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SCIENZE E TECNOLOGIE DELLE PRODUZIONI AGRO-ALIMENTARI

**IMPROVING HAZELNUT QUALITY AT HARVEST
AND NON-DESTRUCTIVE ASSESSMENT OF
POST-HARVEST NUT QUALITY**

DOTTORANDO: ANTONIO PANNICO

TUTOR:

DR. CHIARA CIRILLO, PHD

COORDINATORE:

CH.MO PROF. GIANCARLO BARBIERI

CO-TUTOR:

CH.MO PROF. MARCELLO FORLANI

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ABSTRACT

Italy is the second hazelnut producer country in the world, and more than 30% of the Italian cultivated area is located in Campania (South Italy). Usually hazelnuts (*Corylus avellana L.*) are sold both in-shell and shelled. In-shell hazelnuts are mainly designed for fresh consumption while shelled ones, are employed, as raw material, in confectionary and bakery food companies. Furthermore, due to its beneficial health attributes, hazelnuts have also been valued by cosmetic and pharmaceutical companies. Nuts are source of bioactive compounds such as plant proteins, essential minerals, vitamin E, unsaturated fatty acids, and polyphenols. Fruit quality has gained a key relevance in the processing industry, since companies are attempting to find useful methods to better characterize their own products. However, hazelnut is still little studied and till now many questions on nut quality improvement need to be still addressed. The general aim of this thesis was to increase the knowledge of factors and techniques that can positively affect hazelnut final quality. Therefore, the thesis was organized in three experiments.

In the first experiment the effect of foliar fertilization with nitrogen and micronutrients on yield, nut quality, and kernel composition was evaluated. Thirty ten-year-old hazelnut trees, cv. 'Mortarella' randomly distributed in an private orchard located in Caserta, Southern Italy, were selected. The foliar spray treatments compared were: control (C) (only water), Coryl-Dry 2.5% (CD) (7% CH₄N₂O, 2% N organic, 0.5% B H₃BO₃, 0.5% Zn EDTA, 0.05% Fe EDTA) and boron 300ppm (B) (H₃BO₃). Spraying applications were carried out on mid April, mid May and mid June in 2011, 2012 and 2013. In general, foliar fertilization affected vegetative growth. CD trees showed a higher shoots extension growth, total leaf area, and trunk cross-sectional area than control trees in 2012, while there were no significant effects on yield. Foliar spray significantly improved kernel weight and decreased the incidence of blanks by about 2% compared to control. Differences in fat, protein, and carbohydrate concentration were found in 2012 and 2013 among treatments. In CD and B trees, fat concentration increased from about 60% to 64%, whereas carbohydrate concentration tended to decrease from 23.4% to 19-15.5% compared to control. The total polyphenols content was affected by foliar fertilization in 2013, CD and B trees had a higher values (respectively 153.4 and 147.7 mg/kg) than the control (128.7 mg/kg). Foliar fertilization improved oil composition of the kernels, but fatty acid composition responded differently to nutrients spray. Oleic acid concentration was promoted while palmitic acid concentration was reduced in fertilized plants compared to control trees. Furthermore, CD and B trees were characterized by an increase in

the ratio of unsaturated/saturated fatty acids compared to untreated trees. Finally, foliar spray improved lipid stability compared to control, due to higher total polyphenol concentration.

The second experiment aimed to study the intra-canopy variability in kernel growth and composition. In August 2013, ten 10-year-old hazelnut trees were selected in the same orchard of the first experiment. Tree canopies were divided in six horizontal layers. Each layer was equally divided into inner zone and outer zone, obtaining 12 canopy sectors. The PAR distribution in each canopy sector was measured on 5 September at nine times of the day (hourly measurements, from 10:30 am to 18:30 pm), by horizontally placing a ceptometer in five different points inside each canopy sector. On 24 September, when fruits were physiologically ripen, five infructescences were separately harvested in each canopy sector of sampled trees, and their the original position (height from the ground) was measured. A sample of fifty nuts per sector was used for qualitative analysis. Canopy position affected kernel fresh and dry weight. Height above the ground and kernel dry and fresh weight resulted linearly and positively related. Fat percentage was found to be similar for all the kernel samples harvested at different heights below 2.00 m, averaging 64%, whereas it decreased linearly at 60% in apical position samples (above 2.0 m). On the contrary, protein and ashes content on percentage increased progressively from lower to higher zone. Furthermore, height above the ground strongly affected fatty acid composition. The intermediate productive layer showed higher quantity of oleic acid than bottom and top canopy layer. Among the fatty acid, stearic acid and γ linolenic acid resulted positively related to height above the ground, while palmitoleic acid and linoleic acid were negatively related to it. Finally, the extinction coefficient K_{232} decreased from 1.6 to 1.35 proceeding from the lowest to the highest canopy position.

In the third experiment, the feasibility of NIR spectroscopy in estimating the level of lipid oxidation in unshelled and shelled hazelnuts was investigated. Twenty 5-kg samples of unshelled 'Mortarella' hazelnuts, harvested between 2008 and 2012, were stored in a warehouse at room temperature until the beginning of the experiments in January 2013. Five randomly selected whole hazelnuts per batches were measured by NIR spectroscopy on two opposite sides, first as a whole kernel and then as a shelled kernel. After scanning, kernel oil was extracted and fat oxidation was determined. The best results, expressed as coefficient of determination, were obtained for the K_{232} extinction coefficient for both unshelled ($R^2=0.79$) and shelled ($R^2=0.85$) hazelnuts and also low values for the RMSECV were obtained. The possibility to use the K_{232} variable as predictor of lipid oxidation in both unshelled and shelled hazelnuts is a commercially valuable result.

1. STATE OF THE ART

The genus *Corylus*, belonging to the birch family (Betulaceae), includes cultivated hazelnut (*Corylus avellana* L.) that represents the genetic basis of the most important commercial cultivars. The commonly used terms ‘filbert’ and ‘hazelnut’ are often used interchangeably to define all plants in the genus *Corylus*, including *C. maxima* and *C. colurna* (Turkish hazel) that also have desirable characteristics, as non-suckering growth of the latter. In its natural form hazelnut is a deciduous, monoecious, multi-stemmed bush, but commercially has been grown as a single trunk tree also. Hazelnuts have a number of unique characteristics, which include flowering periods of weeks or even months between pollination and fertilization.

Italy is the second hazelnut producer in the world (22%) followed by United States (5%) and Spain (3%), but behind Turkey, whose huge supply (70%) dominates the world market (FAOSTAT, 2013). Hazelnut production is spread along Italy with main cultivation in Piedmont region (13%) (North Italy), Lazio region (38%) (Central Italy), Campania (37%) and Sicily region (9%) (South Italy) (ISTAT, 2010). In 2013 the total Italian production reached 128,940 MT (FAOSTAT, 2013).

Hazelnuts are sold both in-shell and shelled shape. In-shell hazelnuts are mainly designed for fresh consumption while shelled ones, are employed, as raw material, in confectionary and bakery food companies. Furthermore, due to its beneficial health attributes, hazelnuts have also been valued by cosmetic and pharmaceutical companies (Kris-Etherton et al., 1999). Nearly 90 percent of the harvested yield is addressed to processing companies, whereas fresh consumption represents the 10 percent. Due to their main processing destination, most of the cultivars are selected to obtain uniform high-quality nuts, and the market standards are mainly driven by the requirements of the confectionary industry (Mehlenbacher, 1991). Kernels are commercialized frequently roasted, which provides a more intense flavor and a crispy texture. Roasted hazelnuts are employed for obtaining butter paste or snacks, and also as ingredients for many food products (i.e. cookies, ice cream, breakfast cereals, cakes, chocolates, coffee, bread, liqueurs, spreads).

The recent recognition of nuts as heart-healthy foods by the "Food and Drug Administration" has provided a major boost to the image of hazelnuts (FDA, 2003). Nuts are included in healthy diets such as Mediterranean diet, which is based on generous amounts of fruit, vegetables, legumes, whole grains, cereals, seeds, olive oil and also nuts. It is also known that hazelnuts contain dietary fiber as well as beneficial nutrients, such as plant proteins, essential minerals (potassium, calcium, magnesium, selenium), vitamin E, vitamins of B complex, as well as unsaturated fatty acids, plant sterols and tocopherols (Alasalvar et al., 2003;

Kornsteiner et al., 2006). The presence of phenolic compounds in hazelnut kernels prevents seed oxidation and is related to the slight astringency and bitter taste of fresh nuts (Bignami et al., 2005). Nuts have been shown to contain substances such as tocopherols and polyphenols that significantly reduce coronary heart disease risks, some types of cancer, and several other diseases and physiological syndromes (Richardson, 1997; Yurttas et al., 2000). Among the most common nut species, hazelnut exhibited an intermediate content of total phenols, lower than walnut and pecans but higher than pines and macadamias (Kornsteiner et al., 2006). Furthermore, there is an increasing interest in the lipid characteristics of nut oils as they seem to be a novel source of bioactive constituents and functional nutrients (Kris-Etherton et al., 1999; Shahidi and Miraliakbari, 2005; Maguire et al., 2004). The benefits of inclusion of hazelnuts into the human diet is mainly related to its fat components (around 60%), most of which are highly rich in MUFA (primarily oleic acid). A high MUFA diet tends to raise HDL cholesterol and to lower TAG concentrations (Mensink and Katan, 1987; Rajaram et al., 2001; Mercanligil et al. 2006). Thus, hazelnut is an excellent source of MUFA and may provide beneficial effects. In addition to MUFA, other components found in hazelnut oil, including PUFA (Horrobin and Manku, 1983; Feldman, 2002) and tocotrienol (Qureshi et al., 1991). have been reported to reduce plasma total and LDL cholesterol concentrations.

Fruit quality has gained a key relevance in the processing industry, since companies are attempting to find useful methods to better characterize their own products in an increasingly competitive market, in which wealthy consumers are willing to pay high prices for high quality products.

Nut and kernel size, nut and kernel shape, thin shell, low kernel defect, delicious kernel, and high content of fatty acids and protein are among the main characteristics considered in the evaluation of nut and kernel quality of hazelnut (Botta et al., 1997). Furthermore, Mehlenbacher et al. (1993) reported that nut and kernel defects are serious problems in hazelnut. Among the nut defects blanks, brown stain disorder, doubles, moldy kernels, kernels with black tips, shriveled kernels and poorly filled nuts are of main interest.

Many quality traits such as kernel color, internal brown colour and susceptibility to rancidity in hazelnut are influenced by kernel composition (Ozdemir and Devres, 1999; Ozdemir et al., 2001).

Kernels should meet the stated market type, be free of any misshapen or underdeveloped kernels and be free of any shell or foreign material and off-odor, off-flavor or mold. Water content of kernels should not exceed 6% if shelled or 7% if in-shell, and the total water content of unshelled nuts should not exceed 10 to 12%. Size is specified with grade as a

determinant of quality, and minimum sizes are used for specification of classes “Extra” and “Class I” in international trade. For in-shell markets, larger and particularly rounded types are preferred. Shelled markets accommodate both rounded and oblong types, and size preference is dependent on the destination. Hazelnut kernel oil content ranges from 57 to over 70% and total sugars average about 4% and both vary with variety and growing location (Botta et al., 1994).

Another important quality parameter is the incidence of blanks, since this defect decreases the yield and kernel percentage, causing lower income and increased costs of harvesting and sorting (Mehlenbacher et al., 1993; Silva et al., 1996). The occurrence of blanks is frequent in several hazelnut cultivars. The external appearance of blanks is similar to that of normal nuts. Inside the blank nuts there are two undeveloped ovules at the vertex of the vascular strand or there is simply a rudimentary kernel. For fruit production is essential of both the ovule and the embryo growth. Pollination and fertilization must occur for growth of the embryo and the ovule to develop. Without fertilization, the embryo does not grow and nor does the seed (Lagerstedt, 1977; Germain, 1994).

As in other fruit species, all fertilized flowers of the hazelnut do not produce fruits. Different stages between fertilization and harvest, the growth of the ovule and the embryo may stop. When fertilization does not occur or when the kernel does not grow enough after fertilization, blank fruits may appear. According to Dimoulas (1979), the absence of fertilization may not be responsible for the production of blanks because all those that he observed included at least one fertilized ovule. A study by Crane et al. (1973) in pistachio attributed seedless fruits to the parthenocarpy phenomenon common in many fruit species. The literature suggests several possible causes for blank formation. Cultural practices, namely inadequate fertilization and irrigation, are reasons advanced for the occurrence of this defect.

Romisondo et al. (1983) confirmed that supplying some nutrient reduced the formation of blanks. Boron is considered one of the fundamental nutrients essential for optimum fruit set and for improvement of fruit and nut quality in some species. Shrestha et al. (1987) increased nut set over controls in the cultivar ‘Barcelona’ with foliar B applications. Also, Hanson (1991) determined that fruit set and yield of sour cherries could often be increased by B applications. Stephenson and Gallagher (1987) also improved the quality of Macadamia nuts with B sprays. However, on the Mediterranean coast of Spain near Reus, Ferrán et al. (1997) demonstrated that B sprays did not increase fruit set or production in hazelnut cultivars ‘Negret’ and ‘Pauetet’. The low productivity of hazelnut orchards has been one of the factors responsible for the abandonment of production of this species.

In hazelnut, unlike other fruit trees, the product consumed is not the fruit itself, but the seed that it contains. There is currently no adequate information in the literature about the factors that determine the kernel composition in hazelnut and the techniques that can improve it. It is well known that ecological conditions, cultivar, location, and technical and cultural practices can affect quality traits and fruit composition in many tree species.

One of the key factors that determines the quality and composition of the fruit is the position of these within the canopy. Several studies have demonstrated the intra-canopy variability of the fruit composition in fresh fruit tree species (Smith et al., 1997; Broom et al., 1998; Forlani et al., 2002; Lewallen and Marini, 2003; Barry et al., 2004; Basile et al., 2007), but still nothing is known concerning the effect of the fruit position on the seed composition and in particular on the composition hazelnut kernels. Like fruits, seeds are also subjected to a complex network of source-sink relationships, in particular, local source-sink relationships within the canopy may have important implications for kernel quality and composition, since branches have been demonstrated to be partially autonomous for carbohydrates partitioning (Marsal et al., 2003). Indeed, the intra-canopy variability of fruit characteristics is related to the heterogeneity of light distribution within the canopy (Luchsinger et al., 2002; Lewallen and Marini, 2003). In addition other major factors related to source-sink relationships may affect fruit quality, such as source proximity (Corelli-Grappadelli and Coston, 1991), leaf-to-fruit ratio (Wu et al., 2005), and competition with other growing fruit (Grossman and DeJong, 1995) or with vigorously growing shoots (Caruso et al., 1997).

As reported in literature (Dag et al., 2009; Fernández-Escobar et al., 2006; Leser and Treutter, 2005; Zheljaskov et al., 2012; Verardo et al., 2013) a cultural practice that affects the plant product composition is fertilization. A balanced nutrition program of both macro and micronutrients is essential for enhancing products quality and composition (Sawan et al., 2001). The use of chemically pure fertilizers necessitates the supply of micronutrients in intensive agriculture. The demand for micronutrients in plants is rather low; however, they are essential to prevent morphological, physiological and biochemical deficiencies and to assure optimum fruit quality. In particular, for the higher plants, both boron and zinc are essential elements and known to be involved in photosynthesis, N-fixation, respiration, cell division, synthesis of nucleic acids, translocation of sugars and other biochemical activities (Cakmak and Marschner, 1988; Goldbach et al., 1991; May et al., 1993). Among the different methods of application, foliar fertilization has the advantage of low application rates, uniform distribution and quick plant responses to applied nutrients (Umer et al., 1999; Mengel, 2002). In hazelnut, especially for these two elements, there is considerable potential for imbalances

and many nutrient management questions are unresolved. In other oilseed species, several studies have demonstrated that foliar fertilization with B and Zn improve fruit composition such as protein content, fat content and fatty acids composition and total phenolic compounds (Taheri and Talaie, 2001; Desouky et al., 2009; Ramezani et al., 2010; Saadati et al., 2013; Sawan et al., 2001; Yang et al., 2009; Gobarah et al., 2006; Ashraf et al., 2013), but nothing is known concerning the effect of these nutrients on hazelnut kernel composition.

In traditional practice, hazelnuts develop and ripen naturally on the trees, then fall to the ground where they further dry. Harvesting is begun when nearly all the nuts have dropped (Lagerstedt, 1979). A period of three weeks or more is often elapsed from the start of the fruits drop to the harvest time. Weather conditions lead to variant nut moisture levels and this may impact storage quality. Tree nuts are exposed to high levels of airborne inoculum in the field, and also during harvest and/or processing. In addition, the hazelnut tree is susceptible to alternate bearing, which often results in a large crop every other season. In order to smooth out the supply, the nuts (usually in shell) are stored in warehouses from one season to the next. Since the hazelnut kernel has a low respiration rate it might be expected to be a relatively stable commodity. However, due to large amounts of unsaturated fatty acids (mainly oleic acid), oxidative rancidity is a potential threat to the stability of the kernel.

Mold and rancidity are two factors which can strongly affect the market acceptability of hazelnuts. Often hazelnut quality was reduced due to the high incidence of kernel mold. Extra costs were incurred because of the necessity of carefully controlling in-shell quality and removing large numbers of moldy kernels from shelled nuts. Lipid oxidation is one of the primary mechanisms of quality deterioration in stored foods regardless of whether oil content is high or low. The changes in flavor, color, texture, and nutritive value and the production of toxic compounds are all important quality consideration (Kanner et al., 1988; Ladikos and Lougovois, 1990). Recently, in order to improve the product quality, the harvest is carried out in twice (Massantini et al., 2009). However, the problem persists and remains a high economic burden select a top quality product, which is strongly demanded by industries.

Non-destructive techniques for internal inspection and qualitative evaluation could be considered as an interesting alternative to the traditional method of selection. Near-infrared spectroscopy (NIR) is a very efficient method for high-throughput screening of plant materials for their chemical characteristics. This indirect method is based on vibrational properties of organic molecule chemical bonds and their interactions with infrared radiation. The NIR absorption spectrum is therefore correlated with a sample's chemical composition (Pasquini, 2003). Compared to conventional time-consuming methods, NIR has already

proven its usefulness for estimating various parameters in diverse natural and agricultural products. Indeed, several studies demonstrated NIR efficiency in characterizing moisture content, fat content and fat quality in fruit from various species such as hazelnut (Moscetti et al., 2013; Bellincontro et al., 2005; Bellincontro et al., 2008), peanut (Tillman et al., 2006; Govindarajan et al., 2009; Hirano et al., 1997), walnut (Jensen et al., 2001), macadamia (Guthrie et al., 2004), Shea nut (Davrieux et al., 2010), chestnut (Liu et al., 2010).

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2. AIMS OF THE STUDY

Hazelnut tree is an economically important crop for the European Community, particularly in the Mediterranean area. The increased interest towards this species is related to its excellent nutritional and nutraceutical properties. The cultivation of hazelnut is strongly related to traditional customs and cultural identity of people, but also for its contribution to a suitable use and recovery of marginal lands. In many regions where this crop is not a major agricultural resource, hazelnut represents an interesting source of income for the local sustainable production system, according to a multifunctional concept of agriculture widely supported by the European Union.

However, hazelnut is a nut tree species that is still marginally studied and several questions on quality improvement still need to be addressed. An improved understanding of the intra-canopy parameters that affect kernel quality may help to develop better practices on hazelnut canopy management that can be used to increase nut quality. A correct approach to nutrient management is required to ensure a high production quality at harvest. A balanced fertilization program of both macro and micronutrients can reduce the incidence of blank nuts and enhance kernel quality and composition. Furthermore, the EU requirements for the hazelnut marketability enforce the total absence of rancid kernels. Therefore, it is important to be able to estimate accurately and non-destructively the degree of fat oxidation of fruits in order to obtain a product meeting the requirements. A manual inspection is traditionally performed by trained workers, who select hazelnuts according to the color and the presence of visible defects, but they cannot detect any lipid oxidation by simple observation. NIR spectroscopy would provide an opportunity for the high-throughput analysis of kernel lipid oxidation of large numbers of samples.

