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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

L. Bacchetta[a], M. Aramini[a], A. Zini[a], V. Di Giammatteo[b], D. Spera[b], P. Drogoudic[c], M. Rovira[d], A. P. Silva[e], A. Solar[f], Botta R.[g]

[a]*ENEA, Agenzia Nazionale per le Nuove Tecnologie, l'Energia e lo Sviluppo Economico Sostenibile, Italy*

[b]*Consorzio di Ricerche Applicate alla Biotecnologia (CRAB), Italy*

[c] *National Agricultural Research Foundation – Pomology Institute (NAGREF), Greece*

[d] *Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Spain*

[e] *Universidade de Trás-os-Montes e Alto Douro (UTAD), Portugal*

[f] *Univerza v Ljubljani, Biotehniska Fakulteta, Slovenia.*

[g]*Universita' degli Studi di Torino (UNITO), Italy*

ABSTRACT.

In the frame of SAFENUT AGRIGENRES 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 werethe 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, a-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 nd clustering analyses was developed to identify the most interesting cultivars for breeding programs.

Keywords: Fatty acid composition; α-tocopherol; hazelnut cultivars; PCA; clustering analysis.

Abbreviations

PCA, Principal Component Analysis; PC, Principal Component Axes; SFA, Saturated Fatty Acids; MUFA, Monounsaturated Fatty Acids; UFA, Unsaturated Fatty Acids; PUFA, Polyunsaturated Fatty Acids; UFA/SFA, Unsatured Fatty Acids/ Saturated Fatty Acids; MUFA/SFA, Monounsatured Fatty Acids/ Saturated Fatty Acids; PUFA/SFA, Polyunsaturated Fatty Acids/ Saturated Fatty Acids; O/L, oleic acid/linoleic acid; SI, stability index = (SFA/UFA) x (α-tocopherol content); C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; SC, Site of Cultivation.

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1. 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 lipidic 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. Lipid content and composition is also very important in 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 (Mehelenbacher, 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 (Açkurt et al., 1999; Özdemir and Devres, 1999; Parcerisa, et al. 1993, Pershern et al.,1995; Parcerisa et al., 1995).

In addition, hazelnuts not only provide a definite flavor to food products, but also play a major role in human nutrition and health (Pala et al., 1996; Alphan et al., 1997; Özdemir and Devres, 1999).

Currently, there is a growing interest in developing a healthy diet, which effectively maintains human health and prevents diseases, such as coronary heart disease (CHD) and cancer. During the past two decades, a multitude of human feeding studies have investigated the effect of nut consumption on blood lipids and other cardiovascular disease (CVD) outcomes (Kris-Etherton et al., 1999; Mukuddem, 2005). The findings of these trials support the epidemiologic evidence that frequent nut intake is associated with a reduced risk of coronary heart disease (CHD) (Hu et al., 1999;. Sabaté et al., 2001). Thus consumption of hazelnuts could ameliorates the diet due to their nutritional and nutraceutical value. Hazelnuts are characterized by high contents of mono-unsaturated fatty acids (MUFA) and low amounts of saturated fatty acids (SFA). A diet with high MUFA and low SFA can effectively control blood lipid levels by decreasing total cholesterol and low-density lipid cholesterol and increasing high density lipid cholesterol, thus reducing CHD risk and blood pressure (Fraser et al., 1992; Fraser, 2009; Alphan et al., 1997). In addition, 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 (Awad and Fink, 2000; Bouic, 2001; Dietrich et al., 2006).

Many nut quality characteristics of different hazelnut cultivars and genotypes have been previously identified in Turkey (Ayfer et al., 1997; Bostan, 1997; Balta et al., 1997; Calıs-kan and Cetiner, 1997; Karadeniz et al., 1997; Beyhan and Demir, 2001; Islam and Ozguven, 2001; Okay and Ozenc, 2001; Ko¨ksal, 2002, Balta et al., 2006; Alasalvar et al., 2009), United States of America (McCluskey et al., 1997 Mehlenbacher et al. 1990a and b), Italy (Barata et al., 1994; Tombesi et al., 1994; Botta et al., 1997; Cristofori et al., 2008), France (Germain and Sarraquigne, 1997), Slovenia (Solar and Stampar, 1997), 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; Bacchetta et al., 2012), the fatty acids and the tocopherol profiles of a set of 75 hazelnut accessions were analyses, in order 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 PCA (Principal Component Analysis) 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., *in press*), olive, (Cantini et al., 1999), loquat

