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# AGRONOMIC FACTORS AND QUALITY OF VEGETABLE CROPS


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**ABSTRACT**

Quality has a product- and a consumer-dependent dimension in which the relationship between objective and subjective aspects represents the core of the economic importance of quality.

Globe artichoke, strawberry and baby leaf vegetables were selected as the experimental crops due to their high importance on national and international markets. For instance, in Italy 46,954 and 6,000 ha are produced annually for globe artichoke and strawberry, respectively. Besides Italy is the European leader in leafy vegetables, with about 3000 ha produced annually under protected cultivation and destined to fresh-cut products such as wild rocket, leaf lettuce, lamb's lettuce, spinach, and swiss chard.

Most aspects of food quality perception by consumers are based on experience or credence characteristics, associating local and traditional produce with high-quality and healthy products. The food quality perception leds to an increasing demand for organic products, partially due to the general thought that organic foods are healthier and more nutritive than conventionally produced products; even if scientific evidence is still insufficient to confirm or reject this assumption.

Recently, consumers' and producers' interest is based on the evolution of the life styles and food style. Particularly, the increasing application of products with high content of services is determining a progressive increase of leafy vegetable destined to fresh-cut products.

Epidemiological studies support the increasing attention of consumers to the nutritional quality of fruits and vegetables with elevated health-promoting properties due to the presence of an array of secondary metabolites, collectively called phytochemicals. The quantity and quality of phytochemicals may be affected by diverse biotic and abiotic stresses, the development stage of the produce, plant genotype, growing season and harvest time, field location and soil quality, available nutrients, light, temperature, irrigation, chemical fertilizers and conventional/organic management.

Within this context, particular attention is given to sustainable agriculture and low environmental impact with elevated crop production rich in phytonutrients.

Arbuscular mycorrhizal (AM) fungi have been shown not only to improve plant nutrition and reduced chemical inputs, but also to induce changes in plant secondary metabolism. Beneficial impact of AM fungi on plant mineral and secondary metabolite contents depends not only by AM fungal species or isolates, but also by plant genotype and fertilization.

Farming practices as conventional and organic management are as important as genetic variation in determining various components in a crop.

Desirable and undesirable compounds in plants are affected by sunlight integral and other factors such as temperature, light intensity, photoperiod and carbon dioxide concentration. The genetic

background, nitrate supply, harvesting time and light intensity during the growing season are predominant factors for nitrate content.

Based on this preliminary information, the influence of agronomic, genotypic and environmental factors on nutritional/nutraceutical profile of vegetable crops was performed. The agronomic factor was evaluated showing the effect of arbuscular mycorrhizal inoculation and field locations on mineral composition, antioxidant activity, phenolic acids and sesquiterpene lactones (SLs) content of globe artichoke.

The agronomic and genotypic factors were evaluated showing the effect of farming system and cold storage on quality attributes in strawberry (Fragaria × ananassa Duch.) cultivars *Sabrina* and *Ventana*. The genotypic and environmental factors were evaluated showing the nutritional characterization of selected baby leaf vegetables destined to fresh-cut products belonging to the families of Chenopodiaceae (red chard, spinach, swiss chard), Asteraceae (chicory, green and red lettuce), Brassicaceae (mizuna, rocket, tatsoi), Valerianaceae (Lamb's lettuce), and the variation in visual appearance, proteins content, minerals content, antioxidant activity, ascorbic acid and total phenols content of the selected leafy vegetables in relation to the light intensity (low and high Photosynthetically Active Radiation, PAR) at time of harvest.

Analysis of the obtained data on artichoke's experiment revealed significant variations among field locations for the agronomic and nutraceutical parameters. Mycorrhizal treatments did not significantly affect the mineral content of the crop. The activation of plant secondary metabolism in response to AM fungi resulted in increases of the content of individual molecules, with a general but not significant positive influence due to the synergistic effect of Glomus spp and mycorrhizal helper bacteria. The portional division of the plant and head parts showed the highest SLs content in the leaves and the highest total phenols content in the edible part, without univocal result if related to inoculation. The data obtained for the strawberry cultivars' experiment showed that the organic '*Sabrina*' strawberry had a higher soluble solids content while the organic '*Ventana*' had a higher hydrophilic antioxidant activity. An opposite trend was observed on total phenols and ellagic acid for '*Ventana*' and '*Sabrina*', respectively. Organic farming did not increase the reducing sugars and ascorbic acid contents in both cultivars compared to conventional management farming. The higher nutritional value in terms of hydrophilic and lipophylic antioxidant activities in '*Ventana*' and the higher ellagic acid content in '*Sabrina*' indicated that the choice of cultivar for a specific farming system is essential since it may affect quality attributes of strawberry fruit. The nutritional/nutraceutical characterization of ten leafy vegetable destined to fresh-cut products rewards the Brassicaceae family and leafy vegetables harvested at low in comparison to high PAR, except for total phenols content in chicory, green lettuce, lamb's lettuce, mizuna, red chard, and red lettuce. All factors considered

in this study have influence on nutritional/nutraceutical quality. From the objective point of view, considering also the nutraceutical properties, quality is not determinable in univocal way.