This thesis aimed to: (a) define a fertilization strategy to decrease the occurrence of blank nuts and to improve kernel composition; (b) improve our understanding of the intra-canopy variability in kernel composition; and (c) define a non-destructive method for detecting fat oxidation of kernels. Three experiments were designed to address these objectives.

3 RESULTS AND DISCUSSION

3.1 Effect of foliar fertilization with nitrogen and micronutrients on yield, nut quality and kernel composition

3.1.1 Introduction

Hazelnut is produced commercially in various location in the northern hemisphere under a wide range variety of management practices including nutrient application. There are several reports in the scientific literature concerning the importance of nitrogen and some micronutrients (boron, zinc and iron) fertilization in improving plant growth, fruit set, yield and nut quality. Research on mineral uptake and utilization of hazelnuts in Italy confirmed that the species consumes less mineral nutrients than other deciduous fruit trees (Rovesi and Ughini, 2005). On the other hand, environmental conditions, such as cold, rain, or drought, that lower mass flow in the soil, decrease diffusion and limit transpirational flow in the plant, thus resulting in nutrients deficiency even when the supply in the soil appears to be adequate. Mineral foliar application is particularly useful under conditions when nutrient uptake from the soil is restricted. and offers a supplementary way to provide nutrients during critical phases of restricted nutrient supply (Mengel, 2002). In addition it can alleviate the effects of strong binding of plant nutrients in soils as well as difficulties in the acquisition of nutrients due to particular soil condition, as happens in K^+ fixing soils. Foliar application become more important for perennial fruit crops that are often deep rooting so that fertilizer applied to the soil surface may be of little use and more readily available to the cover crops. Furthermore their rooting systems may deplete the surrounding soil of nutrients potentially available to trees.

Although foliar application may be more frequently adopted for micronutrients, mostly heavy metals, which are often fixed by soil particles and then scarcely available to plant roots, the efficiency of foliar sprays may result high also for some macronutrients. Foliar application of nitrogen plays a major role in fruit trees, and it was reported that in peach leaves ureic N uptake during the season till leaf senescence was appreciable, since 60 -70% of the urea intercepted by the canopy was translocated into the wood and to the roots contributing to the N supply of trees during the following spring (Tagliavini et al., 1998). Basically the most common form of nitrogen as foliar spray is urea, rather than nitrate, and ammonium. Urea is a non-electrically charged molecule which is water soluble, but has also some hydrophobic properties, that make it suitable for foliar application as it can relatively easily penetrate the epicuticular wax as well as the cutin layer (Kirkby and Mengel, 1970). As soon as urea has

reached the cytosol it can be hydrolyzed by urease, present in most of plant cells, and the resulting NH_3 is metabolized in the GOGAT pathway (Freney et al., 1992).

Micronutrient requirements can generally be better met by foliar application than macronutrients since in absolute terms higher amounts of macronutrients are needed. Foliar applications of micronutrients were chosen as preferable to soil applications, as applying the fertilizer directly to the leaves is an efficient method of delivering minerals to the parts of the plant where they are needed, especially for trace elements like B, Zn and Fe (Jurgens, 1987).

Boron in the form of boric acid is a non-charged molecule under the apoplast pH conditions,, for this reason it is relatively mobile in the apoplast and frequently applied as foliar spray (Mengel, 2002). Iron are only to a very low degree phloem-mobile and therefore older leaves do not contribute to the Fe supply of younger ones as is also the case of Fe sprayed on older leaves (Kosegarten et al. 1999). Fe-chelates are widely used for foliar application their effect depending much on the time of application (Tagliavini and Rombolà, 2001). Regarding the zinc, foliar application of Zn-lignosulfate, Zn-EDTA, and ZnSO_4 all are capable to increasing the Zn concentration in the leaves to an adequate level (Mengel, 2002).

In hazelnut it was shown that the tree may efficiently use nitrogen reserves to sustain early-season growth (Olsen, 1997). At the beginning of the vegetative season very little N actually comes from fertilizer applications, whereas main of the nitrogen needing of the new leaves is fulfilled with its remobilization from perennial organ reserves (Olsen, 2001). Therefore, N application timing can have a significant effect on how the applied N is used by the tree. Nitrogen applied in March tends to stimulate vigorous vegetative growth, while split applications (March and June) cause more nitrogen to be moved into the trunks and roots for storage (Olsen, 1997).

In fruit trees foliar applications of urea are used as a way to ensure that nitrogen reserves are adequate to support an increasing crop quality (Sanchez et al., 1995). Nitrogen is recognized as the most important nutrient for nut crops, both for growth of young seedlings and for nut production, therefore additional N may increase growth of young orchards, and productivity and profitability of mature trees (Braun et al., 2005; Cacka and Smith, 2009). In black walnut it was found that N applied during nut filling stage, or shortly before, improved nut growth (Gray and Garrett, 1999). In hazelnut, it has been found that shoot length, yield, nut size, and percentage nut filling increased with N application, while the percentage of unfilled nuts decreased (Painter, 1955; Painter and Hammar, 1962; Bergougnoux et al., 1978; Romisondo et al., 1983; Bignami et al., 2002, Nicolosi et al., 2009).

Boron is an essential element for reproductive growth and development in annual as well as perennial plants. In several fruit trees, including pear (Wojcik and Wojcik, 2003), prune (Callan et al., 1978), sweet cherry (Usenik and Stampar, 2001), apple (Sanchez and Righetti, 2005), olive (Perica et al., 2001), and nut trees as pecan (Wells and Conner, 2008), macadamia (Stephenson and Gallagher, 1987) and almond (Nyomora et al., 1999) foliar B applications increased. In hazelnuts boron is considered one of the essential nutrients for optimum fruit set and for improvement of fruit and nut quality (Alkoshab et al., 1988; Borges et al., 2001). This mineral is known to be involved in cell division, synthesis of nucleic acids and translocation of sugars (Pilbeam and Kirkby, 1983; Parr and Loughman, 1983). On the other hand, boron fertilization is the subject of much discussion. Research in Oregon have been reported to increase fruit set and nut quality in hazelnut (Painter and Hammar, 1964; Baron et al., 1985; Shrestha et al., 1987), although no response was found under Mediterranean conditions (Ferran et al., 1997; Borges et al. 2001; Paula Silva et al., 2003). One explanation for the difference is different cultivars. Another is that B plays a role in nut set under cool spring conditions; Pacific Northwest springs are cool and moist, whereas Mediterranean springs are warmer. Furthermore, plants with lower tissue B concentration before B application responded more significantly to application (Hanson, 1991; Nyomora et al., 1997). Boron is also recognized to cause toxic effects to many crops (Sparks and Payne, 1976; Wilcox, 1960; Oertli and Kohl, 1961). Therefore, it is also important the dosage. In hazelnut, the best results were achieved with concentrations of foliar B spray between 300 and 600 ppm (Shrestha et al., 1987; Erdogan e Aygun, 2009).

Most relevant effect were obtained by applications of foliar boron together with other nutrients. Especially foliar applications of boron and zinc have been successfully used to promote tree vigor, fruit set, fruit size and yield in apple (Amiri et al., 2008), olive (Talaie and Taheri, 2000), macadamia (Huett and Vimpany, 2006), walnut (Keshavarz et al., 2011) and almond (Sotomayor et al., 2001). In hazelnut has been shown that B foliar sprays with macro and micro nutrients such as nitrogen, zinc and iron enhances fruit set, yield and nut quality (Painter and Hummer, 1964; Solar and Stampar, 2001; Tous et al., 2005; Serdar et al., 2005; Olsen, 2007; Cacka e Smith, 2009; Olsen e Cacka, 2009; Nicolosi et al., 2009).

A balanced fertilization program of both macro and micro nutrients for plant nutrition is essential also for enhancing product composition. In higher plants zinc and boron are known to be involved in photosynthesis, N-fixation, carbohydrate metabolism, respiration and other bio-chemical activities (Cakmak and Marschner, 1988; Goldbach et al., 1991). In olive these elements were capable of improve the quantity and quality of oil content such as oleic acid

and phenolic compounds in fruits (Taheri and Talaie, 2001; Desouky et al., 2009; Ramezani et al., 2010; Saadati et al., 2013). In several oilseed species, including cotton (Sawan et al., 2001), rapeseed (Yang et al., 2009), peanut (Gobarah et al., 2006) and pecan (Ashraf et al., 2013), foliar application of B and Zn increased fat content.

Also nitrogen fertilization alone or together with boron and zinc affects the plant product composition such as protein content, fat content and fatty acids composition. N, B, Zn foliar spray, in pecan (Ashraf et al., 2013) and peanut (Abd and Mona, 2013), increase protein and fat content, in olive (Chouliaras et al., 2009) improve fat content, while in olive (Saadati et al., 2013), boron and zinc application increase total phenolic compounds.

3.1.2 Materials and methods

Plant material and experimental site

Thirty ten-year-old open vase hazelnut (*Corylus avellana* L.), cv. 'Mortarella', with homogeneous growth vigor and bloom density were selected in an private orchard located in Caianello, (Caserta, South Italy 41°18'00"N 14°05'00"E) during the winter 2010. Trees were spaced 3m x 4m and tree rows were North-South oriented. Soil fertility management and other agricultural practices were identical for all trees.

Foliar fertilization treatments

Thirty hazelnut trees randomly distributed in the orchard were selected. Nutrients spray treatments were: control (distilled water), Coryl-Dry (7% CH₄N₂O, 2% N organic, 0.5% B H₃BO₃, 0.5% Zn EDTA, 0.05% Fe EDTA) and boron (H₃BO₃).

The experimental design included fifteen randomized blocks of four plants. Each treatment consisted of five replication of four trees and measurements were taken from the two central trees.

Twenty trees (five blocks) were sprayed with a distilled water solution of Coryl-Dry (CD) at 2,5%, other five blocks with a solution 300 ppm of boron (B) and the other five blocks (C) with only water.

The spraying were performed three times a year in 2011, 2012 and 2013 with a backpack pump pressure sprayer, from the fifth leaf stage (14 April 2011, 17 April 2012 and 16 April 2013) every 3-4 weeks (12 May and 17 June 2011; 19 May and 13 June 2012; 12 May and 18 June 2013).

Plant measurements

From 2011 to 2013, trunk circumference was measured twice a year, at the beginning of vegetative growth (18 April 2011, 27 April 2012 and 1 May 2013) and the harvest time (22 September 2011, 25 September 2012 and 27 September 2013). On-tree measurements were taken on eight one year old shoots per tree, to evaluate vegetative growth, such as basal diameter of fruiting shoots, new shoot number and length, on April, May and June in 2011 and 2012 years. At the same dates, a sample of forty shoots per treatment were randomly collect in order to determine shoot length, number of leaves per shoot, leaf area. From May to September, once a month in the years 2011 and 2012, one-hundred fruit per treatment were randomly harvested and transferred to a laboratory to measure fruit fresh weight and dry weight after dried in a ventilated oven at 60 °C until reaching constant weight. At harvest (22 September 2011, 25 September 2012 and 18 September 2013), yield per tree was registered and samples of 1 kg of nuts per tree were collected and stored at -20 °C until analysis, in the years 2011, 2012 and 2013.

Fruit biometrical analysis

At harvest the number of nuts per kilogram was counted to estimate number of fruits per tree. For each treatment, on a sample of 200 hazelnut, equatorial, longitudinal and transversal diameters of fruit and kernel were measured. On the same samples, was detected fruit and kernel fresh weight and dry weight after dried in a ventilated oven at 60 °C until reaching constant weights. Moisture content was calculated as the difference between fresh weight and dry weight. Dried kernels were stored at -20 °C until composition analyses.

Kernel constituents

Ash, total fat, total protein, carbohydrate and total phenols of kernels subjected to different treatment were determined. Total ash was determined by incinerating of the samples (500 mg of finely ground kernels) for 3 h at 550 °C in muffle furnace according to the AOAC method (1995). Total fat was extracted using a Soxhlet extractor (SER 148/3, Velp Scientifica, Italy); 5 g of finely crushed kernels were placed in a cellulose thimble and extracted with 30 mL of petroleum ether (boiling point 40 - 60 °C) for 6 h (AOAC, 1995). Oil extracted was stored at -20 °C until analysis of fatty acid composition and lipid oxidation. Total crude protein ($N \times 5.30$) was determined by the macro-Kjeldahl method (AOAC, 1995). Carbohydrate content was calculated according to Olivera et al. (2008), using the following equation: carbohydrate

content = 100% – (% moisture + % protein + % fat + % ash). Total phenols were determined according to Jakopic et al. (2011) with some modifications. Hazelnut flour (5 g) was extracted for 45 min with 30 ml of methanol/water (80:20 v/v) in a water bath using sonification. The hazelnut extracts were centrifuged at 7000 rpm for 10 min and the supernatant was filtered through a 0.45 µm membrane filter. Five ml of extract was mixed with 5 ml n-hexane for 3 min in a vortex apparatus, than the mixture was centrifuged at 7000 rpm for 5 min, to remove lipid fraction. The procedure was repeated twice with 5 ml of n-hexane. The total phenolic content of the extracts was assessed using the Folin–Ciocalteu phenol reagent method (Singleton and Rossi, 1965). Ten ml of bi-distilled water and 500 µl of Folin–Ciocalteu reagent were added to 2 ml of diluted extract (1:5 in water), then 1 ml of sodium carbonate (20%, w/v) was added after 3 min. The absorbance at 765 nm was measured after 30 min in the dark. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per kg of hazelnut.

Fatty acid composition

Fatty acid composition was analyzed by gas chromatography after derivatization to fatty acid methyl ester (FAME) with a solution of KOH 2N in methanol as described by Romano et al. (2012). A GC Perkin Elmer AutoSystem XL (PerkinElmer, Mass., U.S.A.) equipped with a programmed temperature vaporizer, a flame ionization detector (FID), and a capillary column of 100 m × 0.25 mm ID and a film thickness of 0.20 µm using a stationary phase of 50% cyanopropyl methyl silicone (Supelco, Bellefonte, Pa., U.S.A.). The carrier gas, helium, was introduced at a flow rate of 20 cm/s. The oven temperature program was as follows: 120 °C for 5 min, 5 °C/min ramp-up to 165 °C for 5 min, and then 10 °C/min ramp-up to 240 °C for 20 min. The split ratio was 1/60, and the FID temperature was 260 °C. Fatty acids were identified by comparison with retention times of external standards (Supelco™ 37 component FAME MIX). Fatty acids concentration were calculated by a comparison with the pure standard retention time and were based on response factors to convert peak areas into weight percentages.

Analyses of lipid alteration and oxidation

The detection of primary and secondary oxidation was performed spectrophotometrically (San Martín and García, 2001).

This analysis consisted of measuring two parameters (K_{232} , K_{270}). K_{232} is a measure of the concentration of conjugated dienes, that are primary products of fatty acid oxidation. K_{270} is a

measure of the concentration of conjugated trienes that are secondary products of fatty acid oxidation. UV specific extinction determination permits a good approximation of the oxidation process in unsaturated oils (Gutierrez et al., 1992; Lopez et al., 1997; San Martin and Garcia, 2001). To measure K_{232} , K_{270} , an oil sample of 100 mg was placed in a 10 mL flask and diluted to 10 mL with spectrophotometer grade hexane (Sigma-Aldrich). The sample was then homogenized and the absorbances at 232 and 270 nm were measured with a spectrophotometer (Varian UV-Vis 4000, Varian, Palo Alto, U.S.A.) using pure solvent as blank (Garcia et al., 1996). Free fatty acids (FFA) were measured by direct titration of the nuts oil extract with 0.1 N NaOH, using phenolphthalein as an indicator. Free fatty acid contents of oil samples were determined in accordance with methods no. 2.201 of IUPAC (1987). The peroxide value (PV) was determined using the extracted oil and estimated by iodometric titration assay, which is based on the oxidation of the iodide ion by hydroperoxides (ROOH). A saturated solution of potassium iodide is added to oil samples to react with hydroperoxides. The liberated iodine is then titrated with a standardized solution of sodium tiosulfate and starch as an endpoint indicator. The PV is obtained by calculation and reported as milliequivalents of oxygen per kilogram of samples (meq/kg); the official determination is described by method no. 2.501 of IUPAC (1987). All analyzes were performed in triplicate.

Statistical analyses

Analysis of variance (one-way ANOVA) were performed for all examined parameters to test significant differences among treatments, using Duncan's test ($P < 0.05$) for mean separation, (SPSS version 19.0 software, SPSS, Chicago, IL, USA).

3.1.3 Results

Vegetative growth, fruit growth and yield

Foliar fertilization affected significantly the vegetative growth of hazelnut trees in the second year of the trial, since it was observed that the average length of lateral shoots per branch and the single leaf area were greater in CD trees compared to control trees, while the number of shoots per branch was not affected by fertilization in both the years (Tab. 1). In 2012, the total leaf area per branch was higher in fertilized plants compared to control (Fig. 1). Indeed, it was observed that total leaf area per branch was similar in all the treatments at the beginning of the growing season, whereas in CD sprayed trees on mid-May, it increased more than control and

B sprayed trees (393 cm², 307 cm² and 317 cm², respectively). At the end of shoot growth measurements (mid June) the total leaf area per branch of B sprayed trees had rapidly increased, reaching similar value of CD trees (Fig. 1)

The net photosynthetic rate during the growing season in both the years, showed for all the treatments a similar pattern, reaching the highest levels in mid-May and then decreasing till the harvest (Fig 2). Treatments significantly affected this parameter only on the second date of measurement in 2012 (11 May), when it was higher in CD trees than B and control treatments (9.27 μmol/m²*s, 7.68 μmol/m²*s and 7.42 μmol/m²*s, respectively).

Tree size was very similar in all the treatments at the beginning of the experiment as indicated by the trunk cross sectional area measured on April 2011 (Fig. 3). The vegetative growth of the CD sprayed trees increased, from the beginning of the second year (2012) of the trial, resulting significantly higher in 2013 (Fig. 3).

The cumulative growth of the fresh weight of the fruits showed no significant differences between treatments in the years 2011 and 2012 (Fig. 4). However, the cumulative growth of the dry weight of the fruits results to be higher for fertilized plants in the first ten days of September (Fig. 4). In addition, significant differences were found at the end of June 2011, where the plants fertilized with boron archived a dry weight of the fruits highest then the other two treatments, and at the end of July 2012, where the plants fertilized archived a dry weight of the fruits highest then the control.

Foliar treatments affected yield components in 2013, since both the yield expressed in kilograms and the number of fruit per tree were higher in the CD treated trees than B fertilized plants at harvest time (Fig. 5).

Biometrical variability in fruit and kernel

The biometric characteristics of fruits and kernels at harvest showed significant differences between treatments in the three years of the experiment. The volume of the fruits was higher in fertilized trees compared to the control in the years 2011 and 2013, while it was lowest in the B treatment in 2012 (Tab. 2). The shell weight was greater in CD treatment in the first two years of trial, while in the last years it was higher in CD and B trees compared to control (Tab. 2). The percentage of blank fruits was lower in fertilized trees compared to the control in the years 2012 and 2013, while in the first year of the experiment only the CD plants had the lowest incidence of blanks at harvest. Finally, the dry weight of kernels in the years 2011 was higher in CD plants and in other two years it was greater in CD and B fertilized trees (Tab. 2).

Kernel composition and total polyphenols content

Difference in percentage of fat, protein, carbohydrates and ashes are showed in the years 2012 and 2013 between treatments. The fat content achieved in both years higher percentages (about 64%) in CD and B treatments compared to control (about 60%) (Fig. 6 and 7). In 2013, protein percentage was greater in CD (19.3 %), while it was lower in other two treatments (around 15%). Instead, the control had the highest percentage of carbohydrates (23.4%) , followed by B with 19% and CD with only 15.5%. No difference was archived for the ashes content in both years with values around 2% (Fig. 7). The total polyphenols content was affected by foliar fertilization in the year 2013, the kernels of CD and B treatments had a higher values (153.4 and 147.7 mg/kg, respectively) then the control (128.7 mg/kg) (Fig. 8).