(*Eryobotrya japonica* Thunb. Lindl.) (Martı´nez-Calvo et al., 2008), peach (Nikolic´ et al., 2010), and apricot (*Prunus armeniaca* L.) (Gurrieri et al., 2001). These information are substantial to increase the knowledge on the European hazelnut germplasm diversity, its nutritional and healthy value, and to improve 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". This aspect is also of relevant importance considering that Turkish supply accounts for more than 80% of the world hazelnut trade largely determining the world export prices. Thus, the specific objectives of this study were:

i. The investigation of the variability of total oil content and fatty acid profiles in 75 cultivars sampled in six European Countries, to ascertain the best genotypes from the point of view of oil quality. All variables were jointly examined and their correlations coefficient 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 cultivars.
iii. The development of a chemiometric method based on PCA and cluster analysis to select the most interesting cultivars for breeding programs.

2. Materials and methods

2.1 *Plant material*

Seventy five hazelnut accessions from different European national collections were included in the analysis: 'Barcelona', 'Comun','Da Veiga', 'Grada de Viseu', 'Hall's Giant', 'Molar', 'Provence', 'Purpurea', 'Raul', Tubulosa' from Portugal, National Collection in Viseu, Direcção Regional de Agricultura e Pescas do Centro and Felgueiras – Direcção Regional de Agricultura e Pescas do Norte; 'Bergeri', 'Casina', 'Corabel', 'Corylus maxima à Pellicule Blanch', 'Corylus maxima à Pellicule Rose', 'Cosford', 'Feriale', 'Fertile de Coutard', 'Ferwiller', 'Gunslebert', 'Imperatrice Eugenie', 'Longue d'Espagne', 'Merveille de Bollwiller', 'Rotβlaftrige Lambernuss', 'Segorbe' from France, Conservatoire Végétal Régional d'Aquinatanie, Montesquieu; 'Castanyera', 'Culplà', 'Gironell', 'Grifoll', 'Morell', 'Negret', 'Pautet', 'Trenet', 'Vermellet' from Spain, IRTA –Institut de Recerca i Tecnologia Agroalimentàries, Constantí; 'Argiroupoli', 'Extra Ghiaghli', 'Gr p 03', 'Karydato', 'Palaz', 'Patem', 'Polycarpos wild', 'Tombul Giaghli' from Greece, AthensPomology

Institute - Naoussa; ‘CV/1’, ‘CV/2’, ‘Istrska Dolgoplodna Leska’, ‘Istrka Okrogloplodna Leska’, ‘Pellicola bianca’ from Slovenia, ex-*situ* collection in Maribor; ‘Ada’, ‘Avellana Speciale’, ‘Barrettona’, ‘Barrettona Vico’, ‘Camponica’, ‘Carrello’, ‘Centenaria’, ‘Comune di Sicilia’, ‘Dal Rosso’, ‘Daria’, ‘UNITO-L35’, Ginnasi’, ‘Lunga di Ginnasi’, ‘Meloni’, ‘Nociara’, ‘Nocchione’, ‘Nostrale’, ‘Palla grossa’, ‘Piazza Armerina’, ‘Riccia di Talanico’, ‘San‘Giovanni’, ‘San Vicino’, ‘Tonda bianca’, ’Tonda di Biglini’, ‘Tonda di Calabria’, ‘Tonda Gentile delle Langhe’, ‘Tonda Gentile Romana’, ‘Tonda di Giffoni’, from Italy (collection cured by Agenzia Regionale per lo Sviluppo e l’Innovazione dell’Agricoltura del Lazio ARSIAL, Rome and University of Torino, Cravanzana - CN). Most of them are of important commercial value at European as well as international level; in the analyses were also included minor cultivars and selections with potential interest for local market and breeding. The plants in the collections were between 13 to 20 years old, planted in randomized block design with almost three replicates for accession. The hazelnuts were harvested at maturity in the crop year 2007 (September), 2008 (September), 2009 (September) dried to about 5% kernel humidity and stored at 5°C as in shell fruits in the dark, closed in plastic bags, until analyses. Every year a sample of about 1 kg was randomly chosen for each accession and analysed.

