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Fatty acids and alpha-tocopherol composition in hazelnut (*Corylus avellana* L.): a chemometric approach to emphasize the quality of European germplasm

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Abstract In the frame of SAFENUT AGRI GEN RES Action, which was a European strategy for the recovery, characterization and conservation of genetic resources, the fatty acids and the tocopherol profiles of a set of 75 hazelnut accessions were analyzed. The aim of this study was to assess the genetic differences among the European germplasm, contributing to the definition of nut quality in traditional European areas of cultivation. Significant differences were found between accessions for oil amount and contents of most fatty acids. As expected, monounsaturated fatty acids made up the largest portion (mean 80.85 %) followed by polyunsaturated fatty acids (10.70 %). The saturated ones were the minor components and accounted for only 8.43 % of the total fatty acids. On the basis of Student's test, significant differences between the 2 years of harvest were found for fatty acid content, except for linoleic acid, the ratio of polyunsaturated, α-tocopherol and the stability index. When the oil content was studied in cultivars from the same site of cultivation, the mean values of the genetic pools from central Italy (60.8 %), Slovenia (59.3 %) and Portugal (58.2 %) showed highest values than those of cultivars grown in Greece (56.8 %), Spain (55.9 %) and France (51.5 %). A chemometric approach based on principal component and clustering analyses was developed to identify the most interesting cultivars for breeding programs.

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Introduction

Hazelnut quality is defined by market standards requested by the food industry that processes about 90 % of the European supply. Hazelnuts, like the other nuts, are high energetic food rich in fats and protein; they are valuable sources of fiber, phytonutrients, and antioxidants such as Vitamin E (Bacchetta et al. 2008). The lipid portion is the main component of the hazelnut kernel, and represents a major determinant of kernel flavor particularly following roasting. Lipids may constitute more than 60 % of the hazelnut kernel dry weight; in addition the specific fatty acids of hazelnut are very similar in composition to those of olive oil and generally considered to be desirable for a healthy diet. A high contents of mono-unsaturated fatty acids (MUFA) and low amounts of saturated fatty acids (SFA) in the diet can effectively control blood lipid levels reducing coronary heart disease risk and blood pressure (Fraser 2009). Furthermore hazelnut oil is an excellent source of some bioactive nutrients such as tocopherols. These ingredients exert positive effects in preventing heart disease and various types of cancer by inhibiting tumor growth and enhancing the human immune system (Dietrich et al. 2006). Lipid content and composition is also very important for the confectionery industry (Alasalvar et al. 2009). Thus, both lipid content and the proportion of the component fatty acids (particularly the ratio between oleic and linoleic acids) are considered very important criteria for hazelnut kernel quality evaluation (Mehlenbacher 1990a). Unsaturated fatty acids (UFA), antioxidants, such as α-tocopherol, and mineral elements, in particular iron, manganese and copper, are involved in rancidity. Therefore, cultivars with low unsaturated/saturated ratios, low in pro-oxidant compounds, rich in anti-oxidants and low in enzymatic activities, should be preferred, because they minimize post-harvest quality losses, packaging and refrigeration costs (Pershern et al. 1995; Parcerisa et al. 1995).

Many nut quality characteristics of different hazelnut cultivars and genotypes have been previously identified in Turkey (Balta et al. 2006; Alasalvar et al. 2009), United States of America (Mehlenbacher et al. 1990a, b), Italy (Tombesi et al. 1994; Botta et al. 1997; Cristofori et al. 2008), France (Germain and Sarraquigne 1997), Slovenia (Solar and Štampar 1997, 2011), Spain (Rovira et al. 1997), Romania (Turcu and Botu 1997) and Portugal (Amaral et al. 2006a; Silva et al. 2007). However the results are generally referred to a limited set of accessions or cultivars from specific areas of cultivation. In the frame of SAFENUT AGRI GEN RES Action, which was a strategy for the recovery, characterization and conservation of hazelnut genetic resources (Bacchetta et al. 2008; Boccacci et al. 2008; Bacchetta et al. 2010, 2011, 2012b), the fatty acids and the tocopherol profiles of a set of 75 hazelnut accessions from Spain, Italy, Greece, Slovenia, France and Portugal were analyzed. The aim of the work was to assess the genetic diversity among the European genetic resources contributing to the definition of nut quality in traditional European areas of cultivations. Chemometric characterization is a useful method for describing and classifying plant germplasm. Statistical methods such as principal component analysis (PCA) and cluster analysis can be useful tools for selecting genotypes characterized by high-quality attributes, including almond (Lansari et al. 1994; Garcıa-Lopez et al. 1996; Drogoudi et al. 2012), olive, (Cantini et al. 1999), loquat (Martı′nez-Calvo et al. 2008), peach (Nikolic′ et al. 2010), and apricot (Gurrieri et al. 2001). This information is substantial to increase the knowledge on the European hazelnut germplasm diversity, its nutritional and healthy value, and to promote its utilization by stakeholders and breeders. As stated in the Commission Report Analysis of the nut sector- SEC 2002 797: "Improved quality is one of the key factors in improving the international competitiveness of the tree nut sector". Thus, the specific objectives of this study were:

i. The investigation of the variability of total oil content and fatty acid profiles in 75 accessions sampled in six European Countries (Spain, Italy, Greece, Slovenia, France and Portugal), to ascertain the best genotypes from the point of view of oil quality. All variables were jointly examined and their correlation coefficients determined in order to establish an efficient selection strategy.
ii. The study of the influence of the year of harvest and of the geographic origin on the fatty acid profiles, α-tocopherol content and stability index in 75 hazelnut accessions.

iii. The development of a chemometric method based on PCA and cluster analysis to select the most interesting cultivars for breeding programs.

Materials and methods

Plant material

The cultivars and the local accessions analysed in this study were listed in Table 1. The samples were collected from different European national collections: National Collection in Viseu, Portugal; Conservatoire Végétal Régional d'Aquinatanie, Montesquieu, France; IRTA—Institut de Recerca i Tecnologia Agroalimentàries, Constantí, Spain; Pomology Institute—Naoussa, Greece; collection of Biotechnical faculty in Maribor, Slovenia; Monti Cimini, Viterbo and University of Torino, Cravanzana collections in Italy. The genetic pool considered in this work, includes important commercial cultivars as well as minor accessions and selections with potential interest for local market and breeding which were genetically characterised by SSR markers during the SAFENUT project (Boccacci et al. 2008; Bacchetta and Di Giovanni 2012) and in previous works (Boccacci et al. 2006). The plants, were maintained in randomized block design with almost three replicates for each accession. The hazelnuts were harvested at maturity (September) in the crop years 2007, 2008 and 2009, dried to about 5 % kernel humidity and stored in plastic bags at 5 °C, dark conditions. A sample of about 1 kg was randomly chosen for each accession and analysed.

Total oil content determination

Total oil contents were determined according to AOAC 954.02 Official Methods (http://www.aoac.org/). Before chemical analysis hazelnuts were manually cracked and shelled kernels were chopped finely using a coffee grinder. 0.5 g portion finely crushed kernel was added to 10 ml of HCl (25 %), 2 ml of Ethanol 99 % and the mixture was shacked vigorously and stored for 1 h at 80 °C. 10 ml of 99 % ethanol and 25 ml of ethyl ether were added and the solution was shacked for 90 s. Using 25 ml of petroleum ether as solvent, the solution was shacked for 90 s, centrifuged for 20 s at 600 rpm, and the supernatant was collected. The residue was re-extracted twice with the same volume of solvent which was then removed using a rotary evaporator at 80 °C. Oil content was expressed as percentage of kernel dry weight.

Analytical procedure of fatty acid assessment

The main fatty acids determined by gas-chromatography in the oil were palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acid. In addition, total SFA (palmitic + stearic acid), total MUFA (palmitoleic + oleic acid), total PUFA (linoleic + linolenic), and the ratios of UFA/SFA, MUFA/SFA, and PUFA/SFA were calculated. The oil was re-suspended in 6 ml *n*-hexane and 0.25 ml of methanolic solution KOH 2N and then centrifuged for 5 min at 4,000 rpm at room temperature. A Gas Chromatograph (Perkin-Elmer, autosystem), equipped with a detector FID and a Stabilwax (Restek) column (RTX2330; 30 m × 0.5 mm ID, 1.0 μm df) was used. The temperatures of the injector and detector were maintained at 250 and 270 °C respectively. The column temperature was held at 170 °C for 3 min, then was increased from 170 to 175 °C at 1 °C min^{-1} and from 175 to 225 °C at 10 °C min^{-1}. The injection volume was 0.5 μl. The retention times of the compounds were compared with those of a fatty acid methyl esters mixture standard (Supelco-Sigma, cat. No. 1891-1 AMP; Sigma-Aldrich Corp., St. Louis, MO, USA). The relative amount of each fatty acid was calculated over the total fatty acids content.