Oil fatty acid composition

From hazelnut oil analysis were identified thirteen different fatty acids. The most significant effect of foliar application was the increase of unsaturated fatty acids (UFA) (Fig. 9 and 10). Indeed, in 2012, the fat of the fruits treated with B had a percentage of UFA of 90.6%, while the one treated with CD of 90.3%, compared with only 90.1% of control (Fig. 9). In 2013, the effect was similar but with CD treatment higher than B (respectively 91.8 and 91.4%), also this year both greater than the control (91.1%) (Fig. 10). Further differences were observed in the quantity of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (Fig. 9-10). In 2012, the fruits B treated had the highest percentage of MUFA (83.2 against 82.8% of C), while the one CD treated had highest percentage of PUFA (7.4 vs. 7.3 of C) (Fig. 9). In 2013, the percentage of MUFA in fertilized nuts was higher (84%) than the control (83.5%), while CD treated nuts showed, in general, an increased percentage of PUFA compared to the control (7.8 against 7.4, respectively) (Fig. 10).

Lipid alteration

Foliar fertilization positively affected the stability of the lipid fraction of hazelnuts (Tab. 4). In the last two years of the experiment, it was observed that the fruits CD and B treated had lower values for all parameters considered, such as free acidity, peroxide value and absorption coefficients (K_{232} and K_{270}).

3.1.4 Discussion

Foliar fertilization affected vegetative growth, especially CD treatment showed a greater shoots extension, total leaf area and trunk cross-sectional area compared to control. The highest total leaf area recorded in mid-May (Fig. 1) was mainly due to the higher rate of net photosynthesis that was recorded in the same period for the CD leaves (Fig. 2b). The increased vegetative growth of CD sprayed trees might be due to the larger availability of nitrogen at leaf level which in turn may increase the concentration of chlorophylls. Since nitrogen is an integral part of these pigments, its increase could be related to the higher photosynthetic net rate (Tisdale et al., 1997). These results are in agreement with Tomar and Singh (2007), who reported highest trunk and shoot extension growth following foliar application of urea in walnut. Furthermore, Hussain et al. (2007) also reported that maximum trunk diameter, leaf area and biomass weight were recorded in pecan seedlings sprayed with nitrogen, while in hazelnut, has been found that nitrogen increased shoots length (Painter, 1955; Painter and Hammar, 1962).

Also boron and zinc foliar nutrition has been reported to enhance tree growth, indeed Keshavarz et al. (2011) observed a consistent increase of vegetative growth in walnut trees under foliar application of B and Zn. Such a response it is supposed to be due to zinc and boron roles as activators of several enzymes in plants, since they are directly involved in biosynthetic pathways of endogenous growth regulator substances such as auxins (Barker and Pilbeam, 2010). This was confirmed in pecan, where best results on trunk and shoot extension growth was recorded with combined applications of urea, boron and zinc (Ashraf et al., 2013). In our experiment, foliar treatments did not affect the production in 2011 and 2012, while a slight decrease (2%) on the B treated plants in 2013 was recorded (Fig. 5). These results are in contrast to some research carried out in Oregon (Painter and Hammar, 1964; Baron et al., 1985; Cacka and Smith, 2009), but they agree with the results of experimental works accomplished in the Mediterranean area conditions (Ferran et al., 1997; Borges et al. 2001; Silva et al., 2003, Tous et al., 2005). These differences can be explained by considering the climatic conditions; Pacific Northwest springs are cool and moist, whereas Mediterranean springs are warmer. In addition, in these different geographical areas different cultivars are used.

In general, foliar spray significant increased kernel weight in comparison with the untreated control (Tab. 2), and this result is in agreement with other foliar fertilization trials performed in hazelnut (Borges et al. 2001; Tous et al., 2005; Cacka and Smith, 2009; Nicolosi et al., 2009). This effect support the hypothesis that foliar nutrition influences the development of

shell and embryo. In particular, it is known that boron is important in the development of cell walls and cell membranes functions and metabolic activities (Blevins and Lukaszewski, 1998). Moreover, boron and zinc are involved in the biosynthesis of auxins, which induces more plant cells production and more dry matter accumulation (Barker and Pilbeam, 2010). Foliar fertilization also decreased the percentage of blank compared to the control. This result is fully in agreement with those obtained in other experiments that included B foliar fertilization with or without zinc (Solar and Stampar, 2001; Borges et al. 2001; Serdar et al., 2005; Nicolosi et al., 2009; Cacka and Smith, 2009). The beneficial effect of foliar B application on blank incidence can be partially explained by an increase of pollen tube growth. Nevertheless, since boron is needed in the tissue formation processes, foliar application prior to or immediately following bloom may increase cell division and improve fruit set (Crassweller et al., 1981).

It has also been reported that foliar fertilization affects the complex mechanism of dry matter transport and transformation in the seeds (Li et al., 1988). The increase in elemental constituents of seed may be due to the effect of micronutrients on stimulating biological activities, such as enzyme activity, chlorophyll synthesis, translocation rate of photosynthetic products and increased nutrient uptake through roots after foliar fertilization (Barker and Pilbeam, 2010). Boron is known to be a fundamental element involved in a large number of metabolic pathways (sugar transport, respiration, carbohydrate, RNA, IAA and phenol metabolism) (Parr and Loughman, 1983). Boron and zinc appeared to be capable of increasing oil percentage in olive fruits (Wiesman et al., 2002, Saadati et al., 2013). The increase of fat content in kernels treated with CD and B (Fig. 6 and 7) is in accordance with the results found in several oilseed species, including cotton (Sawan et al., 2001), rapeseed (Yang et al., 2009), peanut (Gobarah et al., 2006) and pecan (Ashraf et al., 2013). These results could be attributed to the impact of boron, with or without zinc, on both functional and structural mechanisms of enzyme activities within plant cell compartments (Parr and Loughman, 1983). The higher protein content of CD kernels (Fig. 6-7), is probably due to the increased availability of nitrogen. In pecan, foliar application of urea increased total nitrogen in plant tissue, which led to higher protein content in seeds due to the fact that nitrogen is a constituent of protein (Salisbury and Ross, 1992). Furthermore, also application of zinc increase seed protein content, and Shchitaeva (1984) found that the synthesis of metabolically active amino acids depends on zinc application, through an increased synthesis of asparagines and tryptophan. Similar results were obtained in pecan (Ashraf et al., 2013) and peanut (Abd and Mona, 2013). The greater carbohydrates content in control kernels (Fig. 6 and 7), can be

explained by the fact that the synthesis of lipids and proteins in the seeds occurs at the expense of carbohydrates. Analogue results have been found in almond (Bi et al., 2004) and in rapeseed (Asare and Scarisbrick, 1995). The higher total polyphenols content in foliar fertilized kernels might be due to the role of boron in the metabolism of phenolic compounds (Parr and Loughman, 1983). Boron is one of nutrients responsible for change in concentration and metabolism of phenolic compounds in vascular plants (Cakmak et al., 1995) and a boron deprivation resulted in an increased activity of polyphenoloxidase, enzyme that catalyses the oxidation of phenolic compounds (Pfeffer et al., 1998). This response is in agreement with what was found in olive after foliar fertilization with boron and zinc (Saadati et al., 2013).

Fat plant metabolism is quite a complex process, which mainly takes place in plastid and endoplasmic reticulum. There is a lack of information in the specific literature on the effect of microelements on fatty acid composition of hazelnuts. In the present experiment, boron application improved oil quality of kernels, but it seems that fatty acid composition responded differentially to different nutrient spraying. Indeed, oleic acid content was increased in both the year by the boron application, whereas the effect of CD treatments was found only in the last year (2013). On the contrary, a detrimental effect on the percentage of palmitic acid on treated plants compared to control was recorded (Tab. 3). These results were in agreement to those reported by Desouky et al. (2009), who showed that foliar boron application, alone or simultaneously to zinc (Saadati et al., 2013), on olive trees can affect fatty acid compositions. The higher linoleic acid content in fatty composition of CD kernels (Tab. 3) is fully in agreement with Dag et al. (2009) and could be explained by the nitrogen fertilization. This fatty acid modification may be due by the enhancement or inhibition of oleate desaturase activity during triacylglycerol biosynthesis (Dag et al., 2009). This effect can be explained by the fact that the nitrogen supply can extend the temporal duration of leaf photosynthetic activity, accelerating the seed maturity achievement. Considering that a highest polyunsaturated fatty acid accumulation is expected in fully ripened seed, this might be one of the reasons explaining the effect of nitrogen on increasing linoleic acid content (Ghasemnezhad and Honermeier, 2008). Since most part of PUFA is due to linoleic acid, its increase also explains the higher PUFA content in the CD kernels (Tab. 3). On the other hand, the higher content of UFA and the lower content of SFA of treated trees compared to control, can be explained by the higher oleic percentage and the lower palmitic percentage of fatty acid in foliar fertilized kernels (Tab. 3).

The higher lipid stability, expressed in terms of free acidity, peroxide value and extinction coefficients (K232 and K270), of fertilized hazelnuts compared to control, may be related to

the higher content of total polyphenols, which, functioning as antioxidant compounds, protect biological system against oxygen radicals (Bendini et al., 2007) and contribute to stabilize lipid content in olive (Servili et al., 2004). In hazelnut, total phenol content and antioxidant activity of several cultivars has been investigated by Jakopic et al. (2011) and their results demonstrated that the phenol content is highly related to the antioxidant activity into seeds.

3.1.5 Conclusions

Foliar fertilization of hazelnut trees has been demonstrated to be an efficient method to reduce the incidence of blank nuts at harvest, which in turn can positively affect the commercial value of yield. Furthermore, foliar application of nitrogen and micronutrients, such as boron and zinc, may improve some qualitative characteristics of nuts at harvest, mostly kernel dry weight. Nevertheless, it was found that also the nut composition may be improved by foliar nutrition, as the fat percentage was increased and also qualitatively improved by an increased percentage of un-saturated fatty acids. An influence of foliar sprayed nitrogen and micronutrients was observed also on the stability of kernel oil and it was related to a higher accumulation of total polyphenols, which antioxidant function tends to preserve the fat quality of nuts during the post-harvest period.

3.1.6 References

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Table 1. Effect of foliar nutrient applications on shoots number, shoots length and leaf area per branch. Different letters in the same column indicate significantly different value ($P<0.05$) by Duncan's test.

Year	Treatment	Shoots number	Shoots length (cm)	Leaf area (cm ²)
2011	C	7.36	2.47	22.71
	CD	7.62	2.38	23.72
	B	7.96	2.27	22.45
2012	C	7.48	2.23 b	23.29 b
	CD	7.60	3.13 a	26.40 a
	B	7.51	2.88 ab	24.13 b

Table 2. Effect of foliar nutrient applications on fruit and kernel quality at harvest time. Different letters in the same column indicate significantly different value ($P<0.05$) by Duncan's test.

Year	Treat.	Fruit			Kernel	
		Volume (mm ³)	Shell weight (g)	Blank (%)	Volume (mm ³)	Dry weight (g)
2011	C	19731 c	1.23 b	5.5 a	8482 c	1.05 b
	CD	21226 a	1.32 a	3.2 b	9175 a	1.10 a
	B	20564 b	1.29 ab	4.5 ab	8885 b	1.08 ab
2012	C	20845 a	1.45 b	5.6 a	8643 a	1.18 b
	CD	20719 ab	1.48 a	4.0 b	8692 a	1.22 a
	B	20276 b	1.45 b	2.1 c	8469 b	1.20 a
2013	C	21111 b	1.38 b	6.4 a	9498	1.20 b
	CD	22444 a	1.47 a	4.9 b	9577	1.26 a
	B	22397 a	1.48 a	4.2 b	9285	1.23 a

Table 3. Effect of foliar nutrient applications on fatty acid composition (%) of hazelnut kernel oil . Different letters in the same column indicate significantly different value ($P<0.05$) by Duncan's test.

Year	Treat.	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1 n9 t	C18:1 n9 c	C18:2 n6 c	C18:3 n6	C20:1	C18:3 n3	C20:3 n6	C23:0
2012	C	6.026 a	0.178	0.043	0.067	3.742 a	0.021	82.436 b	7.023 b	0.147 b	0.132	0.109	0.031	0.045
	CD	5.998 a	0.178	0.043	0.065	3.637 b	0.017	82.461 b	7.128 a	0.152 a	0.119	0.117	0.031	0.052
	B	5.918 b	0.184	0.042	0.065	3.429 c	0.018	82.795 a	7.098 ab	0.142 c	0.113	0.117	0.029	0.050
2013	C	6.182 a	0.205 a	0.041	0.060	2.668 b	0.013	83.170 b	7.220 b	0.116	0.141	0.128	0.028	0.028
	CD	5.609 b	0.169 b	0.033	0.055	2.523 c	0.017	83.595 a	7.576 a	0.102	0.136	0.133	0.023	0.027
	B	5.747 b	0.154 c	0.035	0.055	2.769 a	0.016	83.677 a	7.126 b	0.108	0.156	0.106	0.026	0.026

Table 4. Effect of foliar nutrient applications on free acidity, peroxide value, and extinction coefficients of hazelnut kernel oil. Different letters in the same column indicate significantly different value ($P < 0.05$) by Duncan's test.

Year	Treat.	Free acidity (% Oleic acid)	Peroxide value	K ₂₃₂	K ₂₇₀
2012	C	1.07 a	4.37 a	1.76 a	0.56 a
	CD	1.04 b	0.53 c	1.50 b	0.22 c
	B	1.05 ab	2.03 b	1.57 b	0.29 b
2013	C	0.66 a	7.62 a	2.11 a	1.10 a
	CD	0.28 c	6.57 b	1.47 c	0.58 c
	B	0.33 b	6.93 b	1.91 b	0.94 b

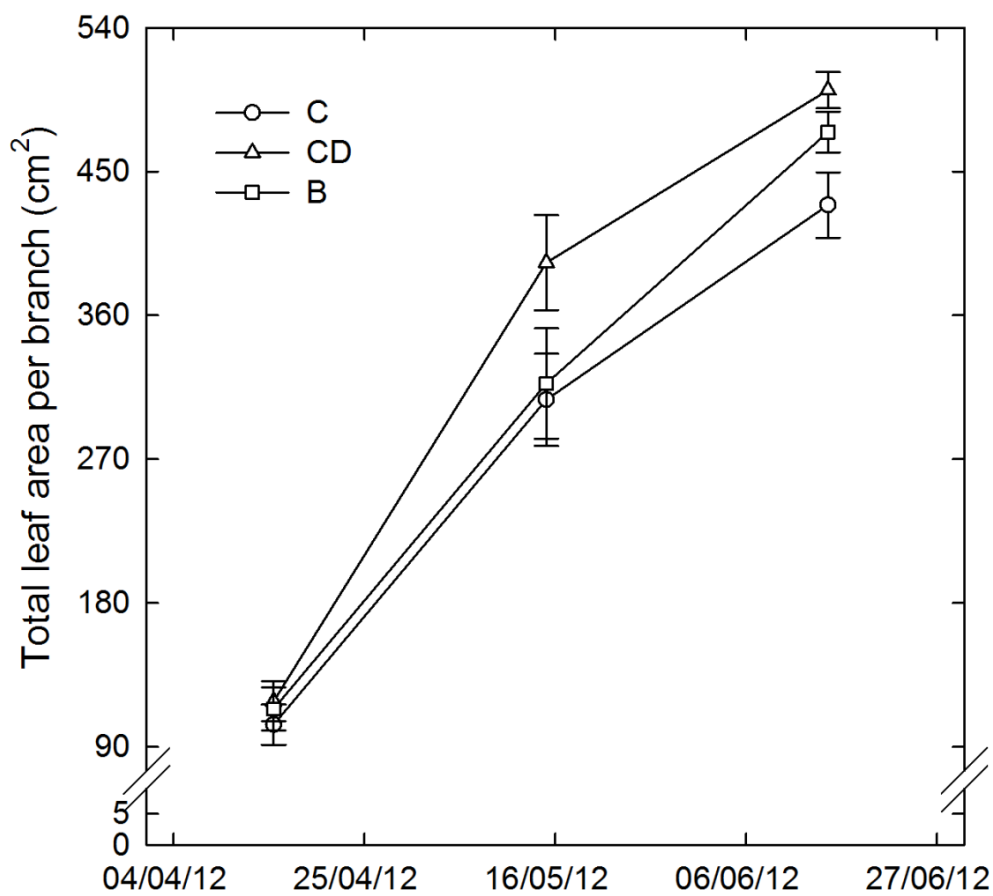


Figure 1. Effect of foliar nutrient applications on total leaf area per branch on three dates during the growing season 2012. Error bars represent the standard errors of mean for each treatment.

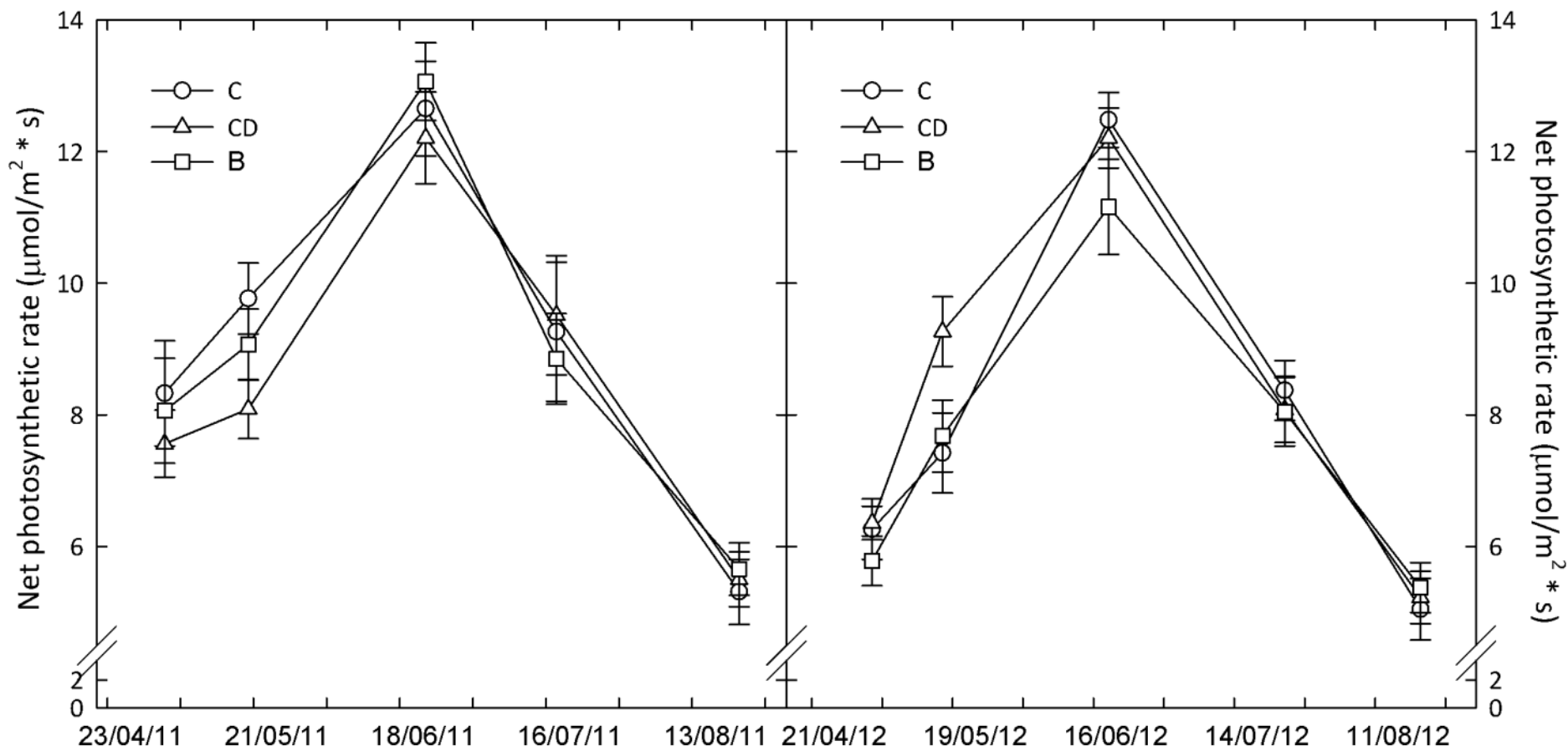


Figure 2. Effect of foliar nutrient applications on net photosynthetic rate during growing seasons 2011 and 2012. Error bars represent the standard errors of mean for each treatment.

