SUPPLEMENTARY MATERIAL

Fatty acid content profile and main constituents of *Corylus avellana* kernel in wild type and cultivars growing in Italy

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Abstract:

The kernel composition (moisture, ash, protein, carbohydrate, calories, fat, monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids) of two hazelnut (*Corylus avellana* L.) cultivars (`Tonda Gentile Romana` and `Tonda Gentile Trilobata`) and of two wild type growing in different climatic conditions (north-west and central Italy) was evaluated. The main kernel component was fatty acid ($65.9 \pm 1.8\%$, mean value), and the most abundant fatty acid in hazelnut was oleic acid (C_{18:1}) ($83.5 \pm 1.0\%$, mean value). The saturated fatty acids are the minor compounds in kernel hazelnut, resulting in a ratio un-saturated fatty acid to saturated (U/S) fatty acid of 9.0 ± 1.6 . Compared to other tree nuts and vegetable oils, hazelnut oil is among the ones with the highest contents of monounsaturated and the lowest content of saturated fatty acid. Thus, hazelnut may be beneficial for the human diet preventing cholesterol-based atherosclerosis and ischemic cardiovascular diseases.

Keywords: *Corylus avellana*; fatty acid; MUFAs; PUFAs

Experimental part

Study Area and Plant Material.

The study was carried out in the period May to September 2014 on *C. avellana* plots (wild plants and orchards) growing in the north-west of Italy (Piedmont and Lombardy, N₁, N₂ and N₃) and in central Italy (Lazio, C₁, C₂ and C₃). In particular, N₁ and N₂ were *C. avellana* cv. 'Tonda Gentile Trilobata' orchards, N₁ growing in Alba (CN, 44°42'13"N; 08°01'13"E) at 172 m a.s.l. and N₂ in Lequio Berra (CN, 44° 36' 49" N; 08° 06' 11" E) at 715 m a.s.l. Both N₁ and N₂ received routine horticultural care suitable for commercial production, including fertilization, weed and pest control. They were not subject to controlled irrigation. N₃ was a wild *C. avellana* populations growing at Zerbolò (PV, 45°12'39"N, 09°03'26"E, 74 m a.s.l.). With regard to the *C. avellana* growing in Lazio, C₁ and C₂ were both *C. avellana* cv 'Tonda Gentile Romana' orchards growing in Nepi (VT 42°14'37"N, 12°20'47"E; 215 m a.s.l.). C₁ and C₂ received routine horticultural care and were subjected to different drip irrigation systems: C₁ was subjected to surface drip irrigation while C₂ to sub-surface drip irrigation, with a drip-line buried at a 30 cm depth. C₃ was a wild population growing in Capranica (VT, 42°14'57"N, 12°09'31"E, 400 m a.s.l.). Two plots (400 m² each) were randomly selected in all of the six *C. avellana* populations. Representative plants (n = 5 per plot) were randomly selected in the center of each plot for measurements.

The climate of Viterbo (VT) was characterized by a minimum air temperature (T_{min}) of the coldest month (January) of 2.6 ± 1.7°C, a mean maximum air temperature (T_{max}) of the hottest month (July) of 30.8 ± 1.4°C, and a mean annual air temperature (T_m) of 14.6 ± 6.6 °C. Total annual rainfall was 824 mm (data from Lazio Regional Agency for Development and Agricultural Innovation; Meteorological Station of Viterbo, data of the period 2004-2014), mainly distributed in autumn and winter. During the study period, T_{min} of the coldest month (March) was 5.1 ± 1.9°C, T_{max} of the hottest month (August) was 28.1±1.6°C, T_m 17.3 ± 4.4 °C and total rainfall from March to September 467 mm (data from Lazio Regional Agency for Development and Agricultural Innovation; Meteorological Station of Viterbo, data of the period March – September 2014).

The climate of Cuneo (CN) was characterized by a T_{min} of the coldest month (December) of -6.4 ± 2.0°C, a T_{max} in the hottest month (July) of 34.6 ± 1.3 °C, and a T_m of 13.1 ± 8.2°C. Total annual rainfall was 659 mm mainly distributed in autumn and winter (data from Piemonte Regional Agency for the Environmental Protection; Meteorological Station of Alba Tanaro, data of the period 2003-2014). During the study period, T_{min} of the coldest month (March) was 0.4 ± 1.2°C, T_{max} of the hottest month (July) was 33.3 ± 1.5°C, T_m was 17.8 ± 4.7°C and total rainfall from March to

September 437 mm (data from Piemonte Regional Agency for the Environmental Protection; Meteorological Station of Alba Tanaro, data of the period March – September 2014).

Kernel composition.