2.2 *Total oil content determination*

Total oil contents were determined according to AOAC 954.02 Official Methods. Before chemical analysis hazelnuts were manually cracked and shelled kernels were chopped finely using a coffee grinder. The oils were extracted from the ground sample. A 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 hour at 80°C. 10 ml of 99% ethanol and 25 ml of ethyl ether were added and the solution was shaked for 90 seconds. 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; the supernatant from the three extractions were combined and the solvent was removed from the extract using a rotary evaporator (Rotovapor) at 80 °C. The resulting oil was weighed and stored at -80°C until use. Oil content was expressed as percentage of kernel dry weight:

% oil content = Weight of oil / Weight of sample X 100

2.3 *Analytical procedure of fatty acid assessment*

The fatty acid profiles of hazelnut oil, obtained by AOAC 954.02 method, were determined by gas-chromatography. 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 4000 rpm at room temperature. A Gas Chromatograph, equipped with a detector FID and a Stabilwax (Restek) column (RTX2330; 30 m x 0.5 mm ID, 1.0 μm df) was used. The temperatures of the injector and detector were maintained at 250 °C, and 270° C respectively. The column temperature was held at 170 °C for 3 min, then was increased from 170° C to 175° C at 1°C min^{-1} and from 175° to 225° C at 10° C min^{-1}. Finally, the temperature was maintained at 225° C for 10 min. 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 which was composed of methyl linoleate, methyl linolenate, methyl oleate, methyl palmitate, methyl stearate (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 FA content.

2.4 *Analytical procedure of tocopherols determination*

Hazelnut oil was extracted from nut samples and tocopherols were determined using 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 Soxhlet extractor 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). Samples of 0.15-0.20 g of hazelnut oil were shacked at 60° C for 45 min with 20 ml 2M 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 Perkin Elmer 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 mm x 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. Standard linearity was verified in each case by analysis of six standards in triplicate, each containing 10-90 mg L^{-1} α-tocopherol and 1-15 mg L^{-1} δ- and γ-tocopherol. For each sample the tocopherol content was calculated as the mean value of two replicates from the saponification process and was expressed as mg kg^{-1} oil.

2.5 *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. Principal Component 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 used to identify groups of similar cases.

3. Results and Discussion

3.1 *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 genotypes 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 genotypes. Thus, based on the statistical tests, significant differences (for $P<0,05$) were found for oil content and for most of fatty acids contents except for C18:3; no significant differences were found for α-tocopherol content and stability index (SI) considering the median test.

The average amount of oil on dry weight found in the hazelnut samples was 56.95%, ranging from 41.96 % in cv 'Gunslebert' to 63.73% in the Italian cultivar 'Piazza Armerina'. The total oil content was >60% in twenty one accessions, including 14 Italian cultivars ('Avellana Speciale', 'Barrettona', 'Carrello', 'Camponica', 'Comune di Sicilia', 'Lunga di Ginnasi', 'Meloni', Nociara', 'Nocchione', '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% (cv 'Corylus maxima pellicule rose', 'Daria', 'Feriale', 'Gunslebert', 'Karydato', 'San Vicino', 'Segorbe', 'Tonda di Biglini').

The main fatty acids identified in the oil included palmitate (C16:0), palmitoleate (C16:1), stearate (C18:0), oleate (C18:1), linoleate (C18:2) and linolenate (C18:3). Among them oleic acid was, by far, the predominant acid in all the hazelnut oils, ranging from 73.31% in 'Longue d'Espagne' to 84.56% in the Italian cultivar 'Avellana speciale' with an average value of 80.63%. Significant

differences were found in the oleic content among the cultivars; 49 accessions were characterized by C18:1 content > 80.00%.

The linoleic acid, whose content was inversely correlated with the C18:1 one, showed a mean value of 10.57% and pronounced differences among cultivars. The lowest content was found in the Italian ecotype 'Meloni' (5.91%) and the highest in 'Longue d'Espagne' (19.01%). Moreover 35 accessions were characterised by a linoleate 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 data are greater than those reported by other authors for hazelnut accessions and hybrids (Cristofori et al., 2008; Xu and Hana, 2010); nevertheless the mean values were in agreement with those indicated in the same references.

The large number of the accessions we considered in our research shows the great variability of fatty acid profiles in the European germplasm, which can be very useful to select genotypes characterized by low SFA content, to be included in the breeding programs aimed at improving hazelnut kernel quality.

However oleic acid and linoleic acid contributed for over 89% to the total fatty acid composition in all of the analysed genotypes. 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%) acids. These fatty acids, even in traces, can negatively affect nut storability because of their low stability (Cristofari et al., 2008).

In addition, total SFA (palmitate + stearate), total MUFA (palmitoleate + oleate), total PUFA (linoleate + linolenate), and the ratios of UFA⁄SFA, MUFA⁄SFA, and PUFA⁄SFA were calculated. 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 & Aygun (2005), Amaral et al. (2006a,b).