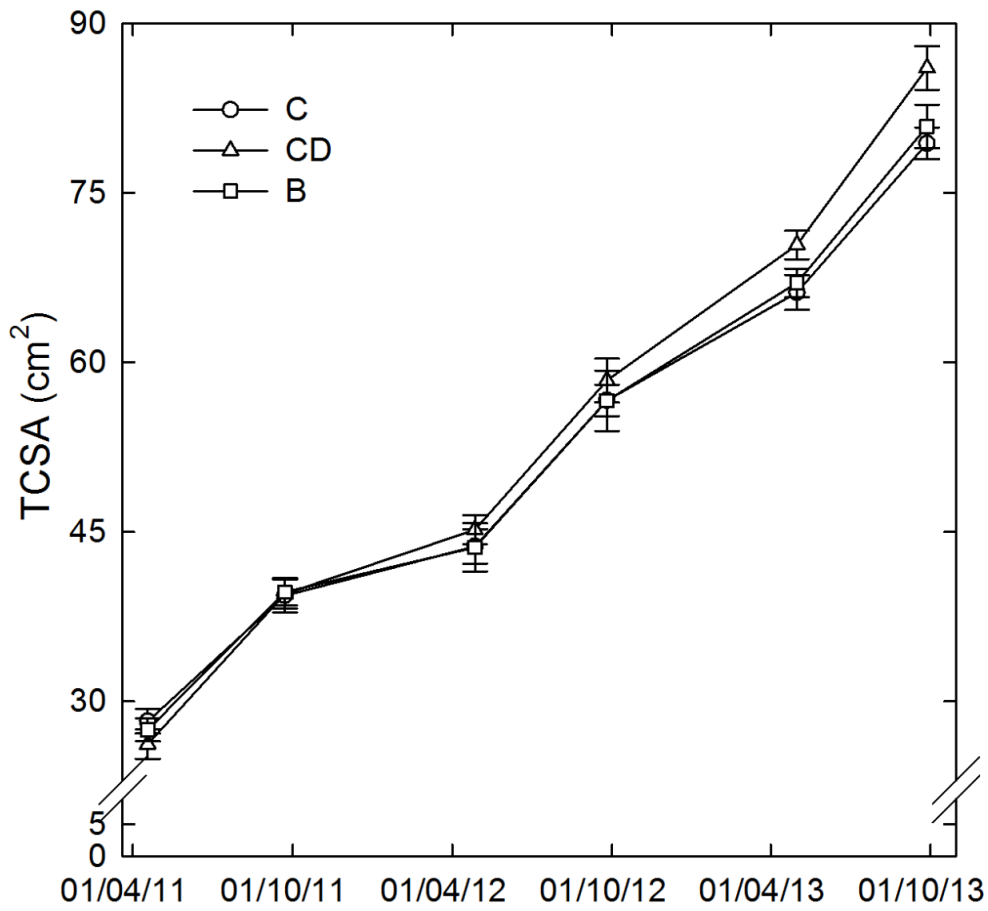


Figure 3. Effect of foliar nutrient applications on trunk cross-sectional area growth (TCSA) from spring 2011 to autumn 2013. Error bars represent the standard errors of mean for each treatment.

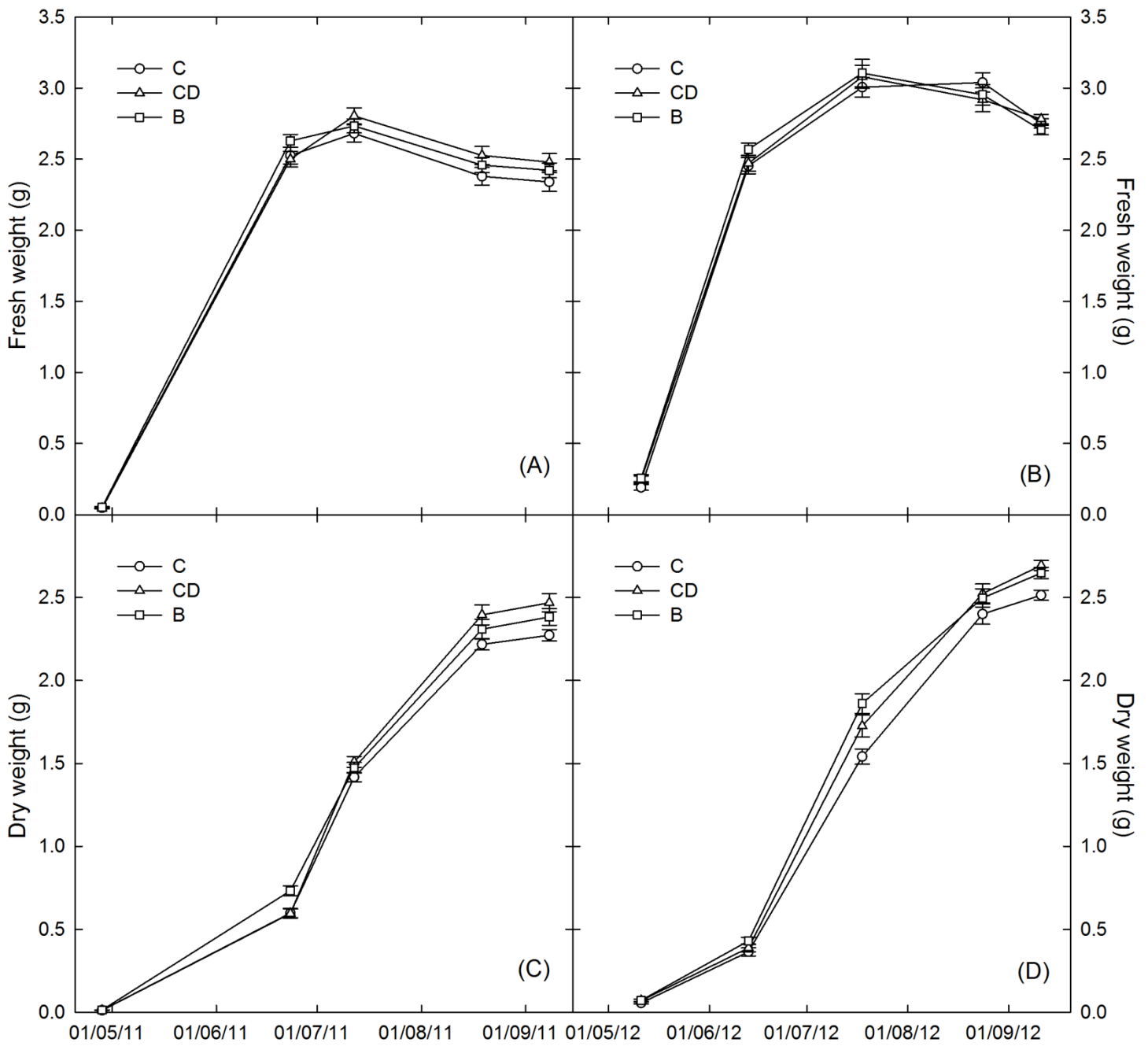


Figure 4. Effect of foliar nutrient applications on fruit fresh (A and B) and dry weight (C and D) during the growing seasons 2011 (A and C) and 2012 (B and D). Error bars represent the standard errors of mean for each treatment.

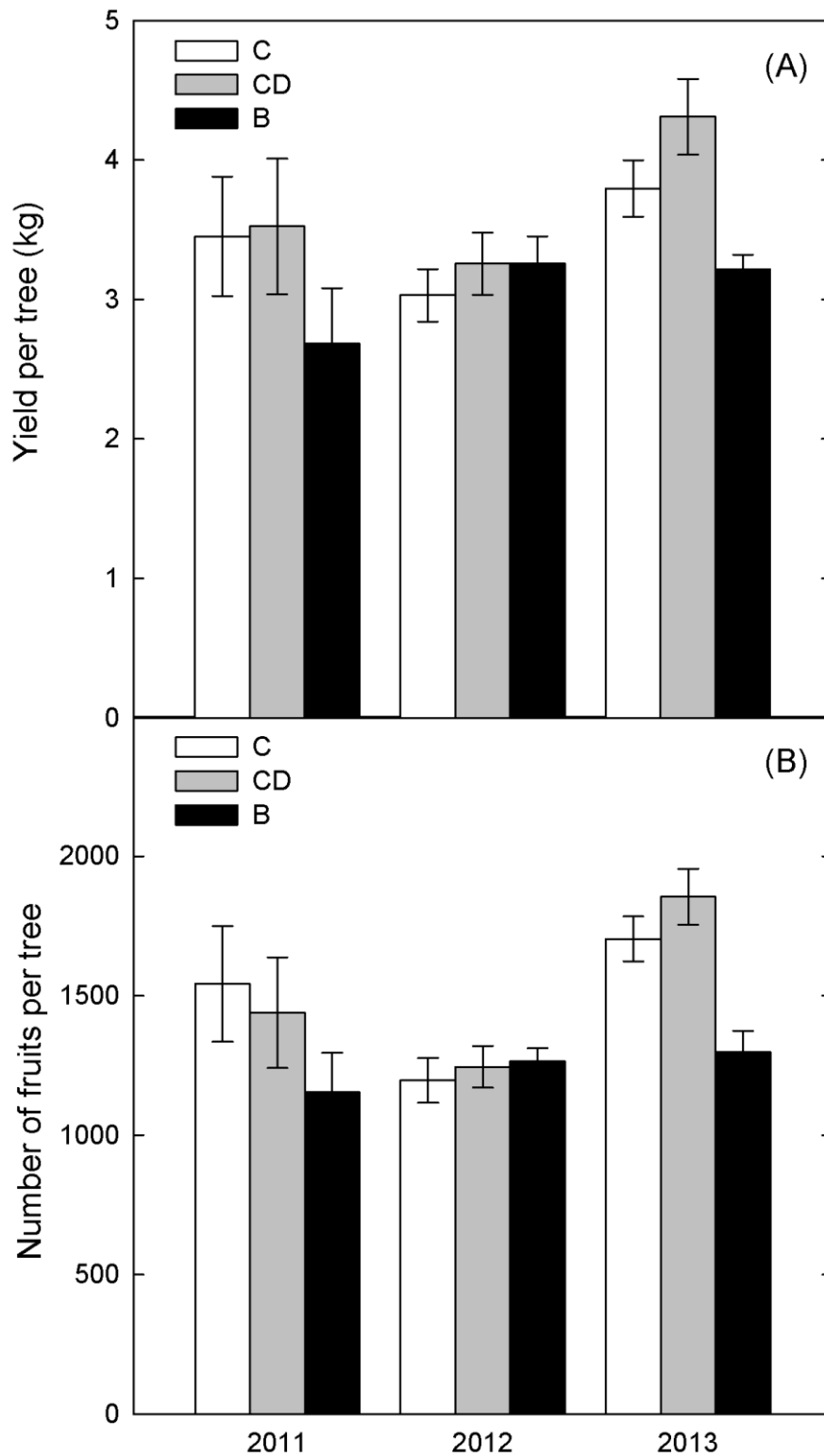


Figure 5. Effect of foliar nutrient applications on (A) yield and (B) fruit number per tree at harvest in 2011, 2012, and 2013. Error bars represent the standard errors of mean for each treatment.

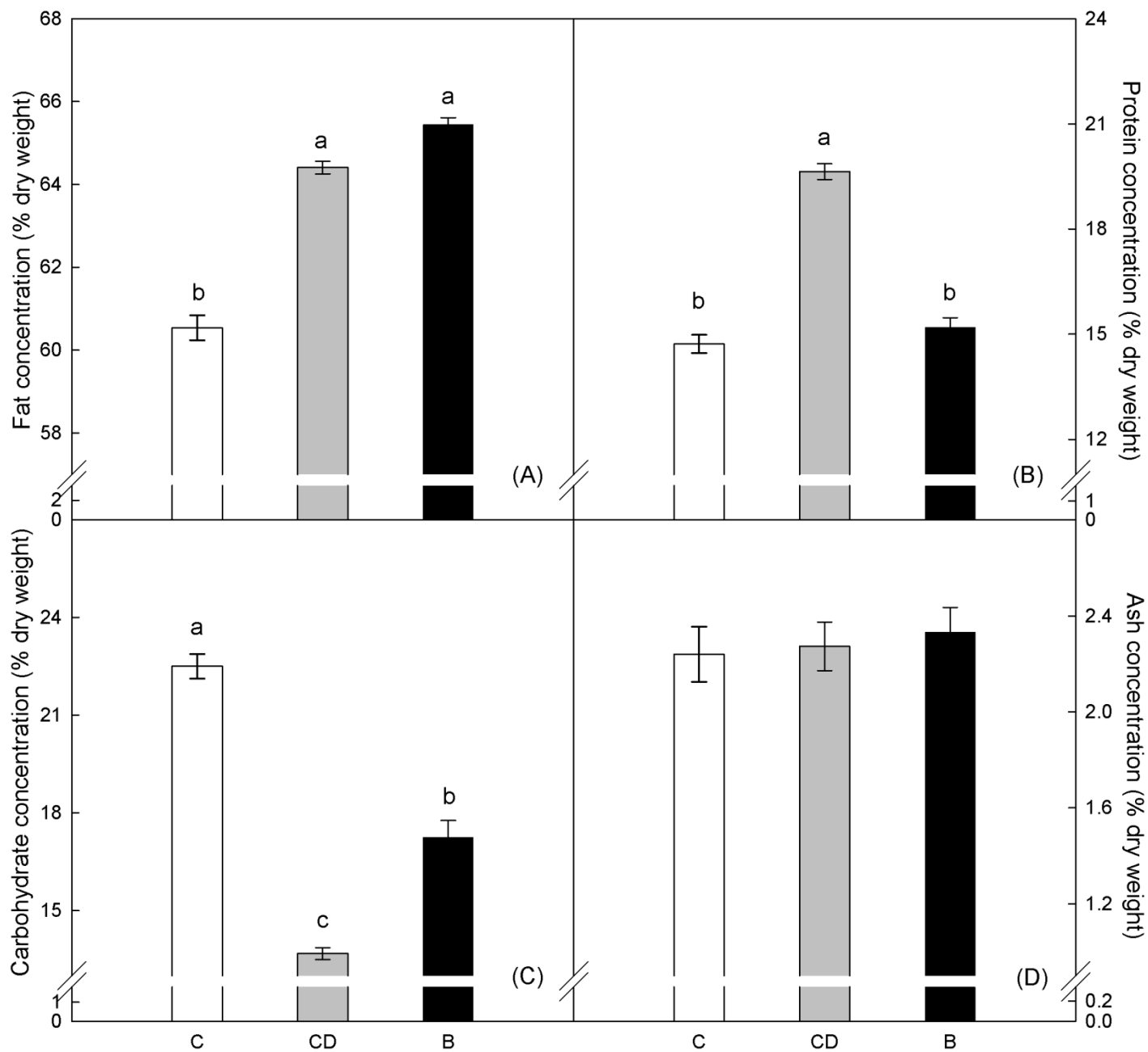


Figure 6. Effect of foliar nutrient applications on kernel composition at harvest in 2012. Error bars represent the standard errors of mean for each treatment, different letters in the same graph indicate significantly different value ($P < 0.05$) by Duncan's test.

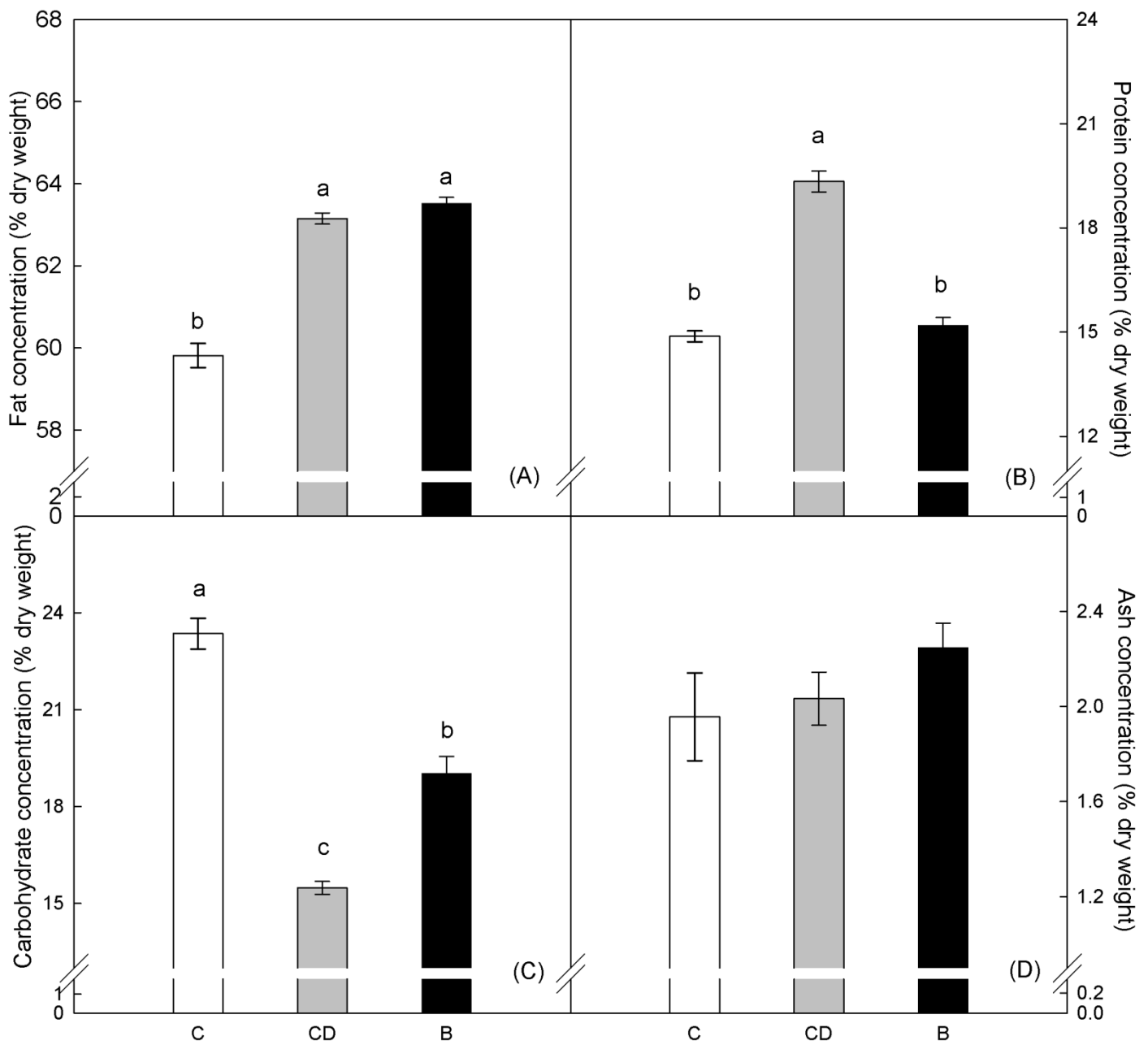


Figure 7. Effect of foliar nutrient applications on kernel composition at harvest in 2013. Error bars represent the standard errors of mean for each treatment, different letters in the same graph indicate significantly different value ($P < 0.05$) by Duncan's test.