Immediately after nut fall (beginning of September in all the populations) randomly selected samples were hand-harvested in order to evaluate the kernel composition in all the considered plots. A sample of 150g of nuts for each of the selected bushes per plot were randomly collected. Kernel moisture content was determined by drying crushed kernels ($n = 3$ per plant per plot) at $100 \pm 5^{\circ}\text{C}$ until they reached a constant weight (AOAC 1990). In order to determine total ash, pre-weighed samples ($n = 3$ per plant per plot) were transferred to a muffle furnace (Metrawatt GmbH, RO-8). The temperature was gradually raised to 550°C and the samples were ashed for 24 h to a white color and then weighed (AOAC 1990). Nitrogen was determined by Kjeldahl method on dried kernel samples ($n = 3$ per plant per plot). The protein percentage was calculated using 6.25 as the protein factor (James 1995).

Total oil was determined by crude oil extraction from 3g of finely chopped dried nuts using 150 mL of n-hexane by Automated SOXTHERM Extraction instrument (Gerhardt Analytical System, Germany). Extraction program was carried out according to AOAC (1990). Temperature was set up at 140°C , with a reduction interval of 4min, and reduction pulse of 4s; hot extraction 30 min; evaporation A 6 x Interval, solvent level about 1-2 cm below the thimble; rinsing time: 1 h 10 min; evaporation B 5 x Interval. The extract was cooled off and evaporated to 1 mL under a gentle nitrogen stream. The extracted oils were kept in amber tubes and stored at -20°C until they were analyzed.

Carbohydrate content was estimated by subtracting the sum of the other components using the following formula (Oliveira et al. 2008):

$$\text{Carbohydrate content (\%)} = 100\% - (\% \text{moisture} + \% \text{protein} + \% \text{fat} + \% \text{ash})$$

Total calories per sample were calculated based on the physiologic caloric value of protein, fat and carbohydrates (Robinson et al. 1986):

$$\text{Energy (kcal)} = 4 \times (\text{g protein} + \text{g carbohydrate}) + 9 \times (\text{g fat}).$$

Fatty acid composition of extracted oil.

The fatty acids composition was determined as methyl esters by gas chromatography (GC) coupled to a mass spectrophotometer, according to AOAC (1990). Fatty acid methyl esters were prepared by vigorous shaking (1h at $70\text{--}80^{\circ}\text{C}$) of a solution of each hazelnut oil sample ($n = 12$) in n-hexane (10 mg in 1 mL 2 N methanolic hydrochloric acid solution). Chromatographic analysis was

performed on a Thermo Scientific GC/MS system (TraceDSQII mass spectrometer, TraceGCUltra gaschromatograph), Xcalibur MS Software Version 2.1 (including NIST Mass Spectral Library (NIST 08) and Wiley Registry of Mass Spectral Data 8th Edition, using a Restek capillary column, Rtx-5MS 30 m x 0.25 mm x 0.25 μ m film thickness. The injector and detector temperatures were maintained at 250 °C. The oven temperature was set at 70 °C x 1 min, 20 °C/min up to 180 °C, 3 °C/min up to 220 °C x 15 min. Helium was employed as the carrier gas with a flow rate of 1 mL/min according to the method of European Regulation 2568/91 (1991). Fatty acids were identified by comparison with retention times of external standards (AOAC 1990). The GC reference standard FAME mixture GLC-10 from Sigma-Aldrich was used to identify the FAMES. An external calibration curve was used for calculating the quantities of analytes extracted. For the considered range of FAMES concentration the response of the mass-selective detector was quadratic.

Gas Exchange

Measurements of gas exchange were carried out in May, during the intense sink activity of fruits, according to Cristofori (2005), by an infrared gas analyzer (ADC LCpro+, UK), equipped with a leaf chamber (PLC, Parkinson Leaf Chamber). Measurements were made on fully expanded leaves at the top of the canopy ($n = 3$ leaves per each of the selected plant in each plot). Net photosynthesis (P_N , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), photosynthetic photon flux density (PPFD, $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and leaf temperature (T_l , °C) were measured. Measurements were carried out under natural conditions, on cloud-free days (PPFD $\geq 1000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, saturating level) from 10.00 to 12.00h (Reich et al. 1995). The showed P_N rates were the mean of the maximum rates for the three days of measurement in May.

Statistical analysis.

All statistical tests were performed using a statistical software (*Statistica*, *Statsoft*, USA). Differences in kernel composition and physiological leaf traits were analyzed by one-way ANOVA. Differences were regarded as significant when the p -values were less than 0.05. The results were expressed as means $\pm$ (standard deviation) S.D. Coefficient of variation (CV) was calculated for each kernel composition traits (i.e. moisture, ash, fat, protein, carbohydrate and energy) separately for central samples (CV_C) and north-west samples (CV_N).

A simple regression analysis was carried out in order to test the relationship between the percentage of carbohydrate and net photosynthetic rates, between fat content and carbohydrate content and between linoleic and oleic acid. Principal component analysis (PCA) was carried out in order to

summarize the main fatty acid compositions (oleic acid, linoleic acid, palmitic acid and stearic acid) into major components to explain kernel sample variations.

Disclosure statement

No potential conflict of interest was reported by the authors.

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