A high level of MUFA and a low quantity of SFA in hazelnut oil enhances its usefulness in food and oleochemical applications (Xu *et al.*, 2007). Moreover a diet with a high MUFA and a low SFA could effectively control blood lipid level and reduce blood pressure (Kris-Etherton, 1999; Perez-Jimènz et al., 2002; Alasalvar et al., 2003).

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 (Köksal, 2002). 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 ratios in the oil of the European germplasm was higher (mean value 11.01) than the value reported by Ozdemir et al., (2001) and Alasalvar (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). The natural isomer (R,R,R) disappears more quickly by the liver after supplementation than the enantiomer (S,S,S) (Shintani and Ajjawi, 2004). This implies the natural stereoisomer is discriminate suggesting the importance of α-tocopherol natural intake (Sivakumar *et al.*, 2005). The median test indicated that no significant differences were found among the α-tocopherol patterns in the European hazelnut cultivars, however this result was in contrast with Kruskal Wallis Test. The average content of 75 European hazelnut cultivars was 195.98 ppm ranging from 102.34 ppm in cv 'Tombul Giaghli' to 390.97 ppm in cv 'Cosford'. About 30% of the analysed accessions showed a α-tocopherol content > 200 ppm.

In this study, the stability index (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 a-tocopherol, and monounsaturated fatty acids and it is inversely correlated to linoleic acid content (Zacheo et al., 2000). A higher SI value

indicates a more stable oil. According to the median test, no significant differences were found among cultivars. This result was in contrast with the 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 (Ozedemir et al.., 2001). However twenty one accessions of the European germplasm were characterised by SI value >20 in particular 3 cultivars ('Riccia di Talanico', 'Gunslebert', 'Cosford') showed SI value > 30 due to their high α-tocopherol content. However SI values appeared to be more related to the presence of natural antioxidant such as α-tocopherol rather than fatty acid composition of the oil as reported by Xu and Hanna (2010).

3.2 *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, as other authors suggested in almond (Font I Forcada et al. , 2011); significant negative correlations were found between linoleic and linolenic acids and among PUFA, PUFA/SFA and Stability Index. Positive correlations were associated to palmitate, palmitoleate and stearate, whereas a negative correlation was observed with oleic acid but not with the ratio oleate/linoleate as reported by Kodad and al. (2011) in almond. The percentage of palmitoleate was inversely correlated with α-tocopherol content which was not related to the other variables. The stearic acid content resulted inversely correlated with linoleate and linolenate contents and thus positively correlated with the ratio oleate/linoleate. 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 fact that the pool of oleic acid appears to be controlled by its conversion to linoleic, as a result of the enzymatic activity of desaturase (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 (Kodak 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 = -.473) would allow to select accessions with high oil content and low in linoleate. A negative correlation was found between SFA and PUFA.

3.3 *Influence of harvest year on fatty acid profiles, α-tocopherol content and stability index.*

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 two years of harvest were found for fatty acids content, except for linoleate (C18:2), the ratio PUFA, PUFA/SFA, α-tocopherol and stability index. The Wilcoxon's test confirmed that PUFA/SFA and α-tocopherol were not significantly different between the two harvests. On the contrary the two tests showed a significant difference in oil content between the two years of cultivation. Evaluating oil content of 73 almond cultivars in the same years, Kodad et al. (2011) did not report any 'year effect' on this variable, underling a significant difference only for the interaction '*year x cultivars*'. The year effect has been reported to be significant in some other studies (Parceisa 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 for pistachio by Arena et al. (2007). In 2009 the temperature were higher than in 2008 especially from May to October (http://www.ecmwf.int/research/era/do/get/index). No significant differences were found for α-tocopherol and SI between the two harvests. Parceisa et al. (1995) reported significant differences in vitamin E 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.

3.5 *Influence of place of cultivation on lipid fraction*

Climate and latitude of cultivation can 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).

In Table 4 the results of descriptive statistical analysis (mean value, index number (mean=100), SD and variation coefficients) performed on the lipid fraction from 75 European hazelnut accessions grouped on the basis of their sites of cultivation are shown. When the oil content was studied in cultivars from the same geographical origin, the results showed that there were differences in the mean values of the genetic pool from Italy (mean value 60.8% ±4.7), Slovenia (mean value 593% ±5.6) and Portugal (mean value 58.2% ±5.4), with the index number > 100 followed by Greece (mean value 56.8% ±4.1), Spain (mean value 55.9% ±5.7) and France (mean value 51.5% ±5.3). The Greek cultivars were characterized by a pronounced palmitic, palmitoleic and stearic acid content. The most important lipidic fraction, the oleic acid, showed a relevant content in Italian

accessions followed by Slovenian ones. It is significant to note that almost all cultivars from Greece, Italy, Slovenia, and Portugal had a mean value of oleic acid higher than 80.0%. French hazelnut varieties were particularly rich in linoleate and linolenatee that are the oxidised forms of oleic acid.