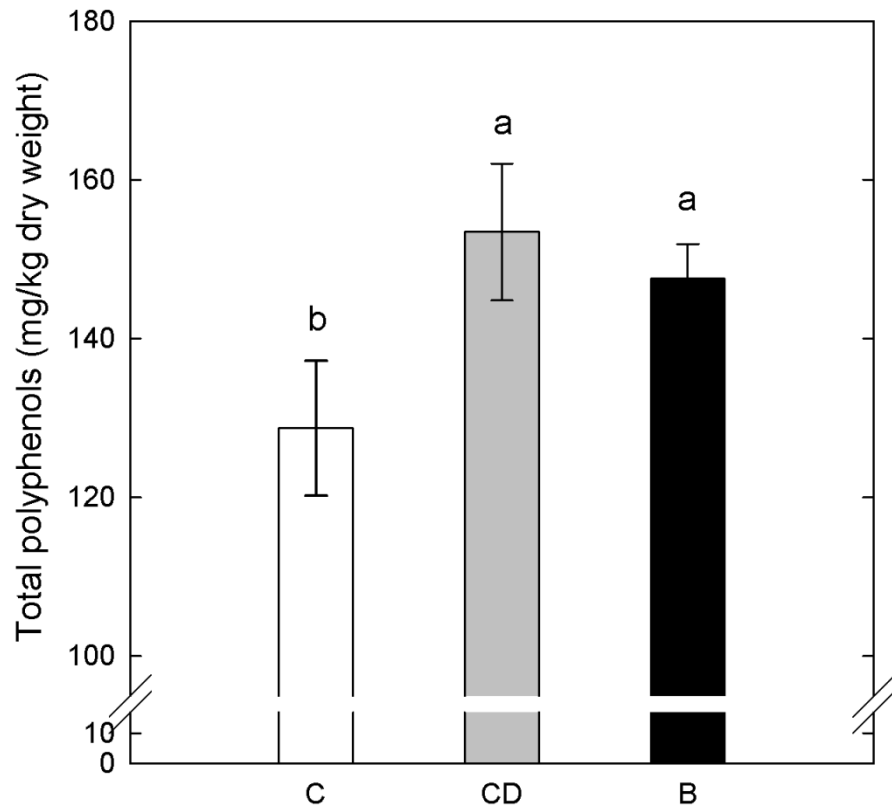


Figure 8. Effect of foliar nutrient applications on kernel total polyphenol concentration at harvest in 2013. Error bars represent the standard errors of mean for each treatment, different letters indicate significantly different value ($P < 0.05$) by Duncan's test.

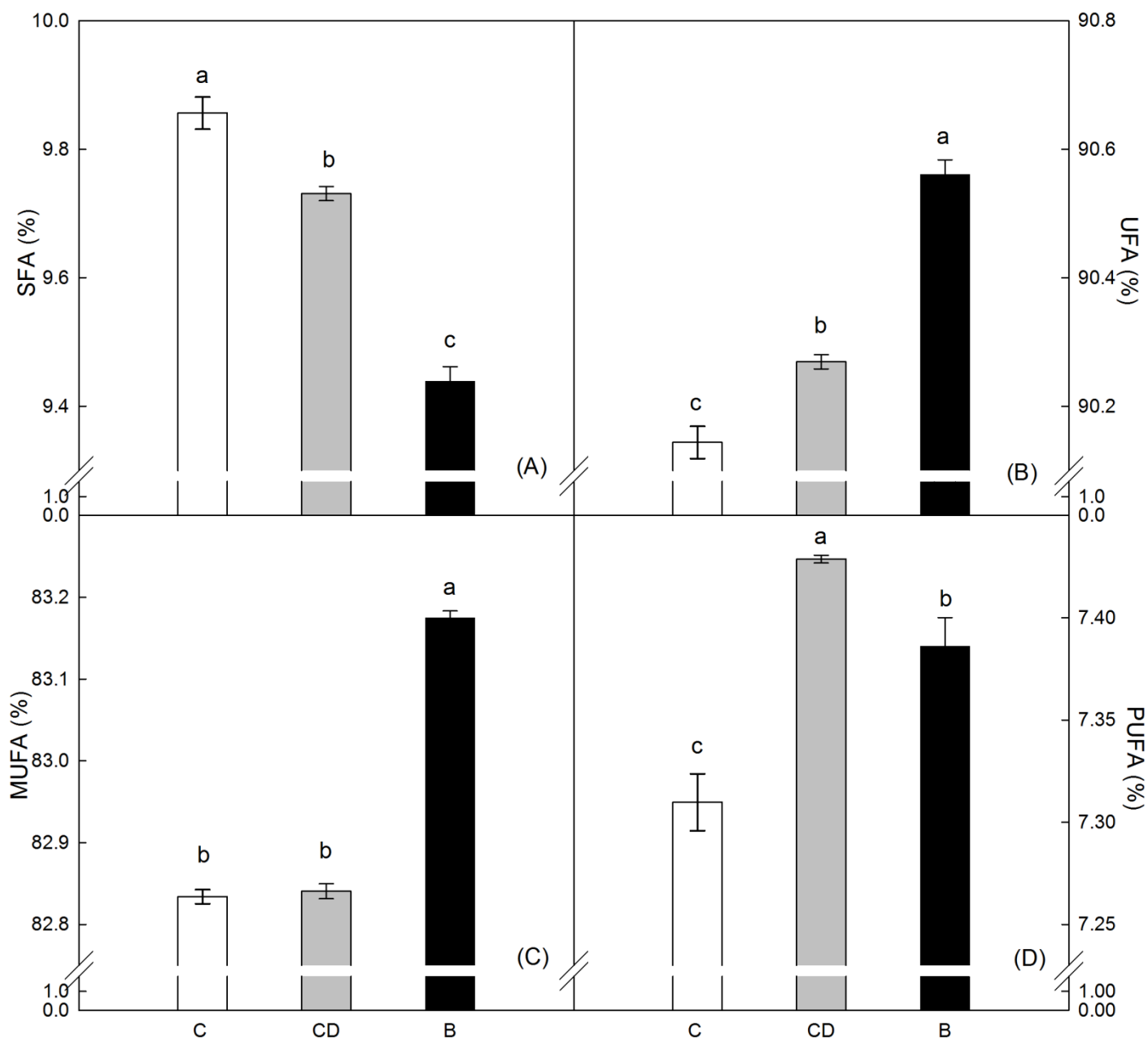


Figure 9. Effect of foliar nutrient applications on fatty acid composition of kernel oil at harvest in 2012. Error bars represent the standard errors of mean for each treatment, different letters in the same graph indicate significantly different value ($P < 0.05$) by Duncan's test.

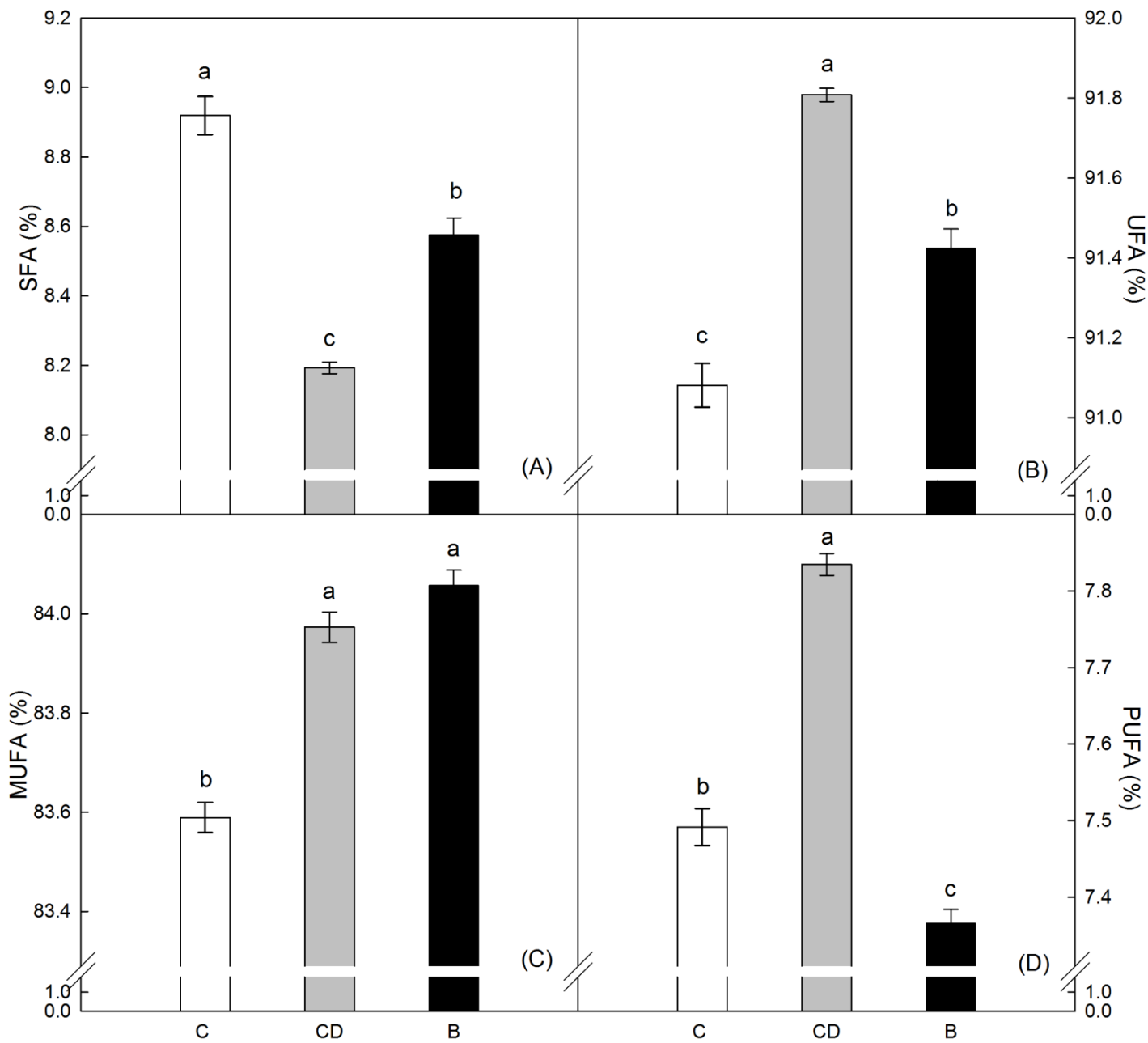


Figure 10. Effect of foliar nutrient applications on fatty acid composition of kernel oil at harvest in 2013. Error bars represent the standard errors of mean for each treatment, different letters in the same graph indicate significantly different value ($P < 0.05$) by Duncan's test.

3.2 Intracanopy variability of fruit composition in hazelnut trees

3.2.1 Introduction

After Turkey, Italy is the second hazelnut producer in the world (128940 Mg per year; FAOSTAT, 2013). More than 30% of the Italian surface area cultivated with hazelnuts is located in Campania, a region in Southern Italy (ISTAT, 2010). Hazelnut production is important for fresh consumption and for confectionery, cosmetic and pharmaceutical industry (Mehlenbacher, 1991; Kris-Etherton et al., 1999). For this reason, the definition of quality in hazelnut production may include differing parameters depending on the destination use of the product. Indeed, confectionary industry may be interested in quality parameters like kernel spheroidal shape, shell/seed ratio, kernel water content, fruit size, and the percentage of empty nuts and of defected kernels (broken, bitter, molded kernels, etc.). On the other hand, consumers and cosmetic and pharmaceutical industries may also be interested in hazelnuts because of the specific seed composition. At commercial harvest the composition of hazelnut kernels is on average the following: 60% of fats, 17% of carbohydrates, 15% of crude proteins, 4% of ashes, and 4% of moisture (Oliveira et al., 2008). Therefore, fats are an important component in seed composition (Savage et al., 1997). The fat fraction of hazelnut kernels is rich of monounsaturated fatty acids (MUFA; oleic acid represents around 81% of total fatty acids) and of polyunsaturated fatty acids (PUFA; linoleic acid represents around 10% of total fatty acids), and poor of saturated fatty acids (SFA; palmitic and stearic acid represent around 5% and 2% of total fatty acids, respectively) (Oliveira et al., 2008). MUFAs and PUFAs are considered to play a positive role in the prevention of cardiovascular disease (Ruxton et al., 2004; Orem et al., 2008; Durak et al., 1999). Moreover, fat concentration and composition are important in defining hazelnut organoleptic characteristics (Botta et al., 1994) and shelf life (Serra Bonvehi and Ventura Coil, 1993; Ozdemir et al., 2001). Despite of its importance, the factors affecting hazelnut kernel composition are still not fully understood. Many studies have focused on comparing different cultivars (Parcerisa et al., 1993; Pershern et al., 1995; Savage et al., 1999; Silva et al., 2007; Oliveira et al., 2008; Petriccione et al., 2010; Milòsevic and Milòsevic, 2012) or growing areas (Greve et al., 1992), demonstrating that genetic and environmental factors can significantly affect kernel composition in hazelnuts. In other fruit tree crops, fruit flesh composition has been reported to vary largely within the canopy of single trees (Smith et al., 1997; Broom et al., 1998; Forlani et al., 2002; Luchsinger et al., 2002; Lewallen and Marini, 2003). For instance, the concentration of soluble solids, ascorbic acid, and mineral content in fruit flesh tends to decrease from the top to the bottom and from the exterior to the interior of the canopy (Campbell and Marini, 1992;

Lewallen and Marini, 2003; Montanaro et al., 2006; Hagen et al., 2007). Similar intra-canopy patterns have been described for fat concentration in the flesh of olive fruits (Gomez-del-Campo and Garcia, 2012). This variability has been often explained by the inhomogeneous distribution of light inside tree canopy (Marini et al., 1993; Luchsinger et al., 2002). Indeed, fruit trees are characterized by partial branch autonomy for carbohydrate (Marsal et al., 2003) and this represents an advantage in terms of carbon partitioning of the fruits located close to well-illuminated leaves compared to fruits positioned in the interior and/or bottom part of the canopy (Lewallen and Marini, 2003). There is no information in the literature about the effect of fruit position within the canopy on seed growth and composition of any fruit or nut tree species. The aim of this research was to study the intra-canopy variability of kernel growth and composition in mature hazelnut trees.

3.2.2 Materials and methods

Plant material and experimental design

In August 2013, ten 10-year-old hazelnut trees (*Corylus avellana* L.), cv. ‘Mortarella’, with homogeneous vigor and crop load were selected in a private orchard located in Caianello (Caserta, Southern Italy). Trees were trained to an open vase and spaced 4 m × 3 m. Tree rows were oriented North-South. Tree canopies were divided in six horizontal layers. Each layer was 45-50 cm tall and was divided equally in an inner zone and an outer zone to have a total of 12 canopy sectors (six interior and six exterior sectors). The availability of photosynthetic active radiation (PAR) inside each canopy sector was measured on 5 September at nine times of the day (hourly measurements, from 10:30 am to 18:30 pm), by placing, horizontally in five different points inside each canopy sector, a PAR sensor connected to the datalogger of a ceptometer (AccuPAR LP-80, Decagon Devices, Pullman, WA, USA).

On 25 August, before the beginning of hazelnut fall, five infructescences (with three hazelnuts each) were selected in each canopy sector in each tree. Since in hazelnuts, the definition of a visual maturity index is more complicated compared to other fruit tree species, to harvest all the selected hazelnuts at similar maturity stages, we used as a maturity index the natural detachment of the fruit from the tree. With this aim, on 25 August each selected infructescence was enclosed in a 20 cm × 20 cm plastic net bag tied to the fruiting shoot bearing the selected fruits. This allowed the fruits to naturally drop without falling on the ground. Every 4-5 days the net bags were inspected to check if the fruit were dropped. On 24

September all the selected fruits resulted naturally dropped inside the net bags and all the fruit separately harvested. At harvest, the height of each selected infructescence from the ground was measured with a semi-rigid measuring tape. On the same date, a sample of eight leaves was collected in each canopy sector per tree (a total of 80 leaves per canopy sector). The area of each sampled leaf was measured with a leaf area meter (LI-3100, LI-COR, Inc., Lincoln, NB, USA). In addition, dry weight of each leaf was measured after being dried in a ventilated oven at 60 °C until constant weight. The specific leaf weight of each leaf was calculated as the ratio of leaf dry weight to leaf area.

Fruit biometrical analysis

Fifty hazelnuts per canopy sector were sampled to measure individual fruit and kernel fresh and dry weight. The latter was measured by drying shells and kernels of the same fruit in a ventilated oven at 60 °C until constant weight. Moisture content was calculated as the difference between fresh weight and dry weight. Dried kernels were stored at -20 °C until composition analyses.

Kernel composition

Following the biometrical analysis, the same kernels were used to measure total ash, fat, protein, and carbohydrate concentration. Total ash was determined by weighting the samples (500 mg of finely ground kernels) after incineration at 550 °C for 3 h in a muffle furnace according to the AOAC method (1995). Total fat was extracted with a Soxhlet extractor (SER 148/3, Velp Scientifica, Italy). Briefly, 5 g of finely crushed kernels were placed in a cellulose thimble and extracted with 30 mL of petroleum ether (boiling point 40-60 °C) for 6 h (AOAC, 1995). The extracted oil was stored at -20 °C until analysis of fatty acid composition and lipid oxidation. Total crude protein ($N \times 5.30$) was determined by the macro-Kjeldahl method (AOAC, 1995). Carbohydrate content was calculated according to Olivera et al. (2008), using the following equation: carbohydrate content = 100% - (% moisture + % protein + % fat + % ash).

Fatty acid composition was analyzed by gas chromatography after derivatization to fatty acid methyl ester (FAME) with a solution of KOH 2N in methanol as described by Romano et al. (2012). A GC Perkin Elmer AutoSystem XL (PerkinElmer, Mass., U.S.A.) equipped with a programmed temperature vaporizer, a flame ionization detector (FID), and a capillary column of 100 m × 0.25 mm ID with a film thickness of 0.20 µm and using a stationary phase of 50% cyanopropyl methyl silicone (Supelco, Bellefonte, Pa., U.S.A.). The carrier gas, helium, was

introduced at a flow rate of 20 cm/s. The oven temperature program was the following: 120 °C for 5 min, 5 °C/min ramp-up to 165 °C for 5 min, and then 10 °C/min ramp-up to 240 °C for 20 min. The split ratio was 1/60 and the FID temperature was 260 °C. Fatty acids were identified by comparison with retention times of external standards (Supelco™ 37 component FAME MIX). Fatty acid concentrations were calculated by a comparison with the pure standard retention time and were based on response factors to convert peak areas into weight percentages.

The detection of primary and secondary oxidation was done spectrophotometrically (San Martín and García, 2001). This analysis consisted in measuring two parameters (K_{232} , K_{270}). K_{232} is a measure of the concentration of conjugated dienes, that are primary products of fatty acid oxidation. K_{270} is a measure of the concentration of conjugated trienes that are secondary products of fatty acid oxidation. UV specific extinction determination permits a good approximation of the oxidation process in unsaturated oils (Gutierrez et al., 1992; Lopez et al., 1997; San Martín and García, 2001). To measure K_{232} and K_{270} , an oil sample of 100 mg was placed in a 10 mL flask and diluted to 10 mL with spectrophotometer grade hexane (Sigma-Aldrich). The sample was then homogenized and the absorbances at 232 and 270 nm were measured with a spectrophotometer (Varian UV-Vis 4000, Varian, Palo Alto, U.S.A.) using pure solvent as blank (García et al., 1996). The analyzes were carried out in triplicate.

