	7
Foothills	322	13.0–13.2	2193	22.7	20.9	6
Tarvisio Alps	718	6.8–7.4	1743	17.4	15.9	2
Canal del Ferro valley	568	9.4	1952	19.4	17.6	1

[a] Annual mean temperature = average of daily mean temperatures recorded during year. The temperature range indicates the presence of more than one meteorological station for each location, with the exception of Canal del Ferro valley where there is only one meteorological station.
[b] Annual rainfall = long-term average (1991–2014) of annual precipitation.
[c] Elevation = average height above sea level where sampled walnut trees grew.
[d] Mean temperature during seed development = starting from the end of walnut flowering and ending at physiological maturity (see text for details).

Table 2. Oil content and main fatty acids of walnut accessions and controls represented by five commercial cultivars

Year (no. accessions)	Oil content (% w/w)	Main fatty acids (%)				
		Palmitic C16:0	Stearic C18:0	Oleic C18:1	Linoleic C18:2	Linolenic C18:3
2013 (58)	65.3 ± 3.2	8.18 ± 0.98	2.85 ± 0.80	16.20 ± 2.82	60.54 ± 2.46	12.00 ± 1.96
	(56.2–71.6)	(6.11–11.37)	(1.54–5.23)	(11.26–25.09)	(53.51–66.53)	(6.89–16.13)
2014 (166)	66.3 ± 3.1	7.24 ± 0.82	2.21 ± 0.71	16.49 ± 2.72	61.28 ± 2.94	12.17 ± 2.02
	(54.2–72.2)	(3.90–9.59)	(1.07–4.42)	(10.05–24.90)	(46.91–68.62)	(7.37–17.57)
Controls	70.2 ± 1.62	7.92 ± 0.98	3.29 ± 0.70	15.63 ± 2.99	58.01 ± 0.87	14.79 ± 2.64
	(68.5–72.9)	(6.95–8.74)	(2.82–3.83)	(12.21–19.72)	(56.94–59.03)	(11.86–18.65)

Values are mean ± standard deviation, with range of variation given in parentheses.

and Koyuncu,[9] Çağlarırmak,[26] Dogan and Akgul[27] and Yerlikaya *et al.*,[28] who analyzed selections collected in Anatolia (57–71%).

Oil content in the walnut kernel is determined by genotype but may also be influenced by environmental conditions and irrigation management.[2,4,29] However, no significant relation was found between altitude and oil content (data not shown).

Fatty acid composition

Data on oil fatty acid composition are reported in Table 2. Walnut kernel oil was mainly composed of five fatty acids, namely palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids. The best represented one was linoleic acid (46.9–68.6%) in both wild accessions and commercial varieties (Table 2), followed by oleic (10.0–25.1%) and linolenic (6.9–17.6%) acids. The data obtained indicate that the accessions harvested in the Friuli Venezia Giulia region showed a higher variability in oil composition compared with commercial cultivars. In particular, some accessions showed a significantly higher oleic acid content (>20%) and lower linoleic (<56%) and linolenic (<11%) acid contents (Table 2).

PUFAs were the main group of fatty acids in walnut oil in both wild accessions (62.9–79.1%) and commercial varieties (70.2–76.5%). On the contrary, SFAs were the lesser group, ranging from 5.8 to 13.8%, with palmitic and stearic acids the two main SFAs present, totaling on average 7.5 and 2.5% of total fatty acids respectively (Table 2). Oleic acid concentration ranged from 11.2 to 25.1% in 2013 and from 10.0 to 23.3% in 2014, with very similar average and range of variation in both years (Table 2). Fifteen accessions of the 35 analyzed in both years showed a negligible variation in oleic acid content between years (~2%); the others showed 5% variation (Table 3).