On these premises, SFA content was predominant in the Greek accessions followed by Italian and Portugese ones. An higher PUFA/SFA and tocopherol amount was observed in French accessions followed by Italian and Slovenian ones, with Portuguese and Greek ones that were the lowest. These results are in good agreement with those reported by Alasalvar et al. (2009) on tocopherol contents of oils extracted from different hazelnuts varieties grown in Turkey, Portugal, Italy, France, Spain and New Zealand. The oils presented a wide range of tocopherol amount, with the highest oil content found in the Turkish 'Tombul' cultivar and the lowest in the Portuguese cultivars. In addition α-tocopherol contents in some hazelnut varieties harvested in Oregon (USA) were reported ranging from 211.4 to 443.5 mg kg^{-1} which were slight higher than those in the European nut sample (Parcerisa *et al.*, 1998).

3.3 *Principal component (PCA) and cluster analyses*

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

The factor loadings of three principal components (PCs) elaborated from the data of kernel composition are shown in table 5. The 3 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 38.395% of total variance), PC2 (accounting for 30.257% of the variance) was highly correlated to palmitic and palmitoleic acids, whereas the third component (accounting for 13.897% of the variance) was associated with α-tocopherol content.

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, including 'Raul', 'Negret', 'Longue d'Espagne', 'Trenet',

‘Casina’ and ‘San Vicino’, had positive values of PC1 and PC2. These cultivars showed relatively low levels of total oil content and oleic acid and high values of linoleate and palmitoleate. Cluster II comprised only cv ‘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, grouping 26 accessions (‘Avellana Speciale’ ‘Barcelona’, ‘Barettona Vico’, ‘Bergeri’, ‘C,a,7’, ‘Comum’, ‘Comune di Sicilia’, ‘CV/2’, ‘CV/1’, ‘Da Veiga’ ‘Grifoll’, ‘Fertile de Coutard’, ‘Feriale’, ‘Gironell’, ‘Hall's Giant’ ‘Istrska dolgoplodna leska’, ‘Imperatrice Eugenie’, ‘Istrska okrogloplodna leska’, ‘Lunga Ginnasi’, ‘Merveille de Bollwiller’, ‘Molar’, ‘Morell’, ‘Pellicola bianca’, ‘Piazza Armerina’, ‘Riccia di Talanico’, ‘Tonda di Giffoni’, ‘Vermellet’), showed positive value of PC1, as a consequence of low total oil and oleate contents, but negative values of PC2 (low level of SFA). Cluster IV included 6 cultivars (‘C. maxima à Pellicule Blanche’, C. maxima à Pellicule Rose’, ‘Cosford’, ‘Rotblaftrige Lambernuss’, ‘Segorbe’) characterized by fair oleic and total oil content an variable amount of SFA. Cluster V comprised 26 cultivars, most of which from Italy (‘Ada’, ‘Barettona’, ‘Camponica’, ‘Carrello’, ‘Centenaria’, ‘Dal Rosso’, ‘Daria’, ‘Incrocio L35’, ‘Lunga di Ginnasi’, ‘Meloni 2007’, ‘Nocchione’, ‘Nociara’, ‘Nostrale’, ‘Pallagrossa’, ‘San Giovanni’, ‘Tonda bianca’, ‘Tonda di Biglini’, ‘Tonda Gentile delle Langhe’ (clone PD6), ‘Tonda Calabrese-Caserta’, ‘Tonda Gentile Romana’), and some from Spain (‘Culpla’ and ‘Pauetet’), Portugal (‘Tubulosa’, ‘Purpurea’, ‘Provence’) France (‘Corabel’) and Greece (‘GR pi 03’). Cultivars of cluster V showed negative values of PC1 and most of them had negative values on PC2. In many cases these cultivars were recovered and conserved “*on farm*”, and showed interesting values for total oil and oleate contents associated to low level of C18:2. Cluster VI, including the Italian ‘Tonda Gentile Langhe’, the Slovenian ‘Polycarpos wild’, the ‘Portugese ‘Grada de Viseu’, the Spanish ‘Castanyera’ and six Greek cultivars (‘Argiroupoli’, ‘Extra Ghiaghli’, Karydato’, ‘Patem’, ‘Palaz’, ‘Tombul Ghiaghli’) had negative values on PC1 and positive on PC2, on the basis of their SFA level.