## 0 PREFACE

Vegetable quality is a complex issue. According to Shewfelt (1999) quality is a term frequently used but rarely defined. This means that different customer groups such as breeders, producers, market participants, food industry workers, and consumers have different views about quality. In recent years, consumers have been paying increasing attention to the quality of vegetables, particularly to the nutritional quality (Gruda, 2005). Objective quality refers to the physical characteristics built into the product and is typically dealt with by engineers and food technologists. Subjective quality is the quality as perceived by consumers. The relationship between the two is at the core of the economic importance of quality. According to Zeithaml (1998) and Olsen (2002) in food, convenience is sometimes named as an example: consumers may say that 'convenience goods are generally of low quality', even though they regard convenience as a desirable property of food products.

The Total Food Quality Model, long commented by Grunert et al. (1996), showed that two major dimensions was used to analyse food quality perception: a horizontal and a vertical dimension.

The horizontal dimension in Figure 1 refers to how quality perception changes over time. It distinguishes quality perception before and after purchase. Most aspects of food quality are either experience or credence characteristics, and the way quality perception changes over time will differ between these. It also integrates research on consumer satisfaction and dissatisfaction and repurchase probabilities (Oliver, 1980).

The vertical dimension integrates the means–end approach (Reynolds and Olson, 2001), that answer to "What motivates consumers to buy one food product rather than another?". The basic assumption of means–end theory is that consumers are not interested in products per se, but in what the product is doing for them. According to Brunsø et al. (2002), food products of good quality answer to four central concepts: taste (and other sensory characteristics) convenience, process characteristics (-for some consumers- such as organic production, natural production, animal welfare, GMO-free, etc.) and health.

Epidemiological studies showed health-promoting properties related to plant fresh foods, especially the intake of fruits and vegetables has been found important to prevent cardiovascular diseases and obesity (Bazzano et al., 2002; Grusak, 1999). The prevention of chronic diseases and the decrease of the risk of mortality from cancer and cardiovascular diseases, leads to consider plant fresh foods "functional foods" or "nutraceutical foods" (Kaur and Kapoor, 2001).

Fruits and vegetables, as a souce of vitamins, mineral nutrients and dietary fibers, possess an array of secondary metabolites, collectively called phytochemicals which play a beneficial and functional role in human health (Cummings and Kovacic 2009; Seeram 2008) and in many

aspects of plant life. Infact, they may serve as signals to aid pollination and seed-dispersion, repellents for insect and vertebrates, preventors of fungal and bacterial diseases by their allelopathic and antibiotic activities (Ames et al., 1993; San Lin, 1999).

The quantity and quality of phytochemicals may be affected by diverse biotic and abiotic stresses, the development stage of the produce , plant genotype, various parts of the crop or specific breeding activities that have been carried out, growing season, harvest time or field location, soil quality, available nutrients, light, temperature, irrigation, chemical fertilizers and conventional/organic management. (Chun et al., 2005; Jiang et al., 2008; Hussain et al., 2012; Konopka et al., 2012; Lee and Steenwerth, 2013).

# 1 STATE OF THE ART

## 1.1 Effects of pre-harvest factors on quality of vegetables and fruits

### 1.1.1 Genotype

A plant's genotype is of primordial importance in the determination of its phytochemical profile, often surpassing the impact of cultural practices such as irrigation and fertilization (Evers, 1994). Studies conducted by Leonardi et al., (2000) and Baslam et al., (2013), reported that plant genotype has been shown to influence the concentrations of ascorbic acid in apple, orange, kiwi, and lettuce, of glucosinolates in broccoli, and of lycopene in tomato.

Fruits and vegetables are currently subject of breeding programs, in the view of development new cultivated lines of quality with phytochemical content as key component and with disease resistant properties.

For instance, Rekika et al, (2005) evaluated 18 strawberry genotype for their phenolic content and antioxidant capacity using several methods. The significant variation in antioxidant capacity and total phenols clearly shows the potential value of certain new cultivars and advanced lines as parents in a breeding program.