Analytical procedure of tocopherols determination

Hazelnut oil was extracted from nut samples and tocopherols were determined with a slight modifications of the method illustrated by Kodad et al. (2006). The dried ground sample (approximately 2.5 g) was placed in a thimble, and the oil was extracted in a Soxhlet extractor (Soxtec system, Bicasa 1047 Model) for 2 h using petroleum ether as solvent with the heating source at 135 °C. Saponification was performed according to a modified EU official method (DOCE L174/39, 13 July 2000, http://www.aoac.org/). Samples of 0.15–0.20 g of hazelnut oil were shacked at 60 °C for 45 min

Table 1 Oil content (% d.w.), fatty acids composition (relative %) and α-tocopherol content (ppm oil) of the kernel in 75 hazelnut cultivars

SC	Cultivars	Oil content	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Total SFA	Total MUFA	Total PUFA	UFA/ SFA	MUFA/ SFA	PUFA/ SFA	α-Tocopherol	SI
FR	Bergeri	57.77	5.69	0.23	2.00	79.57	12.47	0.17	7.69	79.80	12.64	12.03	10.38	1.65	227.93	18.96
FR	Casina	51.91	5.98	0.23	2.35	74.37	16.76	0.15	8.33	74.59	16.90	11.05	8.95	2.10	291.83	27.00
FR	Corabel	53.56	6.27	0.22	2.70	78.70	11.77	0.15	8.97	78.91	11.92	10.14	8.81	1.34	231.05	22.82
FR	Cosford	51.38	5.87	0.22	2.08	79.53	12.01	0.12	7.94	79.76	12.13	11.58	10.05	1.54	390.97	33.64
FR	Feriale	45.93	5.91	0.20	2.23	82.62	8.89	0.13	8.14	82.82	9.03	11.29	10.18	1.11	169.59	15.03
FR	Ferwiller	55.71	5.17	0.16	2.33	84.23	7.97	0.14	7.50	84.38	8.10	12.36	11.28	1.09	316.29	25.60
FR	Fertile de Coutard	61.75	5.50	0.19	2.10	81.87	10.16	0.18	7.60	82.05	10.34	12.17	10.81	1.37	172.57	14.18
FR	Gunslebert	41.97	9.02	0.24	2.33	75.24	12.95	0.23	11.35	75.48	13.18	8.67	7.43	1.23	263.01	33.70
FR	Imperatrice Eugenie	49.48	5.36	0.18	1.94	79.83	12.55	0.11	7.30	80.01	12.67	12.69	10.96	1.73	214.53	16.91
FR	Longue d'Espagne	51.96	5.83	0.21	1.74	73.32	19.01	0.16	7.57	73.53	19.16	12.26	9.73	2.53	202.11	16.85
FR	Merveille de Bollwiller	52.50	5.41	0.18	2.28	79.44	12.48	0.16	7.69	79.62	12.65	12.02	10.37	1.65	200.24	16.69
FR	Provence	59.07	6.59	0.25	2.93	78.30	11.78	0.14	9.51	78.55	11.92	9.55	8.30	1.25	192.59	20.23
FR	Rotblaftrige Lambernuss	57.05	5.56	0.21	2.79	80.20	11.11	0.17	8.35	80.40	11.28	11.00	9.65	1.35	351.75	32.37
FR	Segorbe	47.32	5.17	0.17	2.71	81.09	10.62	0.20	7.88	81.26	10.81	11.69	10.32	1.37	327.92	28.07
GR	Argiroupoli	57.69	6.77	0.30	3.18	78.09	11.57	0.09	9.96	78.39	11.65	9.04	7.87	1.17	130.38	14.42
GR	Extra Ghiaghli	59.50	7.04	0.28	2.92	78.99	10.69	0.09	9.96	79.26	10.77	9.05	7.96	1.08	170.90	18.90
GR	GR pi 03	60.38	6.61	0.22	3.68	81.39	7.38	0.11	10.29	81.61	7.48	8.66	7.93	0.73	126.69	14.63
GR	Karydato	49.20	6.50	0.25	2.73	81.10	9.46	0.10	9.23	81.34	9.56	9.86	8.82	1.04	119.85	12.17
GR	Palaz	59.57	7.55	0.33	3.20	80.35	8.58	0.09	10.75	80.67	8.67	8.32	7.51	0.81	118.57	14.27
GR	Patem	55.37	6.30	0.27	3.10	80.57	9.01	0.09	9.40	80.84	9.11	9.60	8.62	0.98	126.44	13.18
GR	Polycarpos wild	56.88	6.71	0.26	2.62	81.57	8.79	0.13	9.32	81.82	8.92	9.73	8.78	0.96	109.50	11.25
GR	Tombul Ghiaghli	58.36	6.39	0.23	2.71	78.75	12.46	0.08	9.09	78.98	12.54	10.18	8.79	1.40	102.34	10.15
IT	Ada	59.58	6.01	0.19	2.74	83.23	7.76	0.09	8.74	83.42	7.85	10.47	9.57	0.91	263.11	25.29
IT	Avellana Speciale	60.53	5.16	0.16	2.56	84.57	7.42	0.10	7.72	84.73	7.51	11.96	10.99	0.97	185.74	15.61
IT	Barettona Vico	59.68	5.62	0.20	2.73	79.68	11.86	0.09	8.34	79.88	11.95	11.06	9.63	1.43	172.71	15.86
IT	Barettona Le cese	62.72	5.87	0.20	2.75	82.81	8.27	0.07	8.62	83.00	8.34	10.63	9.65	0.97	152.24	14.19
IT	Camponica	62.58	5.79	0.20	3.28	82.55	8.03	0.09	9.06	82.74	8.12	10.04	9.15	0.89	170.95	17.17
IT	Carrello	61.56	5.85	0.20	2.77	82.53	8.52	0.10	8.61	82.73	8.61	10.61	9.61	1.00	163.29	15.40
IT	Comune di Sicilia	60.29	5.23	0.17	2.97	83.19	8.35	0.08	8.21	83.36	8.43	11.22	10.19	1.03	191.46	16.94
IT	Centenaria Ginnasi	59.26	6.15	0.16	2.97	82.98	7.58	0.06	9.12	83.14	7.65	9.96	9.12	0.84	119.92	12.05
IT	Dal Rosso	50.81	6.01	0.21	2.37	83.21	7.95	0.20	8.37	83.42	8.14	10.95	9.97	0.98	249.37	22.84
IT	Daria	49.21	6.14	0.25	2.69	83.09	7.70	0.09	8.83	83.35	7.79	10.34	9.46	0.89	150.93	14.43