The PCA results confirmed that oleic and linoleic acids contents are important are useful parameters for quality characterization of hazelnut cultivars, as reported for almond by Kodak 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 varieties, but also in local ecotypes 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 ecotypes 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.

4. 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 we considered in our 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 (r2=-.934) which agreed with other results reported on similar species such as almond (Kodad et al., 2011), 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 oil stability against oil 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 a-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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Fig 1 – UPGMA dendrogram derived from Euclidea Distances of similarity showing the relationship among 75 hazelnut cultivars on the bases of the 3 PCs axes elaborated by PCA from the kernel composition.

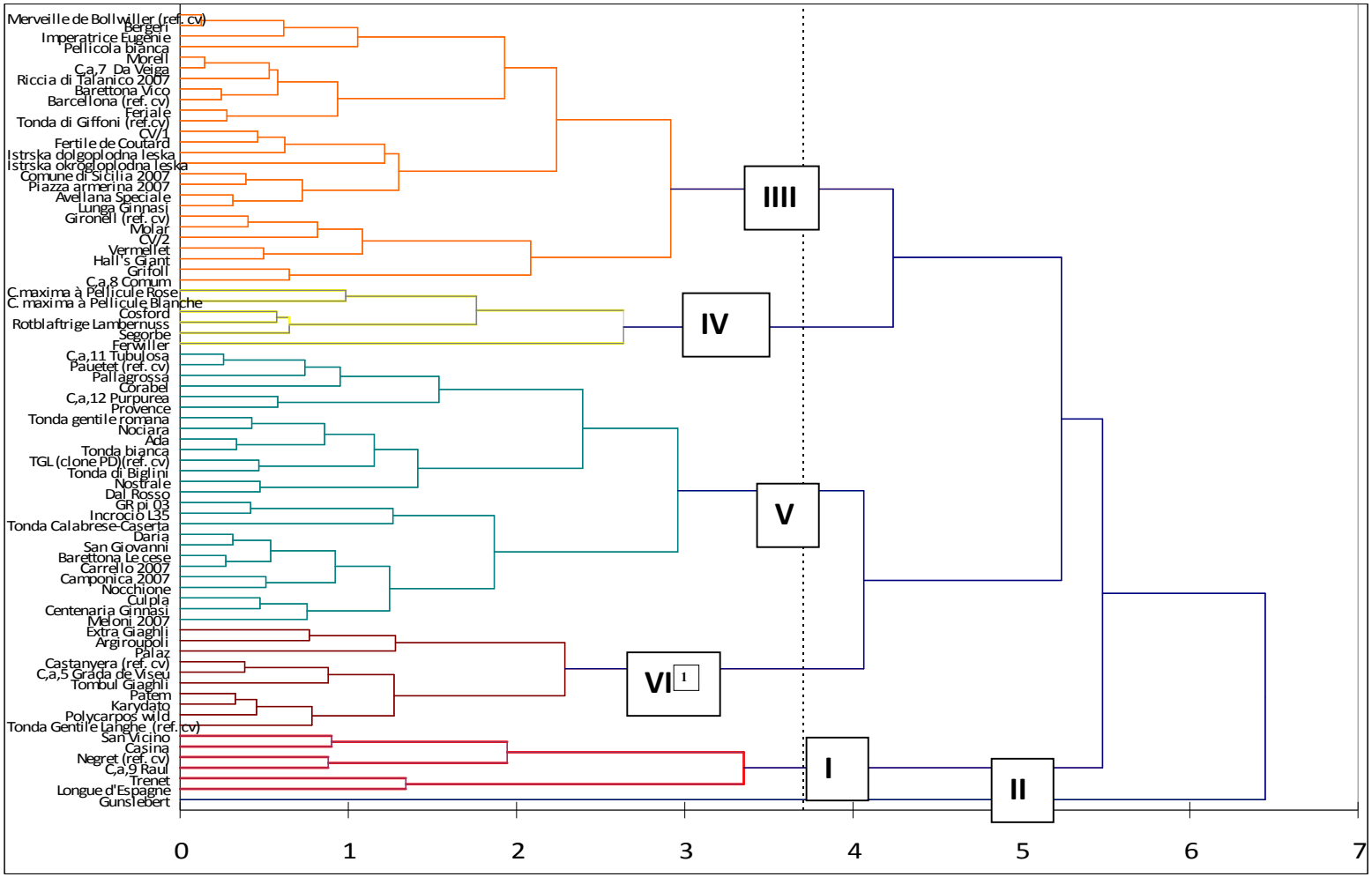
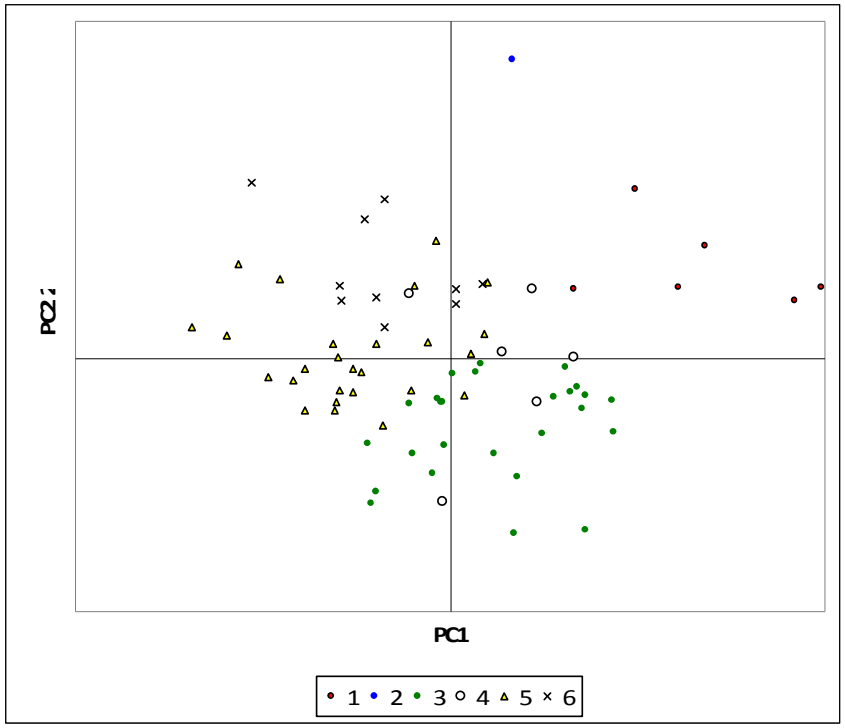