Statistical analyses

The relationship between fruit position within the canopy expressed as height above the ground and daily average of available PAR, specific leaf dry weight, K_{232} parameter and, fat, ash, palmitoleic acid, oleic acid and linoleic acid concentration was assessed by quadratic regression analysis. The relationship between fruit position within the canopy expressed as height above the ground and kernel fresh weight, kernel dry weight and, protein, eptadecanoic acid, eptadecenoic acid, stearic acid and linoleic acid concentration was assessed by linear regression analysis. The significance of effect of canopy layer position on total fat, carbohydrate, protein and ash content per kernel was assessed by one-way ANOVA using Duncan ($P < 0.05$) as post-hoc test for mean separation. The statistical analyses were processed using SPSS version 19.0 software (SPSS, Chicago, IL, USA).

3.2.3 Results

Daily average of PAR increased curvilinearly with fruit height above the ground (Fig. 1A). Specific leaf weight increased from the bottom (around 4 mg cm⁻²) to the top of the canopy (around 11 mg cm⁻²) (Fig. 1C). Both kernel fresh and dry weight increased linearly with fruit height above the ground (ranging between 0.96 and 1.23 g kernel⁻¹ and between 0.92 and 1.18 g kernel⁻¹, respectively) (Fig. 1B and 1D). Seed moisture content was not affected by fruit position inside the canopy (an average of 4%). Total fat, carbohydrate, protein, and ash content per kernel increased progressively from the bottom to the top canopy layers (Tab. 1). Also kernel composition was significantly affected by fruit position inside the canopy (Fig. 2 and 3). Fat concentration decreased progressively from the bottom to the top of the canopy (ranging between 60 and 64%), whereas protein and ash concentration increased with fruit height within the canopy (ranging, respectively, between 16.5 and 20% and between 1.8 and 2.1%). Carbohydrate concentration was not affected by fruit position in the canopy (Fig. 2B). A total of thirteen fatty acids were identified in hazelnut oil. In Fig. 3 we reported only those with concentration higher than 0.02%. Independent of the fruit position in the canopy, oleic acid was the most concentrated fatty acid. The concentration of this acid was maximum (85.5%) in kernels of fruits in canopy sectors located at intermediate height (between 160 and 270 cm) and lower at the bottom and the top of the canopy (Fig. 3F). The concentration of eptadecanoic acid (Fig. 2C), stearic acid (Fig. 2E), and γ linolenic acid (Fig. 3H) increased linearly with fruit height within the canopy, whereas the concentration palmitoleic acid (Fig. 2B), eptadeceic acid (Fig. 2D), and linoleic acid (Fig. 3G) decreased progressively from the bottom to the top of the canopy. The concentration of palmitic acid (average 5.1%) was not affected by fruit position within the canopy (Fig. 3A).

The extinction coefficient K_{232} decreased from 1.6 to 1.4 proceeding from the bottom to the top canopy layers (Fig. 4). Instead, K_{270} was not affected by fruit position within the canopy (average 0.2). All the relationships between the measured parameters and the height of fruit location from the ground were similar for the internal and external canopy sectors.

3.2.4 Discussion

Fruit position inside the canopy appears to be an important factor in determining seed growth and composition in hazelnut trees. Kernel growth and dry matter accumulation in the seed increased progressively from the bottom to the top canopy layers. This is in agreement with what reported for the fruits in other tree species (Campbell and Marini, 1992; Basile et al.,

2007). The intracanalopy variability in kernel fresh and dry weight appeared to be explained by light distribution inside the canopy. Indeed, light availability also increased from the bottom to the top of the canopy (Fig. 1A) as also described previously for other species (Marini and Marini, 1983; Lewallen and Marini, 2003). In addition, in this study we found that specific leaf weight increased from the bottom to the top of the canopy. This is in agreement with what previously reported for peach trees (Basile et al., 2007). Specific leaf weight has been positively correlated with the amount of photosynthetically active radiation intercepted by the leaf in several fruit species (Weinbaum et al., 1989; Marini and Sowers, 1990; Li and Lakso, 2004). In addition, specific leaf weight is highly correlated with leaf photosynthetic capacity (Marini and Marini, 1983), leaf nitrogen per unit leaf area (Weinbaum et al., 1989; Rosati et al., 2000), and daily carbon assimilation (Rosati et al., 1999). Therefore, our results suggest that carbon partitioning to hazelnut seeds is higher in fruit located close to strong source organs (well illuminated leaves characterized by high specific leaf weight) compared to fruits grown close to shaded leaves with low specific leaf weight. In a previous study on peach trees, absolute fruit growth rate was positively correlated with specific leaf weight (Basile et al., 2007). The increase in dry matter accumulation in the kernel of fruit located in the top canopy layers resulted also in an increase in total accumulation per kernel of the single constituents of dry matter (fats, carbohydrates, protein, and ashes) (Table 1).

In addition to the effect on total dry matter accumulation in the seed, fruit position within the canopy also affected differentially the composition of kernel dry matter (Fig. 2). Seed located at the top of the canopy accumulated higher amounts of dry matter, but the dry matter was composed by a higher fraction of protein and ashes and a lower concentration of fats compared to kernels progressively located at lower heights within the canopy. This result was unexpected, because in plants fat synthesis also depend on carbohydrate availability (Ohlrogge and Jaworski, 1997; Conde et al., 2008). Previous studies on hazelnut composition reported that large kernels have a lower fat concentration than small seeds (Balta et al., 2006; Cristofori et al., 2008). Larger investment in the protein fraction may be required when seed growth occurs under non limiting conditions to support large fat biosynthesis in each kernel.

Furthermore, the microclimatic differences brought about by canopy position may influence the accumulation of mineral nutrients in the fruit and thus their ash content. High light and temperature promote fruit transpiration and are positive drivers for mineral nutrient transport to the fruits (White and Broadley, 2003). In our experiment, ash concentration in the kernel was positively correlated with fruit height above the ground (Fig. 2D). This result is in agreement with Morandi et al. (2010) who reported that in exterior canopy sectors higher

transpiration rates during the early stages of fruit development may be a positive driving force for mineral nutrient accumulation in the fruits.

Quality of fatty acid is important to define healthiness of food (Ruxton et al., 2004; Orem et al. 2008; Durak et al., 1999). In addition, fat composition plays an important role in product storability. A concentration increase of unsaturated fatty acids enhances oxidation reactions, that worsen the problems of rancidity during the storage of both nuts (Greve et al., 1992) and oil (Sherwin, 1978). Little is known about the relationship between temperature and/or solar radiation and their effect on the fatty acids synthesis in nut trees, while there are many reports for olive trees and field crops such as soybean, peanut or sunflower (Dornbos and Mullen, 1992; Golombek et al., 1995; Izquierdo et al., 2002). Gomez-del-Campo and Garcia (2012) reported that in olive tree fruits harvested from higher canopy layers exhibited significantly higher stability against oxidation, along with higher palmitic and linoleic acid concentrations, but lower oleic acid concentration. In our experiment fruit position within the canopy affected significantly fatty acid concentration (Fig. 5). The concentration of saturated fatty acids like stearic acid (C18:0) and eptadecanoic acid (C17:0) was positively correlated with fruit height above the ground (Fig. 3E, C). The concentration of unsaturated fatty acids like linoleic acid (C18:2 n6c), palmitoleic acid (C16:1), and eptadecanoic acid (C17:1) was negatively correlated with fruit height above the ground (Fig. 3B, D, G). Temperature plays an important role in fatty acid metabolism (Harris and James, 1969; Wolf et al., 1982). Harris and James (1969) reported that the increase in fatty acid unsaturation that occurs at low temperatures is due to the increase in the oxygen in solution. In plants, low temperature induces the synthesis of unsaturated fatty acid to increase cellular membrane fluidity (Iba, 2002; Upchurch, 2008). Many studies in the literature showed that the concentration of the different fatty acids changes in response to slight variations in temperature (Harwood et al., 1994; Izquierdo et al., 2009; Golombek et al., 1995). In our study we did not measure the temperature of fruits located in different canopy sectors, but it is well known that fruit temperature is positively correlated with exposure to sunlight (Thorpe, 1974; Smart and Sinclair, 1976; Saudreau et al., 2011). Since in our experiment, we found that light availability progressively increased from the bottom to the top of the canopy (Fig. 1A), we can hypothesize that similar intra-canopy patterns occurred also in fruit temperature (at least in some moments of the day). This may explain why the level of fatty acid unsaturation decreased from the bottom to the top of the canopy. An increase in the concentration of unsaturated fatty acids is desirable because it will improve the nutritional value of the hazelnut kernels, but at the same time it makes the fats more susceptible to auto-oxidation reactions (Frankel, 1991). Oxidation of linoleic or

linolenic acid produces hydroperoxides and conjugate dienes that absorb UV light. Hydroperoxides from linoleic acid, as well as conjugate dienes, that may result from their decomposition show absorbance at 232 nm (Gutierrez et al., 1992). This is in agreement with the results of our experiment, since we found that K_{232} decreased from the bottom to the top of the canopy (Fig. 4), following a similar pattern of that of linoleic acid concentration (Fig. 3G). A correlation between K_{232} and the linoleic acid concentration was reported in hazelnut by Bonvehi and Coll (1993). K_{270} indicates the amount of secondary products of fatty acid oxidation (Lopez et al., 1997); in our experiment this parameter was low (around 0.25) and was not correlated with fruit position inside the canopy probably because these molecules (mainly conjugated trienes) are generally produced during storage (Gutierrez et al., 1992).

In our study we found that the concentration in hazelnut kernels of palmitic acid did not change in response to fruit position in the canopy, whereas γ linolenic acid concentration increased linearly from the bottom to the top of the canopy. These results are not in agreement with a previous study reporting in olive trees that (a) the concentration in the fruit flesh of palmitic acid increased from the bottom to the top of the canopy, and (b) the concentration of γ linolenic acid was not affected by fruit position in the canopy. Differences between the results may be due to the fact that different tissues were analyzed in the two studies (fruit flesh for olive trees and seed for hazelnuts).

3.2.5 Conclusions

For the first time we reported on the large variability occurring in seed growth and composition within hazelnut canopies. Dry matter partitioning to kernels increased from the bottom to the top of the canopy and this correlated with a similar increasing patterns in light availability. As a result kernels located in the top canopy layers are larger and contain larger total amounts of fats carbohydrates, proteins, and ashes compared to seeds located in lower layers of the canopy. Dry matter composition was also significantly affected by fruit position within the canopy. Indeed, fat concentration decreased, while protein and ash concentration increased, from the bottom to the top layers of the canopy. Also fat composition significantly changed depending on fruit position within the canopy. The level of unsaturation of fatty acids decreased from the bottom to the top of the canopy. This made the kernels located in the bottom layers of the canopy more interesting from a nutritional point of view, but their fat fraction may be more exposed to oxidation.

3.2.6 References

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Table 1. Total fat, carbohydrate, protein and ash content per kernel for fruit located in six canopy layers. Different letters indicate significantly different value ($P < 0.05$) by Duncan's test.

Canopy layer	Average height above the ground (cm)	Fat content (g/kernel)	Carbohydrate content (g/kernel)	Protein content (g/kernel)	Ash content (g/kernel)
A	351.31	71.00 a	23.25 a	21.35 a	2.47 a
B	302.03	70.72 a	22.54 a	19.93 b	2.32 a
C	250.08	70.71 a	20.25 b	19.20 b	2.01 ab
D	192.59	62.64 bc	17.65 c	17.16 c	1.80 bc
E	150.63	63.77 b	17.89 c	15.69 e	1.78 c
F	96.07	61.02 c	15.66 d	16.71 d	1.75 c

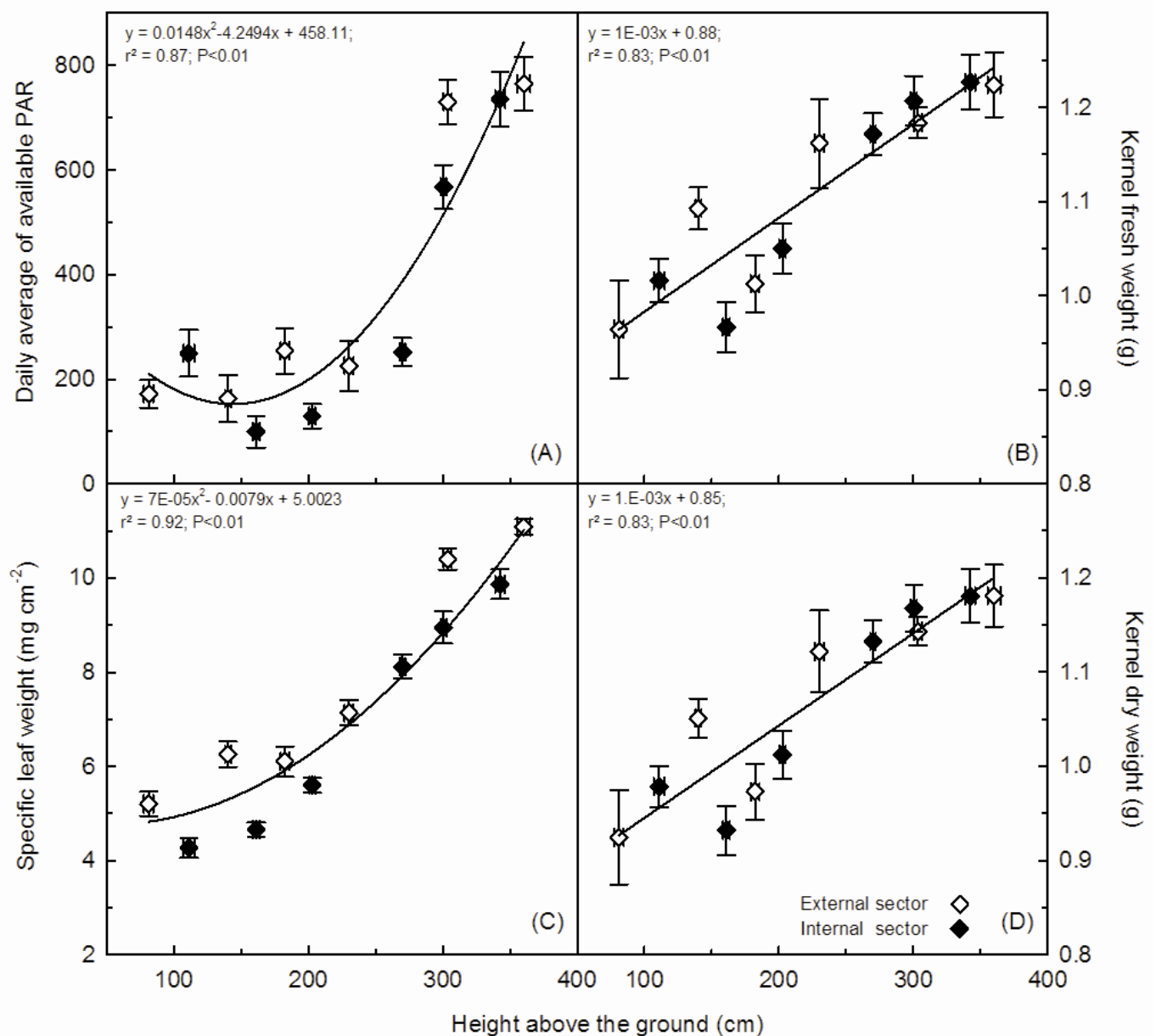


Figure 1. Relationship between fruit position within the canopy expressed as height above the ground and (A) daily average of available PAR, (C) specific leaf dry weight, (B) kernel fresh and (D) dry weight. Different symbols were used for fruits located in external and internal canopy sectors.

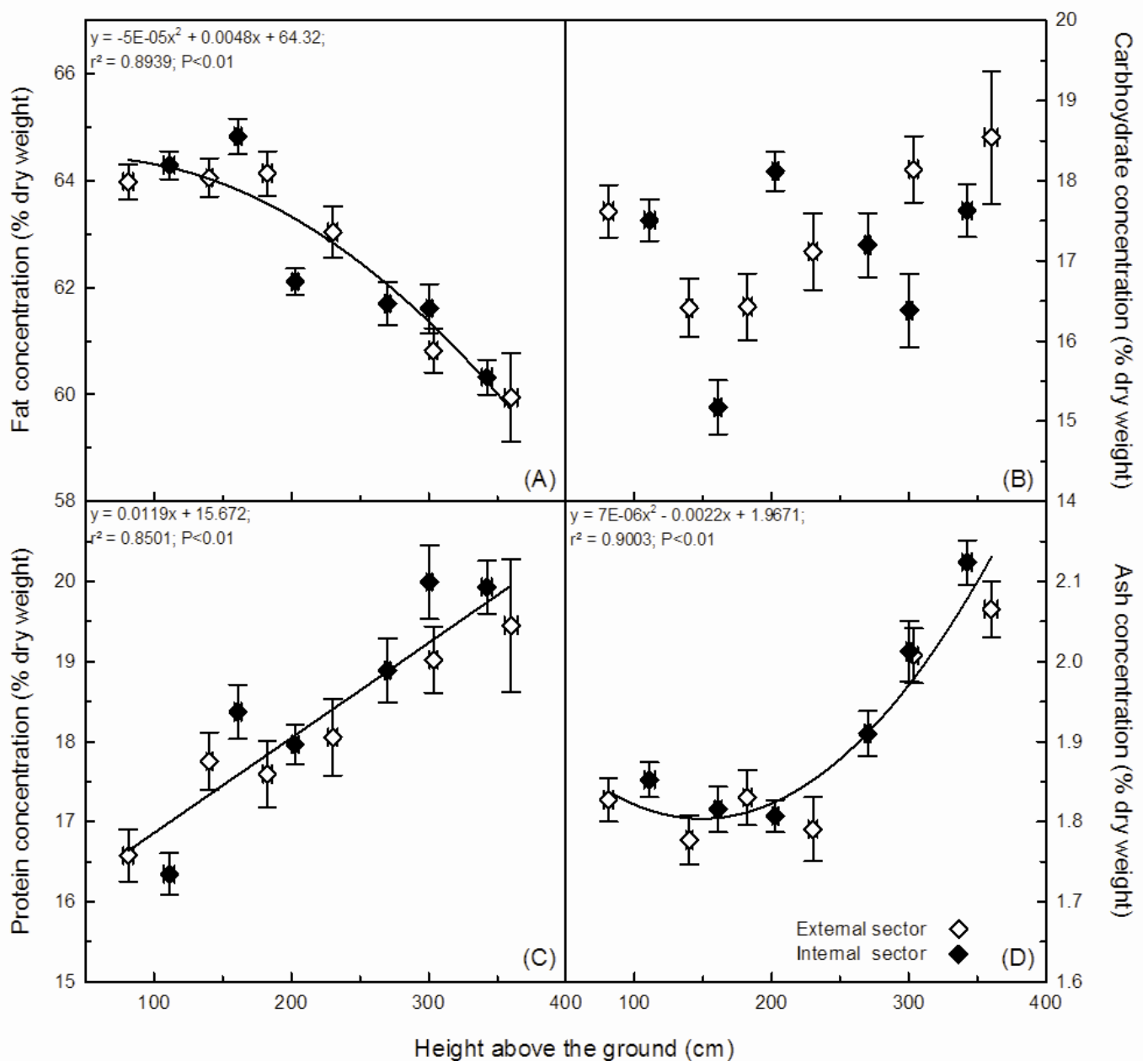
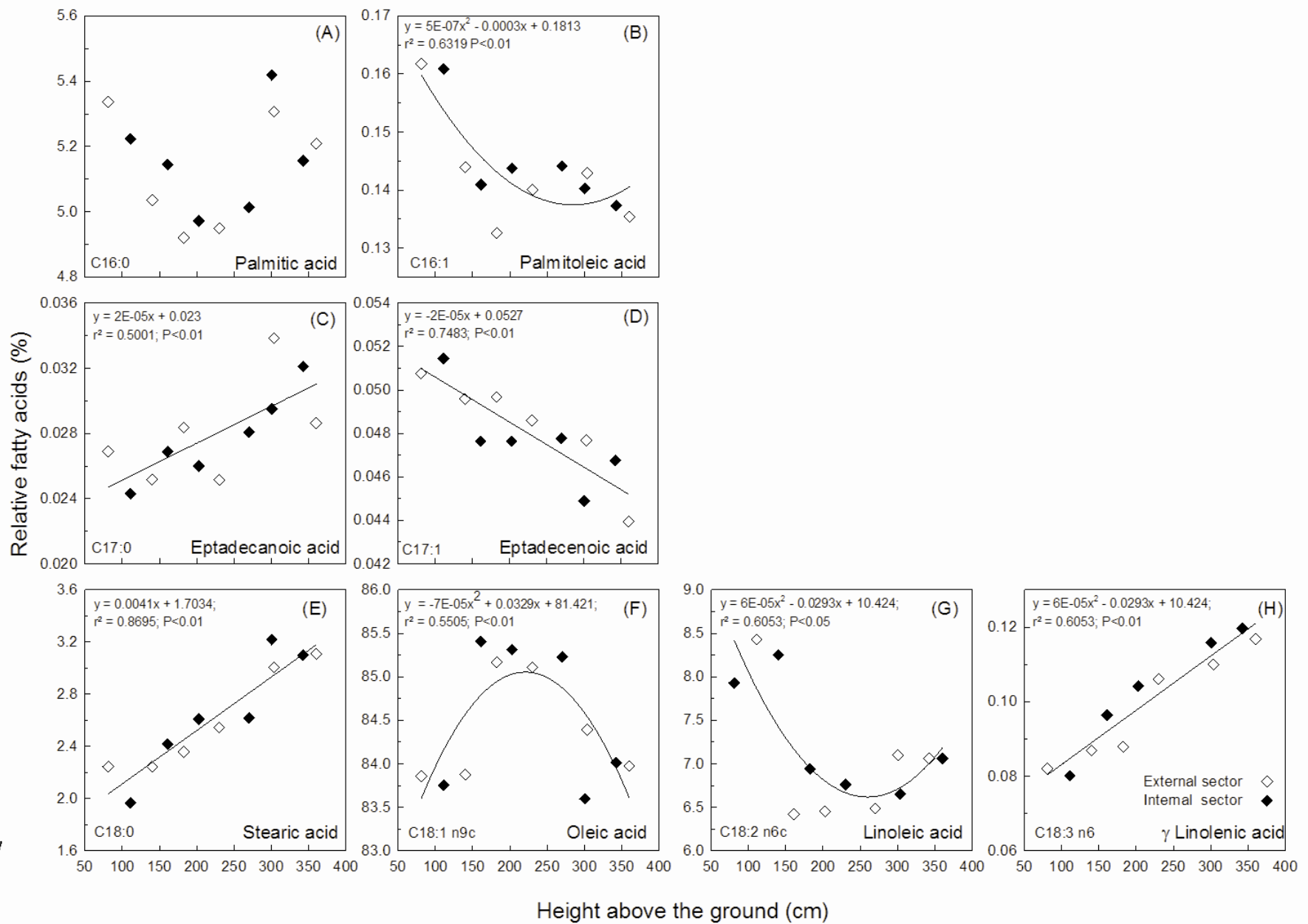