Table 1 continued

SC	Cultivars	Oil content	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Total SFA	Total MUFA	Total PUFA	UFA/ SFA	MUFA/ SFA	PUFA/ SFA	α-Tocopherol	SI
IT	Loc di Piazza Armerina	63.73	5.49	0.19	2.41	83.00	8.97	0.11	7.90	83.19	9.08	11.71	10.56	1.16	217.13	18.35
IT	Lunga Ginnasi	63.00	5.14	0.17	2.65	84.09	7.75	0.13	7.79	84.25	7.87	11.87	10.86	1.01	165.98	14.36
IT	Meloni	61.92	6.98	0.26	2.59	84.17	5.91	0.10	9.57	84.43	6.01	9.46	8.84	0.63	153.16	16.15
IT	Nocchione	62.76	5.83	0.20	2.84	83.94	7.10	0.12	8.68	84.14	7.21	10.55	9.71	0.84	178.61	16.90
IT	Nociara	61.98	5.35	0.18	2.92	82.72	8.71	0.11	8.28	82.89	8.81	11.09	10.03	1.07	224.84	20.19
IT	Nostrale	53.13	5.31	0.17	2.93	81.37	10.07	0.15	8.24	81.54	10.21	11.16	9.91	1.25	257.49	22.79
IT	Pallagrossa	57.32	5.99	0.23	2.70	80.93	9.86	0.14	8.69	81.15	10.00	10.57	9.42	1.15	248.96	23.61
IT	Riccia di Talanico	61.35	5.76	0.20	2.61	80.23	11.00	0.10	8.36	80.43	11.10	10.98	9.65	1.33	149.11	13.82
IT	Tonda bianca	61.93	5.99	0.18	2.94	82.29	8.35	0.14	8.93	82.47	8.48	10.20	9.25	0.96	239.47	23.46
IT	Tonda di Biglini	49.78	6.08	0.23	2.78	82.27	8.51	0.12	8.85	82.50	8.63	10.30	9.33	0.98	195.09	18.93
IT	Tonda Gentile Langhe	59.25	6.46	0.29	2.38	81.54	9.22	0.13	8.83	81.83	9.35	10.48	9.41	1.07	176.24	17.33
IT	Tonda Gentile Langhe- PD6	59.70	6.10	0.26	2.89	82.73	8.09	0.12	8.99	82.99	8.21	10.16	9.24	0.92	237.41	23.38
IT	Tonda Gentile Romana	62.57	5.89	0.21	2.65	83.59	7.57	0.10	8.54	83.81	7.66	10.77	9.87	0.90	240.17	22.18
IT	Tonda di Giffoni	58.60	5.78	0.20	2.53	82.38	9.01	0.14	8.31	82.57	9.14	11.09	9.98	1.11	188.24	16.93
IT	Tonda Calabrese	63.66	6.30	0.21	3.65	83.73	6.03	0.08	9.95	83.94	6.11	9.05	8.44	0.61	170.00	18.78
IT	San Giovanni	63.58	6.30	0.26	2.31	82.62	8.50	0.14	8.61	82.88	8.63	10.64	9.64	1.01	172.34	16.37
IT	San Vicino	47.56	5.99	0.20	3.23	73.85	16.63	0.13	9.22	74.04	16.75	9.86	8.02	1.85	225.53	23.05
IT	UNITO L35	57.89	6.59	0.23	3.38	81.72	7.83	0.16	9.97	81.94	8.00	9.03	8.22	0.80	117.46	13.02
PT	Comum	59.90	4.91	0.18	1.80	81.52	11.54	0.09	6.71	81.69	11.63	13.92	12.19	1.74	176.31	12.65
PT	Da Veiga	60.76	5.85	0.23	2.23	81.70	9.88	0.11	8.08	81.93	9.99	11.40	10.16	1.24	195.55	17.21
PT	Grada de Viseu	54.77	6.24	0.26	2.60	78.63	12.11	0.12	8.84	78.89	12.23	10.33	8.95	1.38	162.19	15.87
PT	Hall's Giant	56.17	5.40	0.21	2.16	79.65	12.47	0.20	7.56	79.86	12.67	12.30	10.63	1.68	123.35	10.23
PT	Purpurea	58.28	6.02	0.23	3.16	79.00	10.83	0.11	9.18	79.24	10.94	9.83	8.64	1.19	173.29	17.63
PT	Raul	44.32	5.88	0.21	2.30	77.16	13.98	0.45	8.18	77.36	14.43	11.24	9.48	1.77	107.53	9.55
PT	Tubulosa	60.41	5.70	0.19	2.82	79.79	11.52	0.12	8.52	79.98	11.64	10.77	9.40	1.38	209.57	19.46
SL	C. max Pellicule Blanche	54.43	6.16	0.20	2.63	78.43	12.36	0.19	8.78	78.64	12.55	10.39	8.96	1.43	304.68	29.43
SL	C.max Pellicule Rose	48.69	5.70	0.18	3.62	80.31	9.89	0.17	9.33	80.49	10.04	9.71	8.64	1.08	243.09	25.12
SL	CV/1	58.90	5.63	0.20	2.24	82.60	9.33	0.14	7.87	82.80	9.46	11.96	10.74	1.23	149.36	13.06
SL	CV/2	56.46	5.61	0.24	1.95	81.21	11.23	0.17	7.56	81.45	11.40	12.72	11.23	1.50	167.76	13.53

Table 1 continued

SC	Cultivars	Oil content	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Total SFA	Total MUFA	Total PUFA	UFA/ SFA	MUFA/ SFA	PUFA/ SFA	α-Tocopherol	SI
SL	Istrska dolgoplodna leska	59.20	5.25	0.20	2.36	83.16	8.74	0.12	7.61	83.36	8.85	12.13	10.97	1.16	188.36	15.48
SL	Istrska okrogloplodna leska	57.49	5.23	0.16	2.17	82.00	10.44	0.13	7.41	82.15	10.57	12.53	11.10	1.43	217.12	17.35
SL	Pellicola bianca	51.08	5.30	0.14	2.60	79.35	12.42	0.14	7.89	79.49	12.56	11.66	10.07	1.59	237.21	20.34
SP	Barcelona	60.27	5.92	0.21	2.39	80.71	10.58	0.20	8.31	80.92	10.78	11.19	9.88	1.31	186.13	16.69
SP	Castanyera	56.07	6.71	0.26	2.06	79.62	11.17	0.09	8.78	79.88	11.26	10.44	9.16	1.28	162.68	15.58
SP	Culpla	59.07	6.38	0.26	2.56	84.11	6.55	0.06	8.94	84.38	6.60	10.21	9.46	0.75	136.80	13.40
SP	Gironell	55.37	5.93	0.22	2.03	79.09	12.62	0.12	7.96	79.31	12.74	11.84	10.15	1.68	179.49	14.91
SP	Grifoll	56.68	5.05	0.19	1.86	83.22	9.68	0.09	6.92	83.41	9.77	13.54	12.12	1.43	184.39	13.51
SP	Negret	60.43	6.20	0.25	2.32	77.27	13.84	0.18	8.52	77.52	14.02	10.96	9.24	1.72	196.56	18.07
SP	Molar	54.75	5.90	0.25	1.73	80.28	11.80	0.13	7.62	80.53	11.93	12.15	10.59	1.56	177.41	14.61
SP	Morell	58.07	6.19	0.20	2.05	81.62	9.76	0.09	8.24	81.81	9.84	11.24	10.04	1.21	172.88	15.40
SP	Pauetet	58.56	6.20	0.27	2.19	80.16	10.98	0.16	8.39	80.43	11.14	10.95	9.62	1.34	245.60	22.71
SP	Trenet	54.54	5.92	0.24	1.56	73.78	18.43	0.15	7.48	74.01	18.58	12.39	9.91	2.49	127.54	10.29
SP	Vermellet	52.33	5.75	0.25	1.62	79.38	12.86	0.15	7.36	79.63	13.01	12.58	10.81	1.77	125.61	9.99
	Minimum	41.96	4.90	0.14	1.55	73.32	5.91	0.55	6.70	73.52	6.01	8.31	7.43	0.61	102.34	9.54
	Maximum	63.73	9.02	0.32	3.68	84.56	19.00	0.45	11.34	84.42	19.16	13.92	12.18	2.53	390.97	33.70
	Mean	56.95	5.95	0.22	2.48	80.63	10.57	0.14	8.43	80.85	10.70	11.01	9.71	1.30	195.98	18.05
	Anova test															
	F	1.70	1.58	1.68	4.15	1.55	1.74	0.79	2.04	1.56	1.73	2.21	2.05	1.77	1.95	1.70
	Sig. at $P < 0.01$	0.005	0.013	0.006	0.000	0.017	0.004	0.858	0.000	0.016	0.004	0.000	0.000	0.003	0.001	0.017
	Median test															
	χ-quadrato	97.45	108.85	101.66	126.05	119.314	125.74	69.33	103.72	115.31	121.74	103.72	97.50	115.51	94.23	85.75
	Sig. at $P < 0.01$	0.042	0.006	0.022	0.000	0.001	0.000	0.663	0.016	0.002	0.001	0.016	0.041	0.002	0.066	0.186
–	Kruskal–Wallis test															
–	χ^2	99.72	105.74	104.97	137.17	112.06	122.70	73.20	118.06	112.27	122.62	117.67	113.43	127.67	102.07	101.28
–	Sig. at $P < 0.01$	0.03	0.01	0.01	0.00	0.00	0.00	0.54	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02

Data are the mean values of 2008 and 2009 years

FR France; *GR* Greece; *IT* Italy; *PT* Portugal; *SL* Slovenia; *SP* Spain

SC Site of cultivation

C16:0 palmitic acid; *C16:1* palmitoleic acid; *C18:0* stearic acid; *C18:1* oleic acid; *C18:2* linoleic acid; *C18:3* linolenic acid

SFA Saturated Fatty Acids; *MUFA* Mounsatured Fatty Acids; *PUFA* Polyunsaturated Fatty Acids; *UFA* Unsatured Fatty Acids; *SI* stability index: (SFA/UFA) x (α-tocopherolcontent).*NB add c also su UFA*

with 20 ml 2 M ethanolic KHO and ascorbic acid (5 ml 0.1 M) using an Incubator 1000/Promasx 1200. The mixture was filtered and treated with 10 ml saturated NaCl, 15 ml *n*-hexane containing 5 mg l^{-1} butylated hydroxytoluene. After separation, the organic phase was collected and filtered through anhydrous sodium sulphate. The aqueous layer was re-extracted with 5 ml *n*-hexane, and added to the first one and dried in Rotovapor R-114 at 50 °C. The residue was then dissolved in 3 ml 100 % methanol and filtered (0.45 µm nylon syringe membrane). Tocopherol isomer determinations were performed using a PerkinElmer Multisolvent HPLC equipped with a double piston pump and an UV detector. The chromatographic conditions were as follows: sample injected, 10 µl; column Phenomenex Luna (3 µm C8 (2), 150 × 2 mm); temperature, 40 °C; mobile phase, acetonitrile water mixture (95:5) at 40 °C with flow rate of 0.4 ml min^{-1}; detection 295 nm for tocopherol isomers and 208 nm for tocopherol acetate. Calibration curves were drawn to quantify all isomers. For each sample the tocopherol content was calculated as the mean value of two replicates from the saponification process and expressed as mg kg^{-1} oil.