Fig. 2 Position of the principal component (PC1 and PC2 position) scores of the hazelnut kernel composition for 75 hazelnut kernel cultivars.



Tab. 1 - Oil content (% d.w.), fatty acids composition (relative %) and α-tocopherol content (ppm oil) of the kernel in 75 hazelnut cultivars. Data are the mean values of 2008 and 2009 years.

Continued in the next three pages.

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-ttocopherol	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

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-ttocopherol	SI**
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
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	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	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 (clone 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

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-ttocopherol	SI**
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
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	Barcellona	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															
	Chi-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	.042	.006	.022	.000	.001	.000	.663	.016	.002	.001	.016	.041	.002	.066	.186
-	*Kruskal Wallis Test*															
-	Chi-quadrato	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

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

Tab. 2 - Correlation between oil content (percentage of kernel dry weight) and the different fatty acids concentrations (percentage of oil total content) in the kernels of 75 hazelnut cultivars 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	.682**													
C18:0	0.21	.256*	0.01												
C18:1	.495**	-.279*	-.239*	0.20											
C18:2	-.473**	-0.03	0.06	-.458**	-.934**										
C18:3	-.480**	0.01	-0.05	-.228*	-.380**	.393**									
O/L	.481**	0.03	-0.07	.443**	.884**	-.948**	-.407**								
SFA	0.02	.851**	.492**	.726**	-0.09	-.268*	-0.12	.264*							
Total MUFA	.496**	-.270*	-.226*	0.21	1.000**	-.936**	-.382**	.886**	-0.08						
Total PUFA	-.479**	-0.03	0.05	-.460**	-.934**	1.000**	.409**	-.948**	-.268*	-.936**					
UFA/SFA	-0.06	-.777**	-.472**	-.783**	0.04	.308**	0.15	-.307**	-.979**	0.03	.308**				
MUFA/SFA	0.08	-.806**	-.517**	-.675**	.344**	0.00	0.03	-0.02	-.940**	.338**	0.00	.951**			
PUFA/SFA	-.411**	-.278*	-0.10	-.643**	-.779**	.947**	.381**	-.888**	-.548**	-.783**	.948**	.584**	.306**		
alfa-tocoferol	-0.23	-0.22	-.331**	-0.06	-0.08	0.13	0.16	-0.16	-0.19	-0.08	0.14	0.18	0.15	0.15	
SI	-.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	.902**

** statistical significance at the 1% level

* statistical significance at the 5% level

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) x (α-tocopherol content).