Figure 2. Relationship between fruit position within the canopy expressed as height above the ground and (A) fat concentration, (B) carbohydrate concentration, (C) protein concentration, and (D) ash concentration in kernels located in external and internal canopy sectors.

Increasing number of carbon atoms



Increasing number of unsaturations

Figure 3. Relationship between fruit position within the canopy expressed as height above the ground and concentration in kernel oil of (A) palmitic acid, (B) palmitoleic acid, (C) eptadecanoic acid, (D) eptadecenoic acid, (E) stearic acid, (F) oleic acid, (G) linoleic acid, and (H) linoleic acid. Different symbols were used for fruits located in external and internal canopy sectors. 66

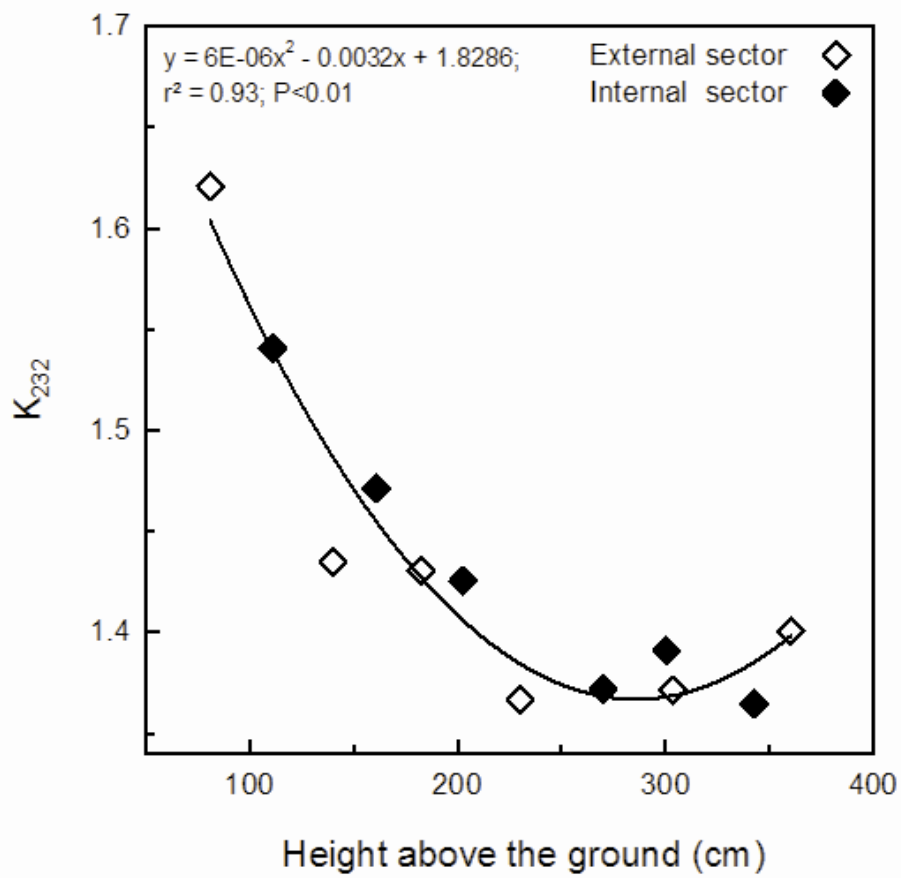


Figure 4. Relationship between K_{232} of kernel oil and fruit position within the canopy expressed as height above the ground. Different symbols were used for fruits located in external and internal canopy sectors.

3.3 Hazelnut lipid oxidation measurement by NIR spectroscopy

3.3.1 Introduction

Hazelnut (*Corylus avellana L.*) fruits are considered throughout the world as raw material for the confectionary, bakery and chocolate industries. The nuts are a valuable source of protein and oil and they are sold both shelled and unshelled, or processed to add color, flavor, texture, and fiber to various foods (Özdemir and Akinci, 2004). Furthermore, they are a source of bioactive compounds such as phytosterols and vitamin E (Delgado et al., 2010). Hazelnut harvest starts, according to traditional cultivation practices, when nearly all the fruits have dropped. Hazelnuts might be drying for up to three to four weeks before harvest is carried out and this can expose the fruits to microbial contamination and to adverse weather conditions that affect the drying process which might affect storability and final nut quality. In addition unshelled nuts are usually stored in warehouses for multiple seasons. During storage nuts might be exposed to microbial contaminations and undergo changes in their chemical composition (Frazer and Lines, 1967). It is known that high moisture content can accelerate the development of micro-fungi that produce specific enzymes breaking down carbohydrates into their monomers. High moisture content can also hydrolyze lipids into free fatty acids (Ayerst and Budd, 1960; De Mello and Scussel, 2007). In nuts, these chemical reactions often result in a bitter taste perception by consumers (King et al., 1983). Another alteration that occurs during hazelnut harvest, processing, and storage is the autocatalytic oxidation of unsaturated fatty acids (Karel, 1985). Indeed, hazelnut have a high oil content (~60%) rich in unsaturated fatty acids (Parcerisa et al, 1993). As a result, this fruit is sensitive to rancidity development and therefore to the progressive loss of its nutraceutical value (Gardner, 1979). Vitamin E stability has been strongly correlated with lipid oxidation (Chun et al., 2006). Unsaturated lipids are sensitive to oxidation when exposed to oxygen, radiant energy and/or organic and inorganic catalysts, such as some metals and enzymes (Pershern et al., 1995; Ory et al., 1984; Karel, 1985). In hazelnut and other nuts (Pershern et al., 1995; Cavaletto et al., 1966) oxidation reactions are also affected by high temperature and water activity, especially during storage (Hadorn et al., 1977). The main products of lipid oxidation are the hydro peroxides, which can break down into secondary products such as aldehydes, alcohols and ketones. These secondary metabolites are often volatile substances and may cause the development of off-odor and off-flavor in foods (Gray, 1978). Furthermore, secondary metabolites, as well as peroxides and lipid-free radicals, can react with proteins and vitamins, causing severe loss in nutritional value (Gardner, 1979).

After harvest, hazelnuts are traditionally inspected manually by trained workers, who select the hazelnuts according to the colour and the presence of visible defects. However, the manual selection is a slow and expensive method, able to detect visible alterations but not lipid oxidation and internal defects of kernels. Moreover, this technique is suitable for shelled fruits and not for those marketed in shell, that amount to 10% of the world hazelnut production (FAOSTAT, 2013).

Near infrared (NIR) spectroscopy is used here as an alternative to the traditional quality evaluation method. Near-infrared spectroscopy (NIRs) is an efficient method for high-throughput screening and it might be able to detect the chemical characteristics of the fruit through the shell. Several studies demonstrated that NIR spectroscopy is able to characterize moisture content, lipid content and lipid quality in dry fruit, including peanut (Tillman et al., 2006; Govindarajan et al., 2009; Hirano et al., 1997), walnut (Jensen et al., 2001), macadamia (Guthrie et al., 2004), shea nut (Davrieux et al., 2010) and chestnut (Liu et al., 2010). In hazelnut, this technique has been used to detect flawed fruits (Moschetti et al., 2013), acidity and water content of kernels (Bellincontro et al., 2005). However, NIR spectroscopy has never been used to classify the degree of kernel defect or to estimate the level of lipid oxidation in unshelled and shelled hazelnuts. The objective of this study was to investigate the feasibility of NIR spectroscopy to estimate the level of lipid oxidation in unshelled and shelled hazelnuts.

3.3.2 Materials and methods

Nut samples

Twenty 5-kg samples (batches) of unshelled ‘Mortarella’ hazelnuts were harvested between 2008 and 2012 in fifteen orchards located in ten production areas in Campania (Southern Italy). Details about the plant material are reported in Table 1. All the samples were stored in a warehouse at room temperature until the beginning of the experiments in January 2013.

A first subsample of nuts was used to estimate lipid oxidation. Five randomly selected whole hazelnuts per batch (Table 1) were measured by NIR spectroscopy on two opposite sides, first as a whole kernel and then as a shelled kernel. After scanning, kernels were stored at -20 °C until lipid extraction.

A second subsample of approximately 20 kg of hazelnuts was collected randomly from all batches (1 kg per batch) (Table 1) and used to classify the nuts for defects. These hazelnuts were shelled and the whole kernels were externally evaluated to select a sample of 120

kernels covering a full range of external quality from healthy (no external mould and no superficial browning) to severely flawed kernels (external mould and dark surface). Each kernel was scanned on two opposite sides by NIR spectroscopy. After the scanning, each kernel was visually examined to evaluate the presence of mold outside and inside the seed, the external and internal color, and the texture. In addition, each kernel was smelled and tasted. For each of these seven attributes a score of 0.5, or 1 was assigned as reported in Table 2. A defect index (DI) was calculated by adding the seven separate scores, ranging from 0 (no defects) to 7 (all defects).

NIR spectra acquisition and analysis

Diffuse reflectance spectra were obtained on a Perkin-Elmer Spectrum One NTS spectrophotometer (Perkin-Elmer, Beaconsfield Bucks, UK) by scanning from 700 to 2500 nm using a resolution of 0.18 nm. Each spectrum was the average of five scans. Reflectance spectral data were transformed into absorbance ($A = \log_{10} R^{-1}$) using the software Spectrum v5.3.1 (Perkin-Elmer, Beaconsfield Bucks, UK). A NIR wavelength standard was used as white reference to spectrophotometer calibration. All spectra were processed and calibration equations were obtained using Unscrambler version 10.2 (CAMO, Oslo, Norway).

Raw spectra were pre-processed using various spectral pretreatments. Because of a high signal to noise ratio at the beginning and at the end of the spectra, the spectral range was reduced to 1100-2400. High frequency noise was filtered by applying a Savitzky–Golay filter. The best results were obtained applying multiplicative scattering correction (MSC) and first derivative with second order polynomial (nine points of smoothing). Similar pre-processing of NIR spectra data was applied in previous studies on hazelnut (Bellincontro et al., 2005; Moschetti et al., 2013). Partial least squares (PLS) analysis was used to develop calibration equations using full cross-validation. The performance of calibration and validation models was evaluated by comparing the coefficient of determination (R^2). The model's predictive performance was assessed by the root mean square error of cross validation (RMSECV) (Nicolaï et al. 2007).

Lipid extraction

Oil was extracted according to a slightly modified protocol of Uysal et al. (2009). Each kernel was finely ground in a mortar with liquid nitrogen. The flour obtained was placed in a test tube and 10 ml of hexane and 1 g of sodium sulphate anhydrous (Sigma-Aldrich) were added. After shaking for 60 minutes, the suspension was centrifuged (6000 rpm, 10 min) and the

supernatant was recovered in a tube and placed in a vacuum concentrator (Thermo Scientific, Waltham, USA) to remove the solvent. The residual oil was stored at -20 °C until analysis.

Analyses of lipid oxidation

The detection of primary and secondary oxidation was performed spectrophotometrically (San Martin and Garcia, 2001). This analysis consisted of measuring three variables (K_{232} , K_{270} , ΔK). K_{232} is a measure of the level of conjugated dienes and which is indicative of the primary oxidation. K_{270} is a measure of the level of conjugated trienes which is indicative of the secondary oxidation. The resulting products (aldehydes and ketones) absorb at wavelengths of 262, 268, and 274 nm. The ΔK coefficient takes into account these absorbencies and is defined as:

$$\Delta K = K_{268} - [(K_{262} + K_{274})/2]$$

UV specific extinction determination permits a good approximation of the oxidation process in unsaturated oils (Gutierrez et al., 1992, Lopez et al., 1997, San Martin and Garcia, 2001). The specific extinction coefficients at 232, 262, 268, 270, and 274 nm were measured according to the following procedure. An oil sample of 100 mg was placed in a 10 mL flask and diluted to 10 mL with spectrophotometer grade hexane (Sigma-Aldrich). The sample was then homogenized and the absorbance was measured with a Varian UV-Vis 4000 spectrophotometer (Varian, Palo Alto, USA) using pure solvent as blank (Garcia et al., 1996). Measurements were carried out in triplicate.

An oil sample of 100 mg was placed in a flask, and 8 mL of a previously neutralized solvent mixture consisting of equal parts of ethanol and diethyl ether was added. Phenolphthalein (1% in ethanol) was added as pH indicator. The resulting solution was titrated with 0.1 N NaOH until the first permanent pink color appeared persisting for at least 10 s (Garcia et al., 1996). The results were expressed as percentage of the free oleic acid. Measurements were carried out in triplicate.

3.3.3 Results and discussions

Estimation of lipid oxidation (Experiment 1)

The original profile of the adsorption spectra for hazelnut is very similar to that of other dry fruit as peanut (Govindarajan et al., 2009), walnut (Jensen et al., 2001), macadamia (Guthrie et al., 2004), and shea nut (Davrieux et al., 2010) (Fig. 1). The spectral region between 1100

and 2400 nm showed an increased absorbance with the degree of lipid oxidation (Fig. 1). A linear relationship between the transmittance ratio in NIR spectra and the degree of lipid oxidation was found in peanuts (Hirano et al., 1998). Nut composition and lipid oxidation was significantly different between the 20 batches (Table 1). The oldest batches showed higher lipid oxidation than the more recent ones, probably due to fatty acid oxidation during storage (Karel, 1985). Several PLSR models were developed for the detection of lipid oxidation both for unshelled and shelled hazelnuts. The variables considered for the detection of lipid oxidation were free acidity, K_{232} , K_{270} , and ΔK .

In a first analysis, the PLS models for the all oil quality parameters considered did not seem usable for predict lipid oxidation due to the low coefficients of determination. The lowest values of RMSECV and the highest coefficients of determination were observed for the K_{232} extinction coefficient (Table 3), however, also for this parameter, the PLS model did not appear suitable for predicting satisfactorily lipid oxidation, for both the unshelled hazelnuts that for ones shelled. A more accurate analysis revealed that the poor performance of the PLS model were due to the flawed kernels, which in turn impeded the correct calibration of the model. Therefore, by removing the flawed kernels from the calibration and validation sets were significantly improved the performance of the PLS model.

The best results, expressed as coefficient of determination, was obtained for the K_{232} extinction coefficient for both unshelled (Fig. 3) and shelled (Fig. 3) hazelnuts and also low values for the RMSECV were obtained (Table 4). K_{232} values rise linearly with the oxidation level (San Martin and Garcia, 2001; Lopez et al., 1997). The PLS models for the other variables do not appear suitable for predicting lipid oxidation due low coefficients of determination (Table 4). Fortunately, K_{232} is considered the most important lipid oxidation variable. K_{232} values larger than 2 are attributed to hazelnuts with taste defects, and K_{232} values larger than 2.5 are deemed rancid (Lopez et al., 1997). K_{232} values are required to be lower than 2.5 according to European Commission Regulation EC no. 1989/2003 (Sinelli et al., 2008).

The finding that the K_{232} variable can also be estimated using both unshelled and shelled hazelnuts is commercially interesting as it indicates that lipid oxidation can also be predicted without shelling hazelnuts.

Detection of flawed kernels (Experiment 2)

Representative spectra of four kernels varying in DI values between 0 and 7 are shown in Fig. 4. Kernels with DI values between 0 and 2 (healthy kernels) had a lower absorbance while

severely flawed hazelnuts (DI between 4 and 7) showed the highest absorbance. The samples with DI between 4 and 7 were characterized by four peaks (1729, 1761, 2307, and 2347 nm), whereas these peaks were almost absent in the spectra of healthy or slightly flawed hazelnuts (Fig. 4). Since hazelnuts contains high levels of lipids in the kernel (Hirano et al., 1998), the degradation of these lipids is likely the main factor for the observed differences in the NIR spectra. Indeed, the peaks at 1729 and 1761 nm corresponds to the C-H bond of the -CH₂ group first overtone stretching band, and the peaks at 2307 and 2347 nm corresponds to the -CH₂ group stretching and deformation combination band specific for lipids (Williams and Norris, 2001; Osborne et al., 1993).

PLSR models were created to predict DI values from the NIR spectra. A high value for the coefficient of determination ($R^2 = 0.89$) and a small value for the root mean square error of cross validation (RMSECV = 0.88) was found in the range 1100-2400 nm using 6 PLS factors (Table 5 and Fig. 5). Satisfactorily PLS models were also created in the range between 2100 and 2200 nm and using only the wavelengths of the four peaks indicated in Figure 4 (Table. 5). The wavelengths between 2100-2200 nm (Fig. 4) corresponds to double C (C=C) bond stretching and deformation combination band (Panford and DeMan, 1990) of lipids (Williams and Norris, 2001; Osborne et al., 1993).

The feasibility of NIR for detection of flawed kernels was demonstrated earlier considering different wavelength ranges. For 'Tonda Gentile Romana' hazelnuts the best PLS model was obtained using wavelengths between 564-600 nm, 1223-1338 nm and 1283-1338 nm (Moscetti et al., 2013). To identify moldy fruits by NIR, the best prediction was obtained using the spectra band between 1818-2085 nm for chestnuts (Liu et al., 2010) and 700 and 1100 nm for peanuts (Hirano et al., 1998). The finding that only four wavelengths are required to detect flawed hazelnuts is commercially interesting as it allows the development of a low-cost NIR instrument that can be used in high-throughput screening.