Statistical analysis

The statistical analyses were carried out with the package program SPSS (version 17), and the software SAS (version 9.1). Parametric tests (One Way Analysis of Variance, paired *t* test) were applied when the assumption of normality and homoscedasticity of the samples were met. However non parametric tests (Kruskal–Wallis *H*, Wilcoxon tests) were used to compare the results reinforcing data interpretation (Lehmann 1975). The correlation coefficients and their statistical significances were assessed by Pearson's correlation analysis. PCA was used to reduce the complexity of the original variables and to detect a reliable structure in the relationship between variables, starting from analysis of correlation matrix among fruit quality measurements and genotypes (Iezzoni and Prittis 1991). Following PCA results, cluster analysis was also applied to identify groups of similar cases on the basis of the new set of orthogonal synthetic variables.

Results and discussion

Genotypic variability of total oil content and fatty acid profiles

The mean values of oil contents and the fatty acid profiles of the oils extracted from 75 accessions of European hazelnut germplasm are summarised in Table 1.

The ANOVA test and the corresponding non parametric tests, the Median and Kruskal–Wallis tests, were performed on the data set to assess the variability among accessions. Thus, based on the statistical tests, significant differences (for $P < 0.001$) were found for oil content and for most of fatty acids contents except for linoleic acid; no significant differences were found for α-tocopherol content and stability index (SI) on the basis of the median test.

The average amount of oil on dry weight was 56.95 %, ranging from 41.96 % in 'Gunslebert' to 63.73 % in 'Locale di Piazza Armerina'. The total oil content was >60 % in 21 accessions, including 14 Italian cultivars ('Avellana Speciale', 'Barrettona', 'Carrello', 'Camponica', 'Comune di Sicilia', 'Lunga di Ginnasi', 'Meloni', Nociara', 'Nocchione', 'Locale di Piazza Armerina', 'Riccia di Talanico', 'San Giovanni', 'Tonda bianca', 'Tonda Calabrese', 'Tonda Gentile Romana'). On the other hand the oil content in 9 % of the accessions was below 50 % ('Corylus maxima pellicule rose', 'Daria', 'Feriale', 'Gunslebert', 'Karydato', 'San Vicino', 'Segorbe', 'Tonda di Biglini').

Among the major fatty acids, oleic was, by far, the predominant one in all the hazelnut oils, ranging from 73.31 % in 'Longue d'Espagne' to 84.56 % in 'Avellana Speciale' with an average value of 80.63 %. Significant differences were found in the oleic content among the samples; 49 accessions were characterized by C18:1 content >80 %.

The linoleic acid, whose content was inversely correlated with oleic, showed a mean value of 10.57 % and pronounced differences among accessions. The lowest content was found in the ecotype 'Meloni' (5.91 %) and the highest in 'Longue d'Espagne' (19.01 %). Moreover 35 accessions were characterised by a linoleic content superior than the mean value. Significant differences were also found for palmitic (mean value of 5.95 %) and stearic acid (mean value

of 2.48 %); which represent the total hazelnut SFA. In our samples their amount ranged from 4.90 to 9.02 % and from 1.55 to 3.68 % respectively. These ranges were larger than those reported by other authors for hazelnut accessions and hybrids (Cristofori et al. 2008; Xu and Hana 2010); nevertheless their mean values were in agreement with those indicated by the cited authors.

Oleic and linoleic acids contributed for over 89 % to the total fatty acid composition in all of the analysed accessions. As suggested by Kodad et al. (2011), their ratio is considered an important criterion to evaluate kernel quality. As compared to other nut and vegetable oils, hazelnut oil has been reported to contain the highest proportion of oleic acid (Alasalvar et al. 2003). On the other hand, the hazelnut oils contain low quantities of palmitoleic (0.14–0.32 %) and linolenic (0.55–0.45 %) acid. These fatty acids, even in traces, can negatively affect nut storability because of their low stability (Cristofori et al. 2008).

As expected, MUFA made up the largest portion, with a mean value of 80.85 % of the fatty acids, followed by PUFA (10.70 %). SFA was the minor component and accounted for only 8.43 % of the total fatty acids. This is in line with the results reported by Parcerisa et al. (1995); Erdogan and Aygun (2005); Amaral et al. (2006a, b). A high level of MUFA and a low quantity of SFA in hazelnut oil enhance its usefulness in food as well as oleochemical applications (Xu et al. 2007).

Oils having higher levels of PUFA are subjected to oxidative change, because the oxidative rates of linoleic and linolenic acids are 100–200 times greater than stearic acid and 10–20 times greater than oleic acid (Pershern et al. 1995). The mean value of the SFA in oil of the European germplasm was very low (mean value 8.43 %) in comparison to Nebraska hybrids (27 %) as reported by Hu and Hanna (2010).

The mean values of the ratios UFA/SFA, MUFA/SFA and PUFA/SFA were 11.01; 9.71 and 1.30, respectively. High UFA/SFA ratios improve the nutritional quality of processed foods when hazelnuts are added. However, it is worth noting that the presence of high amounts of unsaturated fatty acids, especially PUFA in oil, contributes to reduce shelf life. The average of UFA/SFA ratio in the oil of the European germplasm was higher (mean value 11.01) than the values reported by Özdemir et al. (2001) and Alasalvar et al. (2003) in the same commercial and new Turkish cultivars and lower than those reported by Xu and Hanna, (2010) in Nebraska hazelnut hybrids.

Among the three major tocopherols, α-tocopherol was the dominant form (Table 1) representing approximately 97.5 % of the total tocopherols identified as previously reported by other authors (Sivakumar et al. 2005 and Sivakumar and Bacchetta 2006). Moreover α-tocopherol is the most active form in human and animal tissues (Kornsteiner et al. 2006; Alasalvar et al. 2009) due to the substitution pattern of the methyl groups on the chromanol ring making the hydrogen of the C-6 hydroxyl group especially active, i.e., facilitating the transfer of the hydrogen to a peroxyl radical (Sivakumar and Bacchetta 2005; Sivakumar et al. 2005). The median test indicated that no significant differences were found among the α-tocopherol patterns in the European hazelnut cultivars, even if this result was in contrast with ANOVA and Kruskal–Wallis Test. The average content of 75 European hazelnut accessions was 195.98 ppm ranging from 102.34 ppm in 'Tombul Giaghli' to 390.97 ppm in 'Cosford'. About 30 % of the analysed accessions showed a α-tocopherol content >200 ppm.

In this study, the SI (Table 1), was calculated by multiplying the ratio of SFA/UFA and α-tocopherol content, and was used to predict the oxidative stability of the hazelnut oils as reported by other authors (Özdemir et al. 2001). The oil oxidative stability is affected by the presence of high levels of natural antioxidants, especially α, and monounsaturated fatty acids and it is inversely correlated to linoleic acid content (Zacheo et al. 2000). According to the median test, no significant differences were found among cultivars. This result was in contrast with the ANOVA and Kruskal–Wallis Test. SI mean value of hazelnut European germplasm was 18.05 ranging from 9.54 in cv 'Raul' to 33.70 in cv 'Gunslebert'. SI values were approximately two or three times lower than those of the commercial Turkish hazelnuts and their new hybrids which had SI values ranging from 37.5 to 62.69 when the same unit was used for α-tocopherol content (Özdemir et al. 2001). However 21 accessions of the European germplasm were characterised by SI value >20 in particular three cultivars ('Riccia di Talanico', 'Gunslebert', 'Cosford') showed SI value >30 due to their high α-tocopherol content. However SI values appear to be more related to the presence of natural antioxidants such as α-tocopherol rather than

Table 2 Correlation between oil content (percentage of kernel dry weight) and the different fatty acids concentrations (percentage of oil total fatty acids content) in the kernels of 75 hazelnut accessions as mean values of 2008 and 2009 crop years

Variables	Oil content %	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	O/L	SFA	Total MUFA	Total PUFA	UFA/ SFA	MUFA/ SFA	PUFA/ SFA	α-Tocoferol
C16:0	−0.14														
C16:1	0.01	0.682**													
C18:0	0.21	0.256*	0.01												
C18:1	0.495**	−0.279*	−0.239*	0.20											
C18:2	−0.473**	−0.03	0.06	−0.458**	−0.934**										
C18:3	−0.480**	0.01	−0.05	−0.228*	−0.380**	0.393**									
O/L	0.481**	0.03	−0.07	0.443**	0.884**	−0.948**	−0.407**								
SFA	0.02	0.851**	0.492**	0.726**	−0.09	−0.268*	−0.12	0.264*							
Total MUFA	0.496**	−0.270*	−0.226*	0.21	1.000**	−0.936**	−0.382**	0.886**	−0.08						
Total PUFA	−0.479**	−0.03	0.05	−0.460**	−0.934**	1.000**	0.409**	−0.948**	−0.268*	−0.936**					
UFA/SFA	−0.06	−0.777**	−0.472**	−0.783**	0.04	0.308**	0.15	−0.307**	−0.979**	0.03	0.308**				
MUFA/SFA	0.08	−0.806**	−0.517**	−0.675**	0.344**	0.00	0.03	−0.02	−0.940**	0.338**	0.00	0.951**			
PUFA/SFA	−0.411**	−0.278*	−0.10	−0.643**	−0.779**	0.947**	0.381**	−0.888**	−0.548**	−0.783**	0.948**	0.584**	0.306**		
α-Tocoferol	−0.23	−0.22	−0.331**	−0.06	−0.08	0.13	0.16	−0.16	−0.19	−0.08	0.14	0.18	0.15	0.15	
SI	−0.275*	0.17	−0.15	0.15	−0.17	0.08	0.17	−0.11	0.20	−0.17	0.09	−0.18	−0.20	−0.01	0.902**