Tab. 3 - Comparison between mean values (%) of oil content, fatty acids, α-tocopherol and SI in the kernels of 75 cultivars in 2008 and 2009, based on the parametric test of Student and the non parametric test of Wilcoxon.

Variables	Differences 2009-2008	Paired t test		Wilcoxon test	
		t –value	Sig.	Z	Sig.
Total oil (%)	1.66	2.24	0.03	-2.79	0.01
C16:0 (%)	0.44	4.30	0.00	-4.62	0.00
C16:1	0.03	4.13	0.00	-3.77	0.00
C18: 0	0.11	2.96	0.00	-3.02	0.00
C18:1	-1.29	-3.01	0.00	-4.26	0.00
C18: 2	0.52	1.25	0.22	-2.99	0.00
C18: 3	0.02	2.35	0.02	-2.93	0.00
SFA	0.55	4.83	0.00	-5.04	0.00
MUFA	-1.26	-2.96	0.00	-4.25	0.00
PUFA	0.55	1.30	0.20	-3.03	0.00
UFA/SFA	-0.78	-5.60	0.00	-5.17	0.00
MUFA/SFA	-0.77	-6.11	0.00	-5.79	0.00
PUFA/SFA	-0.02	-0.32	0.75	-0.71	0.47
α - tocopherol	4.14	0.50	0.62	-0.50	0.61
SI	1.90	1.95	0.06	-2.19	0.03

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

Table 4 - Mean value, index number (mean=100), SD and variation coefficients of the lipid fraction from 75 European hazelnut accessions. *Continued in the next two pages*

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.	.10	.07	.08	.14	.09	.10	.10	.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	.6	.5	.5	.6	.9	.6	.8
	Variation Coeff.	.19	.08	.09	.08	.11	.16	.10	.13
C16:1	Mean	.21	.27	.19	.23	.23	.21	.25	.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	.1	.0	.1
	Variation Coeff.	.16	.15	.20	.20	.18	.41	.16	.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	.5	.4	.4	.4	.4	.3	.3	.5
	Variation Coeff.	.21	.13	.14	.16	.18	.13	.17	.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.	.05	.02	.04	.02	.03	.03	.05	.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.	.30	.21	.34	.15	.19	.18	.38	.32
C18:3	Mean	.18	.09	.11	.17	.14	.15	.10	.14
	Index Number (Mean = 100)	**131.9**	68.9	79.4	122.9	103.6	110.4	73.3	100
	Standard Deviation	.1	.0	.0	.1	.1	.0	.0	.1
	Variation Coeff.	.57	.31	.44	.62	.80	.27	.45	.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

Variables	Statistical index	Sites of cultivation							
		France	Greece	Italy-ENEA	Italy-UNITO	Portugal	Slovenia	Spain	Total
	Standard Deviation	1.3	.7	.6	.8	1.0	1.0	.8	1.0
	Variation Coeff.	.16	.08	.07	.09	.12	.13	.10	.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
	Variation Coeff.	.05	.02	.04	.02	.03	.03	.05	.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.	.30	.21	.34	.15	.19	.18	.38	.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	.8	.9	1.0	1.4	1.5	1.3	1.3
	Variation Coeff.	.12	.09	.08	.10	.13	.13	.11	.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	.7	.9	.9	1.3	1.4	1.0	1.2
	Variation Coeff.	.13	.08	.09	.09	.13	.14	.10	.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	.6	.3	.4	.2	.3	.2	.7	.5
	Variation Coeff.	.35	.25	.33	.21	.22	.19	.45	.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.	.31	.18	.30	.27	.22	.27	.25	.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.	.45	.21	.30	.25	.25	.34	.21	.40

Table 5- Factor loadings of three principal component (PC) axes of kernel composition after principal component analysis of 75 hazelnut cultivars*.

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	**0.92**	0.343	-0.09
UFA/SFA	0.625	-0.765	-0.065
MUFA/SFA	0.362	-0.913	-0.03
PUFA/SFA	0.98	0.041	-0.125
alfa-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

* Eigen values and their contribution to the total variation are listed at the bottom of the columns.