Accessions were divided into three classes on the basis of their oleic acid content. Classes were defined as follows: <12, 12–20 and >20%. The limits were drawn using the five commercial cultivars as reference. Most of the wild accessions had an oleic acid content that fell into the intermediate class, with the exception of four, namely W037, W046, W089 and W201, which showed a content stably higher than 20% in both years, with little influence of environmental conditions (Table 3). The accessions with high oleic acid content, W037 and W046, were located in the Alpine area, characterized by low mean temperature, whereas accessions W089 and W201 came from the plain. These accessions may be used in breeding programs to enhance oleic acid content in walnut oil and to expand its utilization, by improving stability during long-term storage, allowing it to be used as a frying oil and enhancing the shelf life of walnuts consumed as fruit. In sunflower, breeders have incorporated the high oleic trait into confectionary varieties to increase the shelf life of sunflower kernels.[30]

Significant variations in linoleic and linolenic acids between years were observed in just a few accessions (Tables 2 and 3).

Relationship between fatty acids

The coefficients of correlation between fatty acids of the wild walnut oils are reported in Table 4. A significant and positive correlation was observed between the palmitic and stearic SFAs. The

Table 3. Kernel oil content and main fatty acid composition in 35 accessions sampled in both years

	Oil content (% w/w)		C16:0 (%)		C18:0 (%)		C18:1 (%)		C18:2 (%)		C18:3 (%)	
Accession	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
W005	63.5	66.9	8.07	7.98	2.24	2.58	15.44	14.23	60.78	57.86	13.19	16.18
W012	66.6	70.0	8.39	8.16	2.80	3.97	13.72	15.35	61.96	58.77	13.14	12.68
W027	68.1	66.3	7.81	3.90	2.71	1.87	17.88	15.15	58.96	64.57	12.49	14.11
W028	67.5	70.1	6.86	6.28	2.23	1.73	15.92	16.23	61.44	60.28	13.40	14.10
W034	65.9	63.0	6.11	7.01	5.23	1.41	19.17	16.91	58.82	61.56	10.67	12.41
W037	61.2	62.5	6.65	6.77	2.36	2.52	25.09	23.32	56.20	57.12	9.19	9.84
W042	60.4	61.5	6.31	6.03	2.81	1.66	17.25	15.97	61.74	64.51	11.89	11.69
W045	62.9	65.5	8.32	8.69	2.99	4.42	16.26	14.22	60.64	61.79	11.23	10.86
W046	63.6	65.9	6.97	6.36	2.59	2.29	21.20	20.08	58.26	60.27	10.51	10.54
W051	63.7	64.9	8.11	7.68	2.72	2.56	16.30	12.76	59.60	62.43	13.27	14.43
W079	70.0	70.2	9.30	7.29	2.98	2.13	13.19	16.87	60.48	58.16	13.84	15.37
W087	65.3	64.0	9.16	7.72	2.71	3.38	11.26	11.05	64.87	60.78	11.99	15.60
W089	67.8	65.7	8.45	7.01	3.51	1.47	20.67	17.32	56.01	63.05	11.07	10.91
W097	67.1	62.2	8.53	7.47	3.59	2.27	16.70	14.71	61.87	65.91	9.31	9.55
W100	63.3	68.9	8.12	7.43	3.50	2.74	16.83	21.15	64.42	59.52	6.89	8.11
W102	65.6	67.6	7.96	7.86	3.05	3.58	13.85	15.74	61.50	59.37	13.59	13.01
W104	68.6	68.8	9.11	7.54	2.37	2.70	13.68	12.02	60.72	61.44	13.89	15.99
W136	70.3	68.9	10.81	9.59	5.11	4.19	13.66	17.71	58.10	56.11	12.31	12.39
W140	65.0	66.3	7.72	7.09	2.56	2.87	18.99	13.98	58.22	60.77	12.49	14.22
W141	63.3	66.6	8.05	6.14	2.33	2.48	16.18	17.98	60.80	60.91	12.55	12.17
W142	71.0	62.9	7.29	6.00	ND	1.60	17.45	13.31	63.30	68.35	11.87	10.55
W144	69.7	68.5	7.59	6.71	2.66	3.02	17.04	15.46	61.51	61.34	11.07	12.98
W145	66.8	69.4	8.54	6.87	4.58	1.73	17.91	18.49	57.57	60.25	11.23	12.37
W148	70.1	68.7	11.37	8.07	4.47	3.95	12.20	16.45	58.08	61.03	13.88	10.03
W150	60.0	68.3	7.07	7.63	3.01	2.68	19.18	14.57	59.59	59.49	10.99	14.41
W153	63.9	64.1	9.37	6.45	2.39	1.66	14.93	15.41	60.22	62.22	13.09	13.88
W160	56.2	54.2	8.82	7.59	2.29	2.99	14.47	10.05	65.73	68.62	8.43	9.89
W162	65.2	68.9	7.56	7.08	1.97	1.40	20.24	17.86	59.99	62.69	10.10	10.49
W179	63.5	63.1	8.60	6.81	1.54	2.55	13.63	15.16	61.46	59.41	14.74	15.50
W180	62.2	59.6	9.05	7.25	2.09	1.55	14.49	11.97	60.43	65.71	13.85	13.37
W201	66.6	67.9	8.97	7.49	2.92	2.34	19.33	21.67	58.23	57.94	10.28	9.69
W204	64.7	65.8	8.42	6.70	2.29	1.91	13.90	14.31	62.53	62.86	12.86	13.74
W206	65.1	65.3	7.98	7.73	2.71	3.34	11.42	12.68	66.53	64.44	10.87	11.34
W212	66.3	66.9	8.92	9.18	2.61	2.59	14.43	13.08	58.91	59.19	15.12	15.85
W215	65.6	65.8	8.18	7.08	4.41	3.51	15.92	17.05	61.44	63.62	9.49	7.98
Lara	68.9	71.0	6.02	7.07	2.20	2.30	19.23	19.72	57.90	56.97	13.80	13.14
Franquette	69.0	70.0	9.03	8.51	3.25	3.76	18.06	17.42	57.05	58.03	12.30	11.96
Hartley	69.0	68.8	9.07	8.54	3.55	3.93	14.29	14.82	58.73	58.90	13.44	13.37
Howard	71.4	72.9	7.01	6.95	2.93	2.82	14.02	13.89	56.88	57.31	18.52	18.65
Sorrento	70.3	69.8	8.22	8.65	3.74	3.75	11.95	12.21	59.01	59.03	16.85	16.12