C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; O/L, oleic acid/linoleic acid; MUFA (Monounsatured fatty acids), palmitoleic + oleic; PUFA (polyunsaturated fatty acids), linoleic + linolenic; SFA (saturated fatty acids), palmitic + stearic; SI (stability index), (SFA/UFA) × (α-tocopherol content)

* Statistical significance at the 5 % level, ** statistical significance at the 1 % level

to the fatty acid composition of the oil, as reported by Xu and Hanna (2010).

Correlation among variables

All variables were jointly examined and their correlation coefficients are shown in Table 2. Positive correlations were found between oil content and the percentage of oleic, oleic/linoleic and MUFA content as other authors reported in almond (Font I Forcada et al. 2011). Positive correlations were also associated to palmitic, palmitoleic and stearic acids, whereas a negative correlation was observed with oleic acid but not with the ratio oleic/linoleic as reported by Kodad et al. (2011) in almond. The percentage of palmitoleic acid was inversely correlated with α-tocopherol content which was not related to the other variables. The stearic acid content resulted inversely correlated with linoleic and linolenic acid contents and thus positively correlated with the ratio oleic/linoleic. A highly significant negative correlation was found between the oleic and linoleic acids. As previous reported in other crops such as almond under different environmental conditions (Kodad and Socias i Company 2006), this negative correlation may be explained by the enzymatic activity of desaturase which converts oleic acid to linoleic (Garcia et al. 1992). Furthermore, correlation coefficients greater than 0.71 or smaller than −0.71 have been suggested to be biologically meaningful (Skinner et al. 1999) showing that this correlation is not influenced by climatic and environmental conditions and it is genotype-dependent (Kodad et al. 2011). Thus the selection for one of these fatty acids could negatively modify the amount of the others. The negative correlation between oil content and linoleic acid amount ($r^2 = -0.473$) would allow to select accessions with high oil content and low in linoleic acid.

Influence of harvest year and growing region

Table 3 depicts the differences of mean values of fatty acids, α-tocopherol, SI with pair wise comparison between harvest years 2008 and 2009. On the basis of Student's test, significant differences between the 2 years of harvest were found for fatty acids content, except for linoleic acid, the ratio PUFA/SFA, α-tocopherol and SI. The Wilcoxon's test confirmed that PUFA/SFA ratios and α-tocopherol content were not significantly different between the two harvests. Significant differences in oil amount between the 2 years of cultivation, was confirmed by the two statistical tests. Evaluating oil content of 73 almond cultivars in the same years, Kodad et al. (2011) did not report any 'year effect' on this variable, underlining a significant difference only for the interaction '*year x cultivar*'. However the year effect has been reported to be significant in some other studies (Parcerisa et al. 1995; Cristofori et al. 2008). This discrepancy could be the result of specific climatic conditions of the year tested (Socias i Company et al. 2008). Thus these differences in fatty acid composition should be dependent on climate conditions during the growing season, in particular during the summer months, as reported in pistachio by Arena et al. (2007). In 2009 the temperature were higher than in 2008 especially from May to October

Table 3 Comparison between mean values (%) of oil content, fatty acids, α-tocopherol and SI in the kernels of 75 accessions in harvest years 2008 and 2009

Variables (%)	Differences 2009–2008	Student *t*	Wilcoxon *t*
Total oil	1.66	2.24*	−2.79*
C16:0	0.44	4.30***	−4.62***
C16:1	0.03	4.13***	−3.77***
C18:0	0.11	2.96***	−3.02***
C18:1	−1.29	−3.01***	−4.26***
C18:2	0.52	1.25	−2.99***
C18:3	0.02	2.35*	−2.93***
SFA	0.55	4.83***	−5.04***
MUFA	−1.26	−2.96***	−4.25***
PUFA	0.55	1.30	−3.03***
UFA/SFA	−0.78	−5.60***	−5.17***
MUFA/SFA	−0.77	−6.11***	−5.79***
PUFA/SFA	−0.02	−0.32	−0.71*
α-Tocopherol	4.14	0.50	−0.50
SI	1.90	1.95	−2.19*

C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; MUFA (Mono-unsaturated fatty acids) = palmitoleic + oleic; PUFA (polyunsaturated fatty acids) = linoleic + linolenic; SFA (saturated fatty acids) = palmitic + stearic; SI (stability index) = (SFA/UFA) × (α-tocopherol content)

* $P < 0.050$; ** $P < 0.01$; *** $P < 0.001$

Table 4 Mean value, index number (mean = 100), SD and variation coefficients of the lipid fraction from 75 European hazelnut accessions

Variables	Statistical index	Sites of cultivation							
		France	Greece	Italy-ENEA	Italy-UNITO	Portugal	Slovenia	Spain	Total
Oil content %	Mean	51.5	56.8	60.8	54.9	58.2	59.3	55.9	57.0
	Index number (mean = 100)	90.5	99.8	106.7	96.4	102.1	104.1	98.1	100
	Standard deviation	5.3	4.1	4.7	7.5	5.4	5.6	5.7	6.3
	Variation coeff.	0.10	0.07	0.08	0.14	0.09	0.10	0.10	0.11
C16:0	Mean	5.9	6.7	5.8	6.1	6.0	5.7	6.1	6.0
	Index number (mean = 100)	98.4	113.3	97.1	101.9	100.7	96.0	102.7	100
	Standard deviation	1.1	0.6	0.5	0.5	0.6	0.9	0.6	0.8
	Variation coeff.	0.19	0.08	0.09	0.08	0.11	0.16	0.10	0.13
C16:1	Mean	0.21	0.27	0.19	0.23	0.23	0.21	0.25	0.22
	Index number (mean = 100)	94.2	122.1	88.8	104.0	105.5	96.4	113.7	100
	Standard deviation	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1
	Variation coeff.	0.16	0.15	0.20	0.20	0.18	0.41	0.16	0.23
C18:0	Mean	2.3	3.0	2.7	2.6	2.5	2.4	1.9	2.5
	Index number (mean = 100)	94.0	120.1	110.5	104.4	100.2	95.0	77.1	100
	Standard deviation	0.5	0.4	0.4	0.4	0.4	0.3	0.3	0.5
	Variation coeff.	0.21	0.13	0.14	0.16	0.18	0.13	0.17	0.20
C18:1	Mean	78.9	80.0	82.3	82.2	80.0	81.3	79.3	80.6
	Index number (mean = 100)	97.8	99.3	102.1	102.0	99.2	100.9	98.3	100
	Standard deviation	4.0	1.7	3.1	1.3	2.5	2.4	4.3	3.4
	Variation coeff.	0.05	0.02	0.04	0.02	0.03	0.03	0.05	0.04
C18:2	Mean	12.5	9.9	8.8	8.8	11.1	10.3	12.4	10.6
	Index number (mean = 100)	118.3	93.3	83.7	82.9	105.2	97.2	117.0	100
	Standard deviation	3.8	2.1	3.0	1.3	2.1	1.8	4.7	3.4
	Variation coeff.	0.30	0.21	0.34	0.15	0.19	0.18	0.38	0.32
C18:3	Mean	0.18	0.09	0.11	0.17	0.14	0.15	0.10	0.14
	Index number (mean = 100)	131.9	68.9	79.4	122.9	103.6	110.4	73.3	100
	Standard deviation	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.1
	Variation coeff.	0.57	0.31	0.44	0.62	0.80	0.27	0.45	0.61
SFA	Mean	8.2	9.7	8.5	8.7	8.5	8.1	8.0	8.4
	Index number (mean = 100)	97.1	115.3	101.0	102.7	100.5	95.7	95.2	100
	Standard deviation	1.3	0.7	0.6	0.8	1.0	1.0	0.8	1.0
	Variation coeff.	0.16	0.08	0.07	0.09	0.12	0.13	0.10	0.12
MUFA	Mean	79.1	80.3	82.5	82.5	80.2	81.5	79.5	80.8
	Index number (mean = 100)	97.8	99.3	102.1	102.0	99.2	100.8	98.3	100
	Standard deviation	4.0	1.7	3.1	1.3	2.5	2.4	4.3	3.4