3.3.4 Conclusions

Near-infrared diffuse reflectance spectroscopy is a valid, precise and fast technique to determine some compositional parameters in natural products. In this work, the efficiency to detect the degree of kernels defect and the estimation of lipid oxidation in unshelled e shelled hazelnuts has been proved.

Based on the results of this study, it is possible use NIR spectroscopy to separate the hazelnuts into different quality classes. According to the European Union Regulation No.

1284/2002 less than 3% of flawed kernels is allowed for 'Extra' category, thus the possibility to obtain a flaw-free product represents an high value benefit for hazelnut production. Furthermore, the EU requirements for the marketability consider hazelnuts totally free from rancidity. Therefore, it is important to be able to estimate accurately in a non-destructive way the degree of fat oxidation of fruits in order to obtain a product falling within the limits allowed. Interesting is the capability to predict the oxidation of lipids in unshelled fruits and the ability to detect flaws nuts with measurements at only four wavelengths. The latter result would be useful in the development of a low-cost commercial instrument.

Finally, the use of near-infrared technique requires the utilization of specific equipment and a previous calibration step before the analysis of the real samples is performed. Nevertheless, it may become in the foreseeable future a suitable technique in the food industry selection processes.

3.3.5 References

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Table. 1 Descriptive data of hazelnut samples used for spectrophotometric acquisition. Different letters indicate significantly different value ($P < 0.05$) by Duncan's test.

Sample	Production area (Town /Province)	Producer	Harvest year	Water content (%)	Fat content (%)	K ₂₃₂	K ₂₇₀	ΔK	Free acidity (%)
1	Pontecagnano (SA)	1	2008	4.5	63.7	2.60 c	0.10 cd	0.002 ab	1.19 bcd
2	Pontecagnano (SA)	2	2008	4.6	62.0	2.66 c	0.07 abc	0.001 ab	1.22 cd
3	Pontecagnano (SA)	3	2008	4.5	63.1	3.23 d	0.15 d	0.003 b	1.47 d
4	Monteforte (AV)	4	2008	4.0	64.2	2.75 c	0.09 cd	0.002 ab	1.54 d
5	Monteforte (AV)	4	2010	5.1	62.9	1.69 b	0.05 abc	0.001 ab	0.87 abc
6	Monteforte (AV)	4	2011	5.3	61.3	1.13 a	0.06 abc	0.003 ab	0.64 a
7	Monteforte (AV)	4	2012	4.5	61.3	1.09 a	0.03 ab	0.001 ab	0.55 a
8	Sperone (AV)	5	2012	5.2	64.0	1.04 a	0.03 ab	0.001 ab	0.67 ab
9	Sperone (AV)	6	2012	3.6	60.7	1.05 a	0.03 ab	0.001 ab	0.62 a
10	Sperone (AV)	7	2012	5.2	60.8	1.23 a	0.06 abc	0.002 ab	0.69 abc
11	San Vitaliano (NA)	8	2011	4.9	54.8	1.08 a	0.03 ab	0.001 ab	0.60 a
12	San Vitaliano (NA)	8	2012	5.1	57.1	1.07 a	0.02 a	0.001 ab	0.81 abc
13	Casamarciano (NA)	9	2012	4.5	60.4	1.23 a	0.10 cd	0.001 ab	0.94 abc
14	Casamarciano (NA)	10	2012	5.7	63.0	1.05 a	0.03 ab	0.001 a	0.46 a
15	Saviano (NA)	11	2012	5.7	57.3	1.11 a	0.05 abc	0.001 ab	0.79 abc
16	Nola (NA)	12	2011	5.0	60.6	1.41 b	0.06 abc	0.001 ab	0.86 abc
17	Nola (NA)	12	2012	6.0	55.7	1.21 a	0.08 abc	0.002 ab	0.62 a
18	Lauro (AV)	13	2011	4.7	62.5	1.27 a	0.04 ab	0.001 a	0.63 a
19	Avella (AV)	14	2012	6.4	59.3	1.18 a	0.05 abc	0.001 ab	0.50 a
20	Caianello (CE)	15	2012	5.1	64.6	1.06 a	0.03 ab	0.001 ab	0.55 a
Sig.	-	-	-	-	-	0.001	0.001	0.226	0.001

Table. 2 Evaluation of kernels based on the definition of qualitative scores to several attributes observed. The sum of the scores represents the defect index (DI) of kernel sample.

Qualitative classification of kernels		
Parameter	Value range	Description
Moldy outside	0-1	0=absent 0.5=slightly present 1=present
Moldy inside	0-1	0=absent 0.5=slightly present 1=present
Colour outside	0-1	0=normal 0.5= brown 1= dark
Colour inside	0-1	0=normal 0.5= brown 1= dark
Texture	0-1	0=crispy 0.5=slightly gummy 1=gummy
Smell	0-1	0=good 0.5=poor 1=bad
Taste	0-1	0=good 0.5=poor 1=bad

Table. 3 Coefficient of determination (R^2), root mean square of cross validation (RMSECV) and bias, of NIR cross-calibration for oil quality parameter K_{233} for hazelnuts samples including flawed kernels.

K_{232}	Unshelled nuts	Shelled nuts
R^2	0.570	0.720
RMSECV	0.537	0.519
Bias	0.009	0.024

Table. 4 Coefficient of determination (R^2), root mean square of cross validation (RMSECV) and bias of NIR cross-calibration for oil quality parameters for hazelnuts samples excluding flawed kernels.

Oil quality parameters	Parameter range		Wavelength range (nm)	Unshelled nuts				Shelled nuts			
	Min	Max		PLS factors	R^2	RMSECV	Bias	PLS factors	R^2	RMSECV	Bias
K_{232}	0.961	3.496	1100-2300	10	0.791	0.381	0.071	10	0.846	0.333	0.071
K_{270}	0.014	0.134	1100-2300	5	0.539	0.020	0.001	6	0.613	0.019	0.001
ΔK	-0.001	0.003	1100-2300	6	0.350	0.001	0.000	6	0.363	0.001	0.000
Free acidity (%)	0.267	1.618	1100-2400	5	0.486	0.250	0.003	9	0.591	0.226	0.003

Table. 5 Coefficient of determination (R^2), root mean square of cross validation (RMSECV) and bias of NIR cross-calibration for non-destructive quality assessment of hazelnut kernels.

Parameter range	Wavelength region	Wavelength (nm)	PLS factors	R^2	RMSECV	Bias
	All range	1100-2400	6	0.893	0.880	-0.094
0-7	All range	2100-2200	4	0.811	1.156	-0.030
	4 peaks	1729, 1761, 2307, 2347	4	0.867	0.966	-0.001

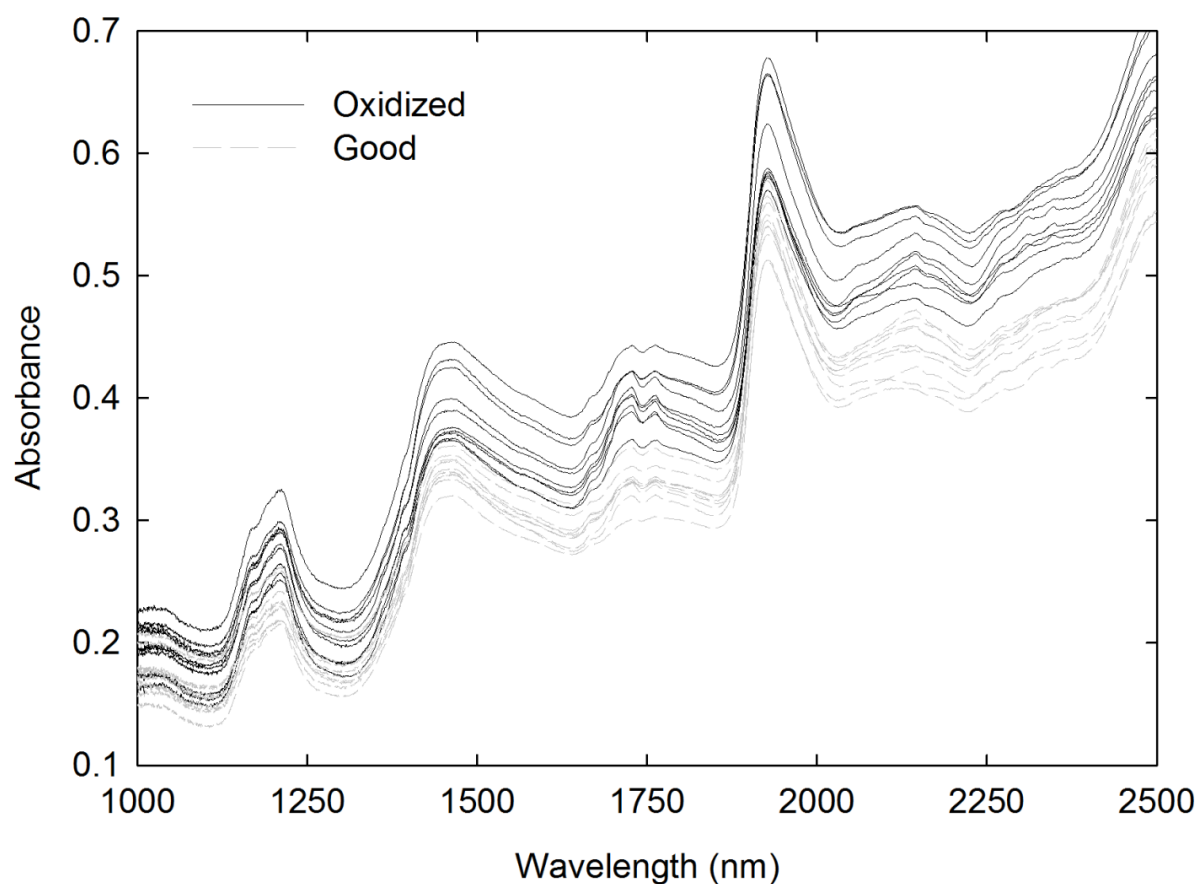


Figure. 1 Original spectra of 20 kernels (10 with non-oxidized fats and 10 with oxidized fats).

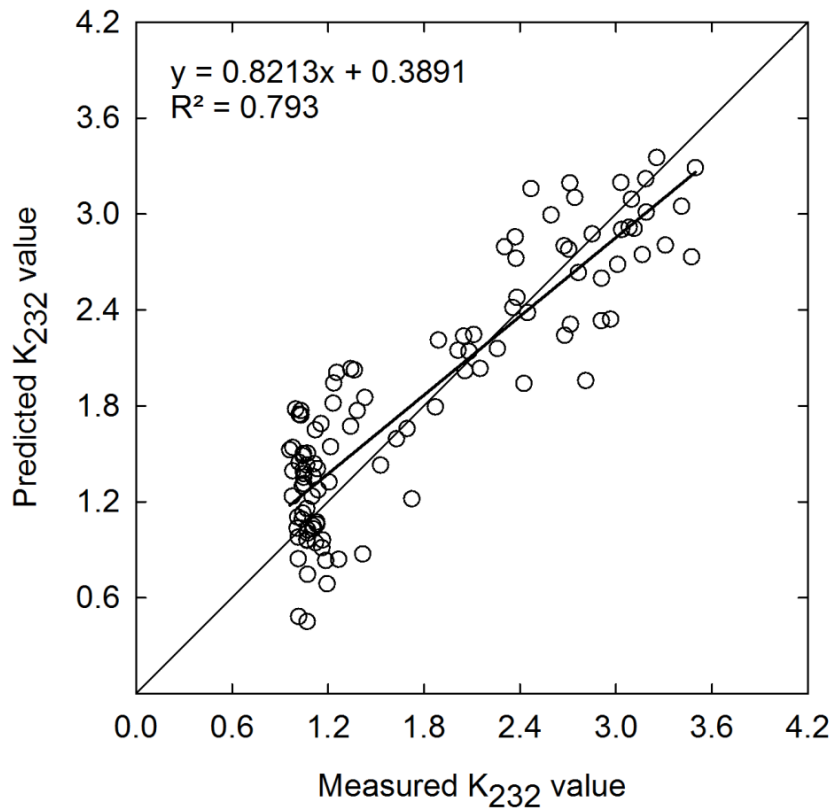


Figure. 2 NIRS prediction results of established PLS model for K_{232} of unshelled fruit.

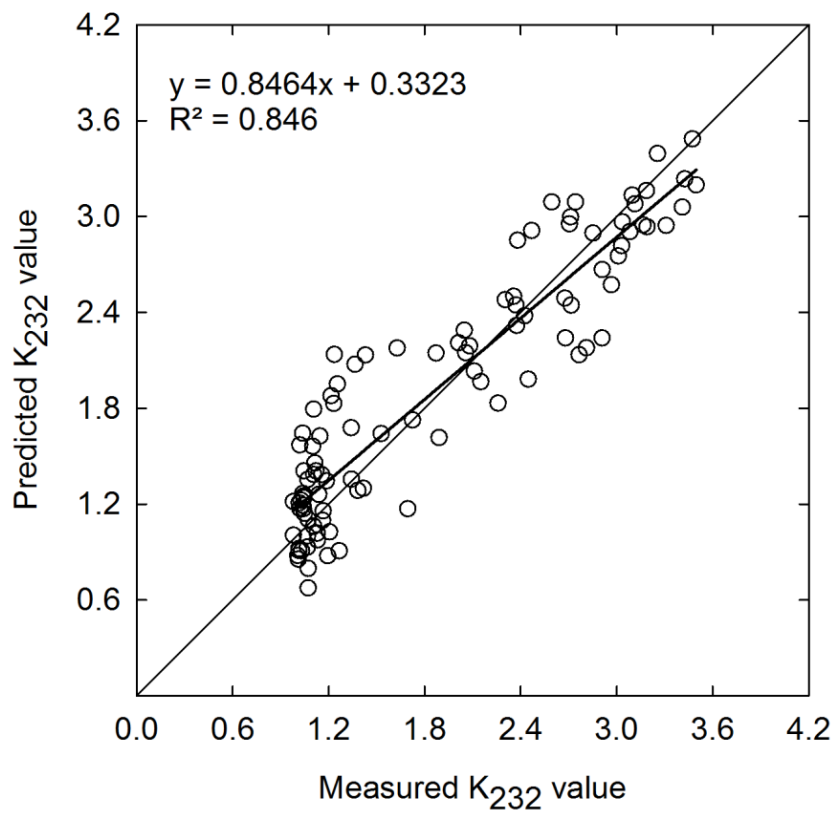


Figure. 3 NIRS prediction results of established PLS model for K_{232} of shelled fruit.

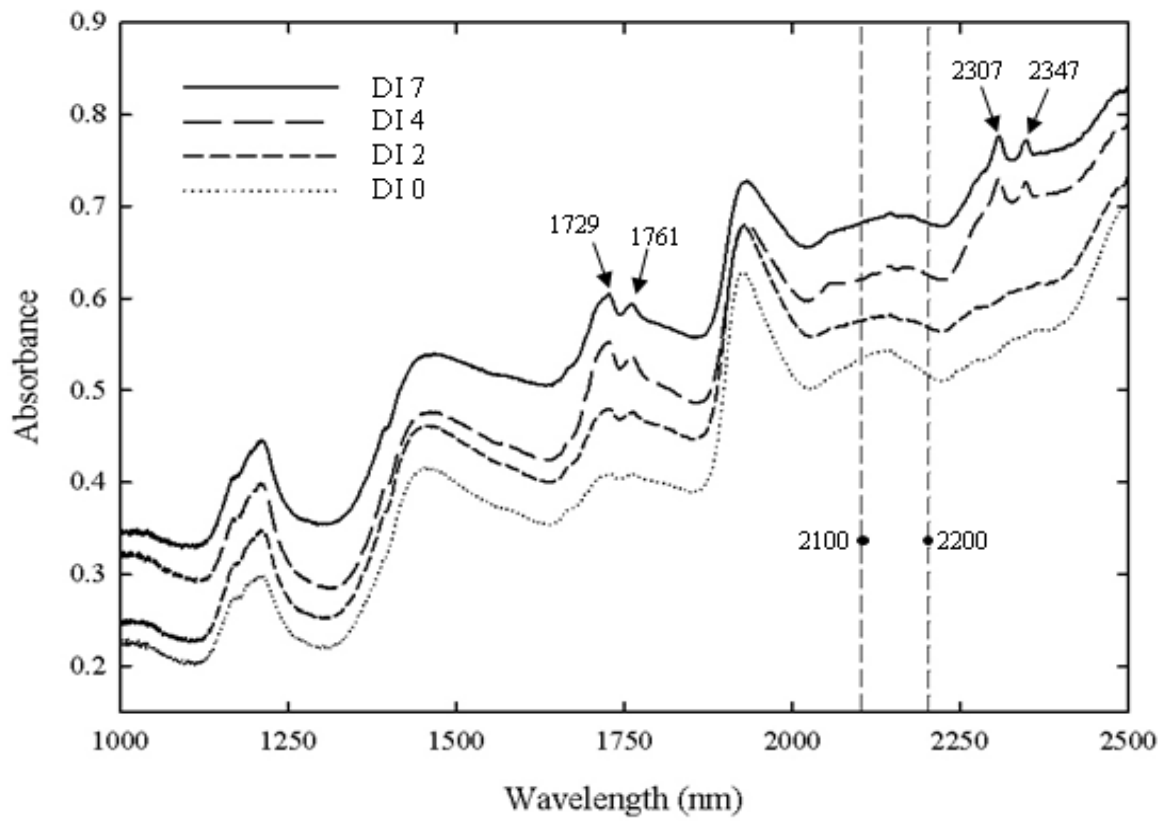


Figure. 4 Original spectra of kernels with four different values of defect index (DI).

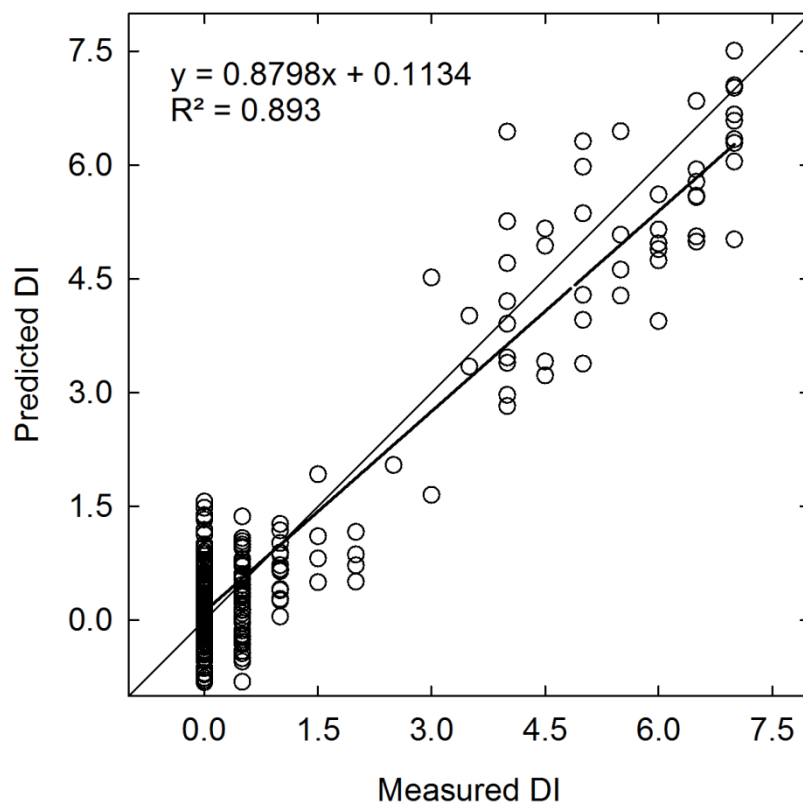


Figure. 5 NIRS prediction results of established PLS model for defect index of hazelnut kernels.

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