ND, not determined.

strongest relationship between fatty acid concentrations was the negative one between oleic acid and PUFAs (Table 4). This relationship can be seen in the linear regression analysis ($P < 0.001$) (Fig. 2). Oleic acid also showed a negative relationship, although of lesser intensity, with linoleic and linolenic acids (Table 4), as already observed in several herbaceous oil crops such as sunflower[15–17] and soybean.[31,32] Our results are comparable to those obtained by Malvolti *et al.*,[24] who reported a significant correlation between oleic and linoleic acid contents ($r = -0.88$) and between oleic and linolenic acid contents ($r = -0.67$). However, in our experiments, the magnitudes of the relationships were generally lower for both linoleic ($r = -0.55$) and linolenic ($r = -0.49$) fatty acid contents, which would suggest interference from environmental factors such as temperature, rainfall and light, as reported elsewhere.[4,10]

Table 4. Correlation coefficients between seed oil content and concentrations of fatty acids in walnut genotypes tested ($n = 35$)

	Palmitic	Stearic	Oleic	Linoleic	Linolenic	PUFAs
Palmitic	1					
Stearic	**0.45****	1				
Oleic	−0.29	−0.08	1			
Linoleic	−0.26	−0.28	**−0.55****	1		
Linolenic	0.08	−0.19	**−0.49****	−0.21	1	
PUFAs	−0.17	**−0.41***	**−0.83*****	**0.74*****	**0.50****	1

Asterisks indicate significance at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