Table 4 continued

Variables	Statistical index	Sites of cultivation							
		France	Greece	Italy-ENEA	Italy-UNITO	Portugal	Slovenia	Spain	Total
	Variation coeff.	0.05	0.02	0.04	0.02	0.03	0.03	0.05	0.04
PUFA	Mean	12.7	9.9	9.0	8.9	11.3	10.4	12.5	10.7
	Index number (mean = 100)	118.5	92.9	83.6	83.4	105.1	97.3	116.5	100
	Standard Deviation	3.9	2.1	3.1	1.4	2.2	1.9	4.7	3.4
	Variation coeff.	0.30	0.21	0.34	0.15	0.19	0.18	0.38	0.32
UFA/SFA	Mean	11.4	9.3	10.8	10.6	10.9	11.6	11.6	11.0
	Index number (mean = 100)	103.7	84.8	98.1	96.7	99.4	105.1	105.2	100
	Standard deviation	1.4	0.8	0.9	1.0	1.4	1.5	1.3	1.3
	Variation coeff.	0.12	0.09	0.08	0.10	0.13	0.13	0.11	0.12
MUFA/SFA	Mean	9.8	8.3	9.7	9.6	9.6	10.3	10.0	9.7
	Index number (mean = 100)	101.2	85.5	100.3	98.8	98.8	105.7	102.8	100
	Standard deviation	1.2	0.7	0.9	0.9	1.3	1.4	1.0	1.2
	Variation coeff.	0.13	0.08	0.09	0.09	0.13	0.14	0.10	0.12
PUFA/SFA	Mean	1.6	1.0	1.1	1.0	1.3	1.3	1.6	1.3
	Index number (mean = 100)	122.2	79.8	81.5	80.6	103.7	100.5	123.6	100
	Standard deviation	0.6	0.3	0.4	0.2	0.3	0.2	0.7	0.5
	Variation coeff.	0.35	0.25	0.33	0.21	0.22	0.19	0.45	0.37
α-Tocoferol	Mean	263.5	126.4	195.3	209.6	160.7	198.4	156.4	196.0
	Index number (mean = 100)	134.4	64.5	99.7	107.0	82.0	101.2	79.8	100
	Standard deviation	82.7	22.7	59.5	55.6	35.0	53.6	39.8	69.6
	Variation coeff.	0.31	0.18	0.30	0.27	0.22	0.27	0.25	0.36
SI	Mean	24.1	13.7	18.1	19.6	14.9	17.6	13.5	18.1
	Index number (mean = 100)	133.3	75.7	100.5	108.8	82.6	97.5	74.8	100
	Standard deviation	10.9	2.9	5.4	4.9	3.7	6.0	2.8	7.3
	Variation coeff.	0.45	0.21	.30	0.25	0.25	0.34	0.21	0.40

(http://www.ecmwf.int/research/era/do/get/index). No significant differences were found for α-tocopherol and SI between the two harvests. Parcerisa et al. (1995) reported significant differences in vitamin E content among three consecutive years of production (1990–1991 and 1992) of four hazelnut cultivars. Anyway some evidences (Sivakumar et al. 2005; Lotti et al. 1985; Izquierdo et al. 1985) even in this case, suggested that the differences in tocopherol content should be dependent on climatic conditions during the growing season, in particular during the summer months.

In Table 4 the results of descriptive statistical analysis (mean value, index number (mean = 100), SD and variation coefficients) on the lipid fraction from 75 European hazelnut accessions, grouped on the basis of their sites of cultivation, are shown. The results showed that there were differences in the mean values of the genetic pool from Central Italy (mean value 60.8 % ± 4.7), Slovenia (mean value 59.3 % ± 5.6) and Portugal (mean value 58.2 % ± 5.4), compared to those from Greece (mean value 56.8 % ± 4.1), Spain (mean value 55.9 % ± 5.7) and France

(mean value 51.5 % ± 5.3). Oleic acid and MUFA were abundant in accessions grown in Italy and Slovenia with a low coefficient of variation among cultivars from the same place of cultivation. These results are in agreement with other studies, where climate and latitude of cultivation affect the degree of fatty acid unsaturation and the oil content in olive (Lotti et al. 1985), almond (Kodad et al. 2010) and other oilseed plants such as sunflower (Izquierdo et al. 1985).

Principal component (PCA) and cluster analyses

In this work both PCA and cluster analyses were applied on fatty acids profiles from 75 hazelnut accessions in order to study their associations and to assess an efficient method of selection.

The factor loadings of three principal components (PCs) elaborated from the data of kernel composition are shown in Table 5. The three PCs account for the 82.5 % of the total variability at *P* value, with a confidence level higher than 95 %. Total oil content, oleic and linoleic acids were primarily responsible for the separation on PC1 (accounting for 38.39 % of total variance), PC2 (accounting for 30.26 % of the variance) was highly correlated to palmitic and palmitoleic acids, whereas the third component (accounting for 13.90 % of the variance) was associated with α-tocopherol content and SI.

Using PC1, PC2 and PC3 as synthetic variables the accessions were clustered in six clusters (Fig. 1). Cluster I and IV included 6 cultivars, cluster II only 1, cluster III and V were the largest ones with 26 cultivars, the last (VI) contained 10 cultivars.

When samples were plotted for PC1 and PC2 (Fig. 2) accessions were separated on the basis of their lipidic profile. Cultivars of Cluster I, which had positive values of PC1 and PC2, showed relatively low levels of total oil content and oleic acid and high values of linoleic and palmitoleic acids. Cluster II comprised only 'Gunslebert' shown as an out-liner on the basis of its low level in total oil content and high level of C16:0. Cluster III, grouped accessions which showed positive value of PC1, as a consequence of low total oil and oleic contents, but negative values of PC2 (low level of SFA). Cluster IV included cultivars characterized by fair oleic and total oil content an variable amount of SFA. Cluster V comprised cultivars with negative values of PC1 and PC2. Accessions with negative values on PC1 and positive on PC2, on the basis of their SFA level, were grouped in cluster VI. The PCA results confirmed that oleic and linoleic acids contents are useful parameters for quality characterization of hazelnut cultivars, as reported for almond by Kodad et al. (2010). On the bases of our results, groups V and VI included germplasm of interest for the fatty acids profile, and useful for future breeding programs aimed at increasing oil stability and nutritional value in hazelnut kernels. Furthermore this work allowed the identification of interesting traits not only in the most important widespread cultivars, but also in landraces present at low frequencies in the major areas of cultivation and conserved on farm, such as 'Dal Rosso', 'and 'Tonda di Biglini', surveyed in Northwestern Italy, 'Ada', Barrettona', 'Centenaria di Ginnasi', and 'Meloni' in Central Italy (*Latium*). Thus the recovery and exploitation of landraces imply not only the enlargement of the genetic basis of the cultivated germplasm providing useful genes but also the offer of new economic possibilities for local markets and potential industrial implementations.

Table 5 Factor loadings of three principal component (PC) axes of kernel composition after principal component analysis of 75 hazelnut accessions[a]

Variable	PC1	PC2	PC3
Oil content %	−0.514	−0.329	−0.259
C16:0	−0.302	0.833	−0.102
C16:1	−0.16	0.597	−0.433
C18:0	−0.68	0.381	0.24
C18:1	−0.737	−0.649	0.068
C18:2	0.918	0.343	−0.095
C18:3	0.485	0.178	0.185
SFA	−0.585	0.800	0.058
Total MUFA	−0.742	−0.642	0.062
Total PUFA	0.921	0.343	−0.09
UFA/SFA	0.625	−0.765	−0.065
MUFA/SFA	0.362	−0.913	−0.03
PUFA/SFA	0.980	0.041	−0.125
α-Tocoferol	0.258	−0.084	0.910
SI	0.087	0.259	0.922
Factor loadings	5.759	4.538	2.085
Proportion of total variation (%)	38.395	30.257	13.897

[a] Eigen values and their contribution to the total variation are listed at the bottom of the columns