Many researchers as Lindsay (2000), Khanizadeh et al. (2006, 2008) and Tsao et al. (2006) noted that phytochemical studies are important to provide critical information to the breeding programs in which, naturally, the paths depend on final objective. Infact, Khanizadeh et al., (2006) reported the development of apples with reduced phytochemical levels, which make them suitable for fresh apple slices because of their non-browing properties.

Many authors have also highlighted the possibility of increasing the level of antioxidants in tomatoes changing biosynthetic pathway of flavonoids and other phenolics compounds (Schijlen et al., 2004). Even in pepper the presence of carotenoids showed high variability in relation to genotype (Howard et al., 2000). Overall, the choice of the cultivar is a crucial factor that contributes significantly the definition of the organoleptic and nutraceutical properties of vegetables.

### 1.1.2 Benificial microorganisms: Arbuscular mycorrhizal inoculation

Several studies have been conducted in order to assess the influence of soil health and fertility on the phytochemical content of fruits and vegetables, especially that the former parameter plays a key role in the production of safe plant foods. For instance, muskmelons grown on fine sandy loam soils produced less β-carotene than those on salty clay loam soils (Lester and Eischen; 1996). Many ecosystem services have been provided by microbial populations colonizing soil, surfaces, and internal tissues of plants. Cavagnaro et al. (2005) and Wilson et al. (2009) reported that the fungal symbionts that grow out from the mycorrhizal root to develop a complex, ramifying network into the surrounding soil, can reach up to 30 m of fungal hyphae per gramme of soil, improving soil structure and contributing to soil stability and water retention (Bedini et al. 2009). Furthermore, many phosphate fertilisers are a major source of soil contamination by cadmium in agricultural systems. Arbuscular mycorrhizal fungi (AMF), through their mycelium network, not only improve Pi uptake by roots but they also have a buffering effect on the cadmium uptake, reducing the toxic effect of cadmium on plant growth (López-Millán et al. 2009; Rivera-Becerril et al. 2002). Overall, the greater tolerance of mycorrhizal plants against root pathogens provides bioprotection against biotic stresses. Several studies have reported that AM can increase Zn uptake by plants even under field conditions (Cavagnaro 2008).

So, arbuscular mycorrhizal fungi (AMF), have been shown not only to improve plant nutrition, but also to induce changes in plant secondary metabolism, leading to enhanced biosynthesis of health-promoting phytochemicals, such as polyphenols, carotenoids, flavonoids, phytoestrogens, and to a higher activity of antioxidant enzymes (Cardarelli et al., 2010; Seeram, 2008). Many studies indicate that activation of plant secondary metabolism in response to AM fungi can result in increases in essential oil concentration of plant tissues or in the content of individual molecules.

It is however important to note that the beneficial impact of AM fungi on plant mineral and secondary metabolite contents depends not only on AM fungal species or isolates, but also on plant genotype and fertilisation (Chaudhary et al. 2008; Gianinazzi et al. 2008; Khaosaad et al. 2006; Perner et al. 2008; Sailo and Bagyaraj 2005; Toussaint et al. 2007).

### 1.1.3 Fertilization

The effects of fertilization on the quality of product vary in relation to the element and its chemical form, the genotype, the environmental conditions and cultivation techniques**.**

Lee and Kader (2000) observed on fruit and vegetables, including cauliflower, broccoli, various citrus crops and some cultivars of potato, the increasing in nitrogen fertilizer resulted in a

decrease in ascorbic acid content. The negative effect of high nitrogen availability on the accumulation of phytochemicals can be also caused by the indirect effects associated with increased expansion of the leaf that causes shadowing of the fruits. Dorais et al., (2008) found in tomato fruits an increase of the content of lycopene, β-carotene, phenols and flavonoids in conditions of limited availability of nitrogen.

The increasing availability of phosphorus generally increases phytochemicals such as ascorbic acid, anthocyanins, flavonoids and lycopene (Bruulsema et al., 2004; Lester, 2006).

N/P/K and minor element fertilization can affect the phytochemical content of fruits and vegetables.

According to Wang and Lin (2003), the use of compost reduced the amount of fertilizer required for enhancing the concentration of ascorbic acid and glutathione of strawberry.

A recent research has shown a favorable effect of sulfur fertilization on the content glucosinolates, flavonoids and hydroxycinnamic derivatives in broccoli (Vallejo et al.,2003a, b).