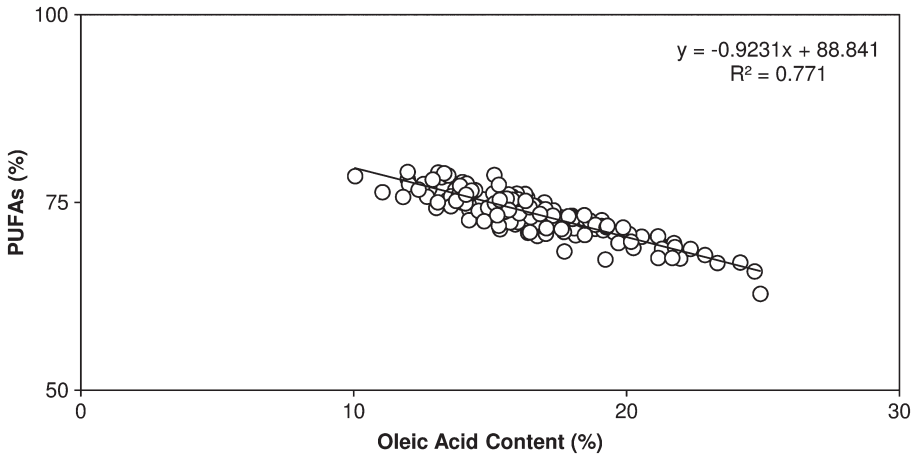

Figure 2. Relationship between oleic acid content and PUFAs (sum of linoleic and linolenic acids) in walnut oil.

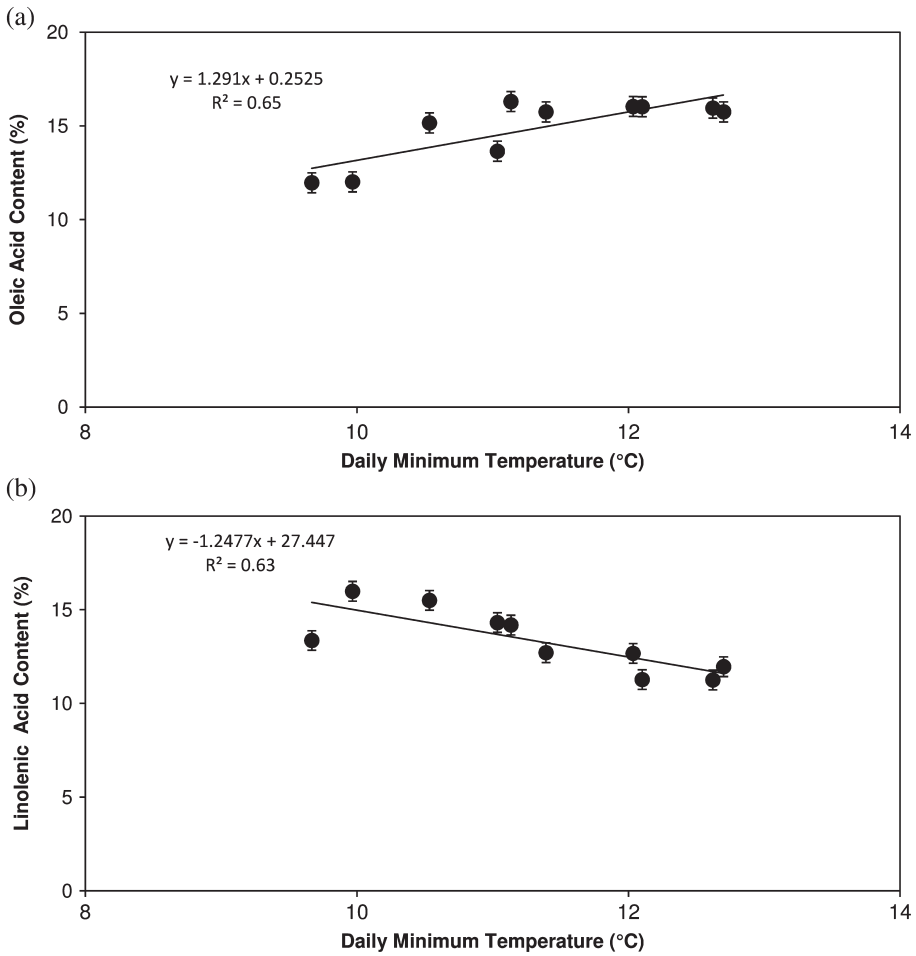

Figure 3. (a) Oleic acid concentration as a linear function of daily minimum temperature during fruit development in walnut ($n = 10$; $P < 0.01$). (b) Linolenic acid concentration as a linear function of daily minimum temperature during fruit development in walnut ($n = 10$; $P < 0.01$). Bars represent standard error of the means.