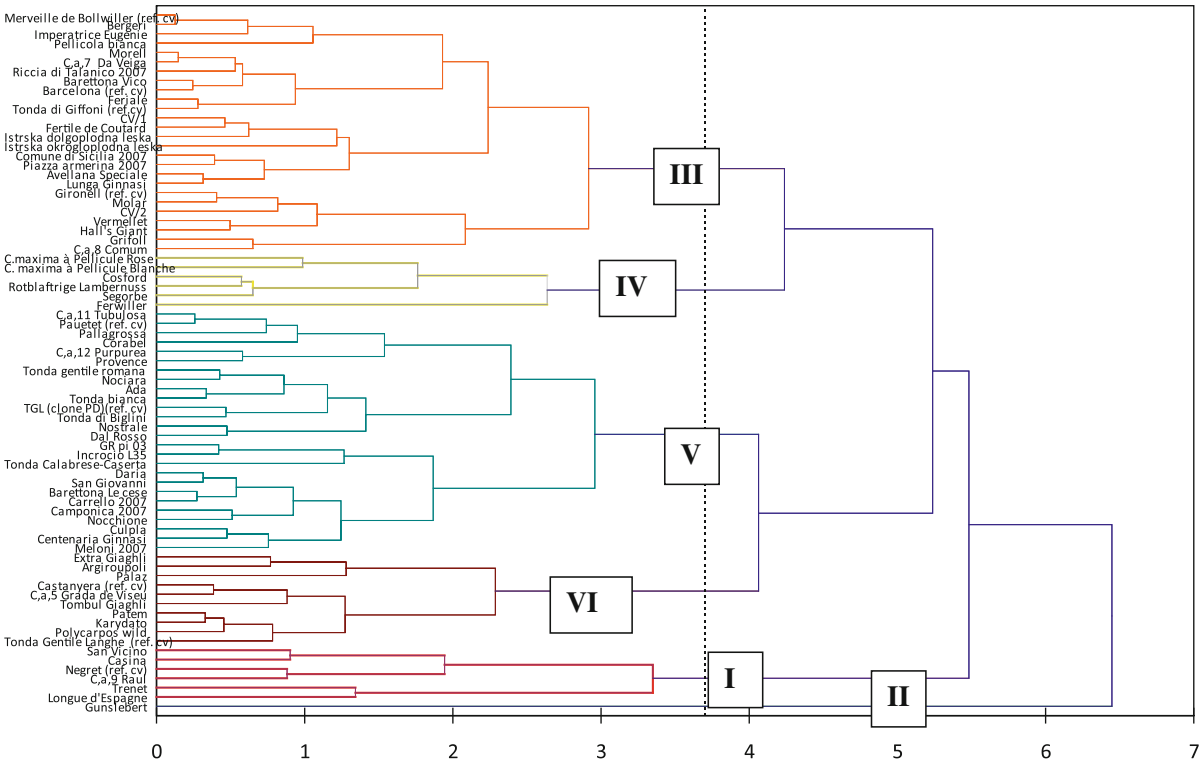

Fig. 1 UPGMA dendrogram derived from Euclidea Distances of similarity showing the relationship among 75 hazelnut accessions on the bases of the 3 PCs axes elaborated by PCA from the kernel composition

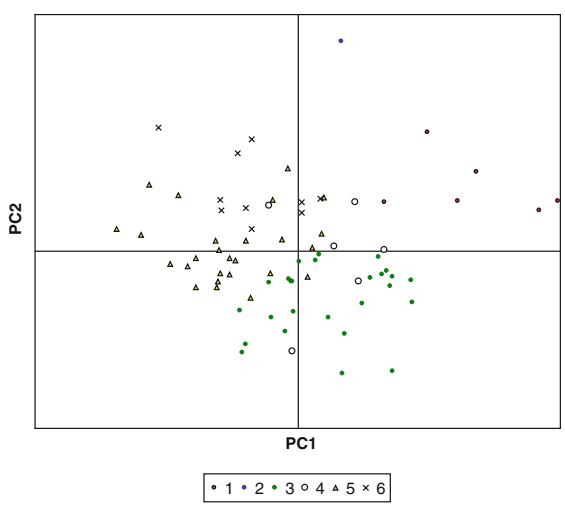

Fig. 2 Position of the principal component (PC1 and PC2 position) scores of the hazelnut kernel composition for 75 hazelnut kernel accessions. The numbers refers to the 6 clusters of the dendrogram

Conclusions

The lipid fraction is a key factor in determining the hazelnut quality and storability, affecting the taste and the nutritional properties of the ready-to-eat products as well as those from further processing. Moreover numerous evidences highlighted its beneficial effect on human health. Thus the enhancement of cultivars with high-quality attributes meet the demand of hazelnut confectionary industries and consumers with positive implication on the competitiveness of the European products in the international market. This aspect is of relevant importance considering that Turkish supply accounts for more than 80 % of the world hazelnut trade largely determining the world export prices.

The large number of the accessions considered in this research showed the great variability in the European germplasm, which can be very useful to identify genotypes characterized by high oil content

and stability in fatty acids profiles. This information is also valuable for the industry to choose the adequate cultivars for confectionary, cosmetic and pharmaceutical processes. Correlation coefficients, which are bivariate relations, provide the basic information on the direct and indirect selection of traits allowing the establishment of a proper strategy for the breeding programs. The high negative correlation between oleic acid and linoleic acid found in our germplasm ($r^2 = -0.934$), indicated the possibility of an indirect selection for high oleic acid and low linoleic acid. This aspect is very important in hazelnuts to evaluate kernel stability against oxidation.

The integrate application of both PCA and clustering analyses, discriminated the cultivars allowing the identification of homogenous groups characterised by different fatty acids profiles. This procedure is very useful, not only to identify the most interesting cultivars and their proper uses, but also as first step towards the definition of a reference 'core collection' (Brown 1989). Nevertheless since several authors have shown the influence of environmental factors on fatty acids composition of oilseeds and nuts, a further step will be to confirm the results studying the expression of lipid profiles in cultivars grown in the same environment conditions. However the results of this research proving data on the fatty acid profiles and α-tocopherol content of a large number of cultivars from different countries, lay the basis for further research on these issues contributing to enhance the quality and the commercial value of the hazelnuts in Europe.

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References

Alasalvar C, Shahidi F, Liyanapathirana CM, Ohshima T (2003) Turkish Tombul hazelnut (*Corylus avellana* L.). 1. Compositional characteristics. J Agric Food Chem 51: 3790–3796

Alasalvar C, Amaral JS, Satir G, Shahidi F (2009) Lipid characteristics and minerals of native Turkish hazelnut essential varieties (*Corylus avellana* L.). Food Chem 113(4): 919–925

Amaral JS, Casal S, Citova I, Santos A, Seabra RM, Oliveira BPP (2006a) Characterization of several hazelnuts (*Corylus avellana* L.) cultivars based on chemical, fatty acid and sterol composition. Eur Food Res Technol 222:274–280

Amaral JS, Cunha SC, Santos A, Alves MR, Seabra RM, Oliveira BP (2006b) Influence of cultivar and environment conditions on the triacylglycerol profile of hazelnut (*Corylus avellana* L.). J Agric Food Chem 54:449–456

Arena E, Campisi S, Fallico B, Maccarone E (2007) Distribution of fatty acids and phytosterols as a criterion to discriminate geographic origin of pistachio seeds. Food Chem 104(1): 403–408

Bacchetta L, Di Giovanni B (2012) The enhancement of hazelnut and almond genetic resources through the European AGRI GEN RES SAFENUT Action. The ENEA experience as project coordinator. Rivista on line EAI N1:15–17

Bacchetta L, Avanzato D, Botta R, Boccacci P, Drogouti P, Metzidakis I, Rovira M, Silva AP, Solar A, Spera D, Di Giovanni B (2008) First results of SAFENUT: a European project for the preservation and utilization of Hazelnut local genetic resources. Acta Horticulturae 845:30–34

Bacchetta L, Spera D, Avanzato D, Botta R, Boccacci P, Di Giovanni B, Drogoudi P, Duval H, Metzidakis, I, Rovira M, Silva AP, Socias R, Solar A (2010) European Hazelnut and Almond genetic resources: results and perspectives of networking SAFENUT AGRI GEN RES activities. In: IHC Science and Horticulture for people—28th International Horticultural Congress, Lisboa (Portugal), 22–27 Aug Abstract vol 2, Symposia. p 546

Bacchetta L, Di Giovanni B, Rovira M, Spera D, Avanzato D, Botta R, Boccacci P, Drogoudi P, Duval H, Metzidakis I, Silva AP, Socias R, Solar A (2011) The AGRI GEN RES 'SAFENUT' Action: a European strategy for the preservation and utilization of hazelnut and almond genetic resources. *Nucis* N 15

Bacchetta L, Avanzato D, Di Giovanni B, Botta R, Boccacci P, Drogoudi P, Metzidakis I, Rovira M, Sarraquigne JP, Silva AP, Solar A, Spera D (2012) The re-organization of European hazelnut genetic resources in the SAFENUT (AGRI GEN RES) Project. VIII International Congress on Hazelnut Chile

Balta MF, Yarilgac T, Askin MA, Kuçuk M, Balta F, Ozrenk K (2006) Determination of fatty acid compositions, oil contents and some quality traits of hazelnut genetic resources grown in eastern Anatolia of Turkey. J Food Compos Anal 19:681–686

Boccacci P, Akkak A, Botta R (2006) DNA-Typing and genetic relationships among European hazelnut (*Corylus avellana* L.) cultivars using microsatellite markers. Genome 49: 598–611