In an interesting recent study, according to Barbieri et al., (2009) in a pre-post harvest experiment, ready-to-eat friariello quality may be improved with an enhanced antioxidant activity and reduced nitrate content by combining, respectively, increased sulfur availability during plant growth and exposure to light during storage.

### 1.1.4 Water management

Water management influences plant nutrient and phytochemical content; the latter is related also to the growth rate of the crop, in a phenomenon known as ‘a diluition effect’, as reported by Davis et al., (2004).

According to Miguel Costa et al., (2007) the use of strategies based on reducing in water supplies (deficit irrigation) allows to contain the consumption of water and to increase the quality of the product without necessarily cause a decrease significant production.

Esteban et al., (2001) and Servili et al., (2004) reported that drought improved the quality of grape and virgin olive oil by increasing the concentration of phenolic compounds.

Measurement of biochemical parameters showed that mild drought did not significantly affect chosen stress indicators of apples trees (*Malus domestica* Borkh.) (Šircelj et al., 2005)

De Pascale et al., (2001) reported improvement in carotenoid content and antioxidant activity in tomato, at the expense of yield, by irrigating with saline water containing NaCl up to 0,25% (w/v). Although lower productivity is expected under saline conditions, higher vitamin C content contributed to the nutritional content of tomantoes.

Dorji et al. (2005) have shown that the water deficit checked on peppers grown in greenhouses reduces the fruit set and their soluble solids content increases of 20% on. Experimental tests conducted in open field on watermelon have shown that reduction of irrigation results in a decline of productivity by 15 to 36% without changing the content of lycopene in fruits (Bang et al., 2004).

### 1.1.5 Environmental factor: Light, Temperature and growing season

Among environmental factors, light, as the sole source of photosynthetic energy and a vital environmental signal, plays an important role in driving photosynthetic biosynthesis and photomorphogenesis (Matsuda et al., 2007; Walters, 2005). Sæbø et al., (1995) and Wu et al., (2007) reported that phytochemical biosynthesis and accumulation in plants are well-correlated with the amount of photosynthates. Poiroux-Gonord et al., (2010), in a recent study on different fresh fruits and vegetables, reported that high light exposure or intensity increased the concentration of ascorbate, phenolic compounds, carotenoids, and glucosinolates, as the result of enhanced photooxidative stress or increased photosynthesis.Currently, horticultural cultivation facilities can be divided into two main categories according to their light source: greenhouses, which use solar light as the main light source, and entirely enclosed protected facilities, which use artificial light sources such as fluorescent lamps and LEDs as the main light source (Wheeler 2008).Treatments with specific wavelength irradiation, such as red light, blue light, and UV-B, also showed variable effects on carotenoids concentration in tomato (Becatti et al., 2009).Many studies have demonstrated that light conditions and nitrogenous fertilisers are two of the main factors affecting plant nitrate levels (Fytianos and Zarogiannis 1999; Lips et al., 1990). Lin et al., (2013) found that under the same photosynthetic photon flux density (PPFD) (210μmol $m^{-2}$ $s^{-1}$) and photoperiod (16 h), a mixture of red, blue and white LED light was more effective in reducing nitrate concentrations in hydroponic lettuce than a mixture of red and blue LED light. Based on the results, it was assumed that the broad spectral energy of red and blue light in the mixed red, blue and white light may contribute to a significant reduction of nitrate concentration in lettuce. Low levels of light radiation on spinach, in addition to promoting the accumulation of nitrate in the leaves, cause the increase of the content of oxalic acid (anti-nutritional factor that limits the Ca bioavailability in the human body) and the decrease in vitamin C, resulting in further deterioration of nutritional quality of the product (Proietti et al., 2004).In rocket, it was found that by changing the ratio of lengths wave of red and far red increases the nitrate content and antioxidants in the leaves (Magnani et al.,2008). Dorais *et al.* (2001), reported that low light