Temperature and fatty acid composition

We analyzed the effect of temperature (daily average, maximum and minimum) on fatty acid composition throughout the fruit development period from the end of flowering to physiological maturity. In the Friuli Venezia Giulia region, this period lasts about 2 months, beginning in June and ending in August, likely with a few days of difference according to altitude. No significant relationship was found between any daily temperature and SFA, linoleic acid and PUFA contents (data not shown). On the other hand, R^2 values of the linear functions between oleic acid concentration and temperature ranged from 0.08 to 0.65, depending on the temperature used. The best positive linear function was obtained considering daily minimum temperatures ($R^2 = 0.65$), evidencing that, in the range 9.5–13.0 °C, an increase of 1 °C in

Table 5. Bootstrap analysis of linear relationship, coefficient of determination (R^2), for daily mean temperature (T_{med}) and daily minimum temperature (T_{min})

Fatty acid	Bootstrap samples	Parameter	Mean R^2	Standard deviation	Median R^2	Minimum R^2	Maximum R^2
Oleic	10 000	T_{min}	0.55	0.05	0.54	0.12	0.88
	10 000	T_{med}	0.24	0.10	0.22	0.01	0.56
Linolenic	10 000	T_{min}	0.45	0.07	0.46	0.14	0.68
	10 000	T_{med}	0.25	0.11	0.25	0.03	0.55

minimum temperature determined an increase in oleic acid content of 1.5% (Fig. 3(a)). Average and maximum temperatures gave worse correlation coefficients ($R^2 = 0.46$ and 0.08 respectively). The latter, owing to the very low correlation value, was discarded from further investigations. R^2 values of the linear functions between linolenic acid concentration and temperature ranged from 0.18 to 0.65, depending on the temperature used, and again, daily minimum temperature gave the best linear fitting function ($R^2 = 0.65$), but in this case the linolenic acid content decreases when temperature increases (Fig. 3(b)). The relationship between oleic acid and maximum temperature was once again low ($R^2 = 0.18$) and therefore disregarded in further investigations. The biological and physiological mechanisms through which daily minimum temperature influences oleic and linolenic acid concentrations are still unknown and would deserve investigation, given that the knowledge of environmental factors affecting fatty acid accumulation might help during germplasm selection and new genotype development.[15]

Bootstrap samples

Table 5 reports the results of bootstrapping on the coefficient of determination (R^2) of linear functions between average and minimum temperatures and fatty acid content. The goal of bootstrapping is to determine how the fatty acid content is influenced by different temperature regimes and to compare the different types of temperature (minimum *versus* average). The oleic and linolenic fatty acid contents were affected more by the daily minimum temperature than by the daily average. Several authors have reported comparable results for herbaceous oil crops.[20,33] For instance, Izquierdo *et al.*[16] reported that, in sunflower, oleic acid percentage was positively related to minimum night-time temperature.

CONCLUSIONS

A considerable phenotypic variability in kernel oil content and oil fatty acid composition was found within walnut accessions sampled throughout the Friuli Venezia Giulia region in two years of observations. Kernel oil content recorded for some wild accessions was comparable to cultivars on the market. These and other accessions also showed significant differences in oil fatty acid composition compared with commercial cultivars.

It would be possible to develop cultivars with a higher oleic acid content (>20%) and lower PUFA content than commercial varieties, since the two characters are negatively and strongly related. In this case, the first step in a breeding program would be to select against PUFAs rather than linoleic or linolenic acid individually or against SFAs. Secondarily, the selected new genotypes, before being recommended to growers or selected as donor parents, have to show a high and stable level of oleic acid across years and environments. These genotypes could be of great interest for the market since they would combine unchanged nutritional characteristics with an increased oxidative stability and shelf life of their oils.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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