Boccacci P, Rovira M, Botta R (2008) Genetic diversity of hazelnut (*Corylus avellana* L.) germplasm in Northeastern Spain. HortScience 43(3):667–672

Botta R, Gianotti C, Me G (1997) Kernel quality in hazelnut cultivars and selections analysed for sugars, lipids and fatty acid composition. Acta Horticulturae 445:319–326

Brown AHD (1989) The case for core collections. In: Brown AHD, Frankel OH, Marshall DR, Williams JT (eds) The use of plant genetic resources. Cambridge University Press, Cambridge, Stralinger P, pp 135–156

Cantini C, Cimato A, Sani G (1999) Morphological evaluation of olive germplasm present in Tuscany region. Euphytica 109:173–181

Cristofori V, Ferramondo S, Bertazza G, Bignami C (2008) Nut and kernel traits and chemical composition of hazelnut (*Corylus avellana* L.) cultivars. J Sci Food Agric 88(6):1091–1198

Dietrich M, Traber MG, Jacques PF, Cross CE, Hu Y, Block G (2006) Does γ-tocopherol play a role in the primary prevention of heart disease and cancer? A review. J Am Coll Nutr 25(4):292–299

Drogoudi PD, Pantelidis G, Bacchetta L, De Giorgio D, Duval H, Metzidakis I, Spera D (2012) Protein and mineral nutrient contents in kernels from 72 sweet almond cultivars and accessions grown in France, Greece and Italy. Int J Food Sci Nutr. doi:10.3109/09637486.2010.523415

Erdogan V, Aygun A (2005) Fatty acid composition and physical properties of Turkish tree Hazelnuts. Chem Nat Compd 41(4):378–381

Font i Forcada C, Kodad O, Juan T, Estopañan G, Socias i Company R (2011) Genetic variability and pollen effect on the transmission of the chemical components of the almond kernel. Span J Agric Res 9(3):781–789

Fraser GE (2009) Vegetarian diets: what do we know of their effects on common chronic diseases? Am J Clin Nutr 89:1607S–1612S

Garcia JM, Agar IT, Streif J (1992) Fat content and fatty acid composition in individual seeds of pistachio varieties grown in Turkey. Gartenbauwissenschaft 57:130–133

Garcıa-Lopez C, Gran′e-Teruel N, Berenguer-Navarro V, Garcı′a-Garcı′a JE, Martı′n-Carratala ML (1996) Major fatty acid composition of 19 almond cultivars of different origins. A chemometric approach. J Agric Food Chem 46:963–967

Germain E, Sarraquigne JP (1997) Hazelnut training systems: comparison between three systems used on three varieties. Acta Horticulturae 445:237–245

Gurrieri F, Audergon JM, Albagnac G, Reich M (2001) Soluble sugars and carboxylic acids in ripe apricot fruit as parameters for distinguishing different cultivars: use of principle component analysis to characterize apricot fruit quality. Euphytica 117:183–189

Hu YX, Hanna MA (2010) Composition and oxidative stabilities of oils extracted from hybrid hazelnut grown in Nebrasca, USA. Int J Food Sci Tecnol 45:2329–2336

Iezzoni AF, Pritts MP (1991) Applications of principal components analysis to horticultural research. HortScience 26:334–338

Izquierdo NG, Aguirrez′abal LAN, Andrade FH, Cantarero MG (1985) Modelling the response of fatty acid composition to temperature in a traditional sunflower hybrid. Agron J 98:451–461

Kodad O, Socias i Company R (2006) Phenotypic correlation between some agrochemical traits of the almond kernel. Acta Horticulturae 726:259–264

Kodad O, Socias i Company R, Pratsd MS, Lopez Ortiz MC (2006) Variability in tocopherol concentrations in almond oil and its use as a selection criterion in almond breeding. J Hortic Sci Biotechnol 81(3):501–507

Kodad O, Estopañán T, Juan G, Molino F, Mamouni A, Messaoudi Z, Lahlou M, R. Socias i Company (2010) Plasticity and stability in the major fatty acid content of almond kernels grown under two Mediterranean climates. J Hortic Sci Biotechnol 85:381–386

Kodad O, Alonso JM, Espiau MT, Estopañán G, Socias i Company R (2011) Chemometric characterization of almond germplasm: compositional aspects involved in quality and breeding. J Am Soc Hortic Sci 136:273–281

Kornsteiner M, Wagner KH, Elmadfa I (2006) Tocopherols and total phenolics in 10 different nuts types. Food Chem 98:381–387

Lansari A, Iezzoni AF, Kester DE (1994) Morphological variation within collections of Moroccan almond clones and Mediterranean and North American cultivars. Euphytica 78:27–41

Lehmann EL (1975) Nonparametric: statistical method based on ranks. Holden-Day, San Francisco

Lotti G, Izzo R, Riu P (1985) Effects of climate on acid and sterol composition of olive oil. Riv Soc Ital Sci 11:126

Martı′nez-Calvo J, Gisbert AD, Alamar MC, Hernandorena R, Romero C, Lla′cer G, Badenes L (2008) Study of a germplasm collection of loquat (*Eriobotrya japonica* Lindl.) by multivariate ana lysis. Genet Resour Crop Evol 55: 695–703

Mehlenbacher SA (1990a) Advances in hazelnut breeding. Annu Rep North Nut Grow Assoc 80:30–34

Mehlenbacher SA (1990b) Hazelnut varieties for the kernel market. In: Proceedings of Annual Meeting Nut Growers Society of Oregon, Washington and British Columbia, vol 75. p 59–66

Nikolic′ D, Rakonjac V, Milatovic′ D, Fotiric M (2010) Multivariate analysis of vineyard peach (*Prunus persica* (L.) Batsch.) germplasm collection. Euphytica 171:227–234

Özdemir M, Filiz Açkurt F, Kaplan M, Yıldız M, Löker M, Gürcan T, Biringen G, Okay A, Seyhan FG (2001) Evaluation of new Turkish hybrid hazelnut (*Corylus avellana* L.) varieties: fatty acid composition, α-tocopherol content, mineral composition and stability. Food Chem 73(4):411–415

Parcerisa J, Rafecas M, Castellote AI, Codony R, Ferràn A, Garcia J, Gonzalez C, Lopez A, Romero A, Boatella R (1995) Infuence 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 Chem 53:71–75

Pershern AS, Breene WM, Lulai EC (1995) Analysis of factors influencing lipid oxidation in hazelnuts (*Corylus* spp.). J Food Process Preserv 19(1):9–26

Rovira M, Romero M, Clave J (1997) Clonal selection of Gironell and Negret hazelnut cultivars. Acta Hoticulturae 445:145–150

Silva AP, Santos A, Cavalheiro J, Ribeiro C, Santos F, Goncalves B (2007) Fruit chemical composition of six hazelnut cultivars grown in Portugal. J Appl Hortic 9:120–123

Sivakumar G, Bacchetta L (2005) Determination of natural vitamin E from Italian hazelnut leaves. Chem Nat Prod 41(6):654–656

Sivakumar G, Bacchetta L (2006) Alpha-Tocopherol from Italian hazelnut germoplasm. Chem Nat Compd 42(1): 845–848

Sivakumar G, Bacchetta L, Gatti R, Zappa G (2005) HPLC screening of natural vitamin E from mediterranean plant

biofactories a basic tool for pilot-scale bioreactors production of a-tocopherol'. J Plant Physiol 162:1280–1283

Skinner DZ, Bauchan GR, Auricht G, Hughes S (1999) A method for the efficient management and utilization of large germplasm collections. Crop Sci 39:1237–1242

Socias i Company R, Kodak O, Alonzo JM, Gradziel TM (2008) Almond quality: a breeding perspective. Hort Rev 34:197–238

Solar A, Štampar F (1997) First experiences with some foreign hazelnut cultivars (*Corylus avellana* L.) in Slovenia. Acta Horticulturae 445:83–89

Solar A, Štampar F (2011) Characterisation of selected hazelnut cultivars: phenology, growing and yielding capacity, market quality and nutraceutical value. J Sci Food Agric 91(7):1205–1212

Tombesi A, Preziosi P, Boco M (1994) Selection of Tonda Romana and Tonda di Giffoni cross pollinated hazelnut seedlings. Acta Horticolturae 351:119–122

Turcu E, Botu I (1997) Behaviour of some hazelnut varieties in the subcarpathian area of Oltenia-Romania. Acta Horticulturae 445:135–144

Xu YX, Hanna MA (2010) Evaluation of Nebraska hybrid hazelnuts: nut/kernel characteristics, kernel proximate composition, and oil protein properties. Ind Crops Prod 31:84–91

Xu YX, Hanna MA, Josiah SJ (2007) Hybrid hazelnut oil characteristics and its potential oleochemical application. Ind Crops Prod 26:69–76

Zacheo G, Capello MS, Gallo A, Santino A, Capello AR (2000) Changes associated with postharvest ageing in almond seeds. Lebensmittel-Wissenschaft und Tecnologie 33: 415–423