intensity reduced pigment synthesis by tomatoes, resulting in uneven fruit coloring. According to Kleinhenz *et al.* (2003a), shading affected anthocyanin levels of lettuce. The temperature variability depends by many factors including the luminous intensity. The control of temperature acquires high importance within the greenhouses where this parameter varies greatly in different seasons. In tomato, red is attributed to lycopene content, which is produced through the biosynthetic pathway of carotenoids that, according Helyes et al. (2003), is conditioned by the temperature levels. Robertson et al. (1995) believe that temperatures between 18 and 26 ° C favor the biosynthesis of these compounds, while Gross (1991) reports as optimal for the formation of lycopene values between 16 and 21 C. The cherry tomato grown in the greenhouse at 16 ° C accumulates lycopene levels up to three times higher compared to plants maintained at 25 ° C (Helyes et al., 2003). Values below 10 ° C inhibit completely the synthesis of lycopene (Robertson et al., 1995; Dorais et al., 2001). Regarding the organoleptic characteristics, bitter taste of watermelons was observed at lower temperatures (Kano and Goto, 2003), while thermal levels sub-optimal have changed positively tomato quality in which it has been observed greater accumulation of carbohydrates (fructose and glucose) (Islam and Khan, 2001). In some cases, reduced levels of temperature can induce a higher production of bioactive molecules; for example, the cabbage broccoli accumulates in the leaves up to 44% more glucosinolates when cultivated at 12 ° C rather than at 22° C (Charron and Sams, 2004). Investigating the influence of growing season on some quality parameters of melon (*Cucumis melo* L.) plants cultivated in nutrient film techniques in greenhouse Mediterranean climate, Pardossi *et al.* (2000) showed that lower solar radiation was responsible for reduced leaf carbon assimilation. This shortage of photo-assimilate supplies and inadequate sucrose synthesis may have led to the production of fewer fruits of larger size and poorer quality due to reduced sucrose content in summer compared with spring. Sucrose concentration represented 76% and 54 % of total soluble sugar in spring and summer, respectively. Seasonal influences have been shown on strawberry "*Fragaria vesca* L." cv. "Regina delle Valli," cultivated under plastic double-tunnels in NFT-systems in Italy. Caruso *et al.* (2004) reported higher sucrose, acids (citric, malic, succinic, and ascorbic), vitamin C as well as calcium and chlorine in fruit harvested in spring, whereas carotenoids, magnesium, copper, nitrates, phosphorus and sulphur accumulated to a greater extent in autumn. Furthermore, tomatoes and strawberries produced in fully sunlight contained more sugar and dry matter than those grown in shade (Caruso *et al.*, 2004; Weston and Barth, 1997; Winsor, 1979). Schreiner *et al.* (2002) investigated the color development of radish throughout the whole year, partly cultivated in the greenhouse and partly in the field and found positive correlations between light intensity and color of radish. Moreover, according to Awad et al., (2000), not all phenolic synthesis respond to sunlight. In apple samples, even though anthocyanins and quercitin

compound were much higher in sun exposed ones, the contents of other major apple phenolics, as catechins and phloridzin, were not significantly different for apples from the sun-exposed or shaded portions of the same tree.

### 1.1.6 Conventional and organic management

Organic foods as defined in the EU regulation (EC) No 834/2007 "are products produced under controlled cultivation conditions characterized by the absence of synthetic fertilizers and very restricted use of pesticides". Organic food is one of the fastest growing sectors of the food and agriculture industry worldwide (Ballester-Costa et al., 2013), since they are perceived by consumers to be healthier, of better quality, more nutritious than conventionally produced foods (Magnusson et al., 2001). Nevertheless, an improved in nutritional and sensorial quality and food safety of organic versus conventional crops has not been proved (Gennaro and Quaglia, 2003), and scientific research on organic products is still contradictory (Kahl et al., 2012). Concerning the conventional and organic management, previous investigations reported that environmental variations including farming practices have been found as important as genetic variation in determining various components in a crop (Anttonen and Karjalainen, 2005; Johansson et al., 2008).

Studies on total carotenoids, certain carotenoids and/or vitamin A content in organically and conventionally grown wheat, green cauliflower, tomatoes, sweet red bell pepper, grapefruit, grapes, apples and carrots, reported no significant differences in content of carotenoids of any type from the two different farming systems (Cardoso et al., 2011; Konopka et al., 2012; Lo Scalzo et al., 2013; Ordonez-Santos et al., 2011; Roose et al., 2009). The relationship between tocopherols content as related to organic versus conventional farming showed no difference in the crops dependent on the farming system applied (Cho et al., 2012; Hussain et al., 2012; Konopka et al., 2012; Perretti et al., 2004; Roose et al., 2009). Also, no increased plasma levels of lycopene were noted from consumption of organically produced tomatoes compared to conventionally produced ones (Caris-Veyrat et al., 2004). In general, weather, environment and genotype seem to play a larger role for the carotenoid content than the farming system (Smith-Spangler et al., 2012). Zhao et al. (2007) did not observe differences in the phenolic content in conventional and organic lettuce.

Dorais (2007) attributed the higher licopene content in organic tomato than those obtained through soiless cultural system because an indirect effects due to organic nutrition.

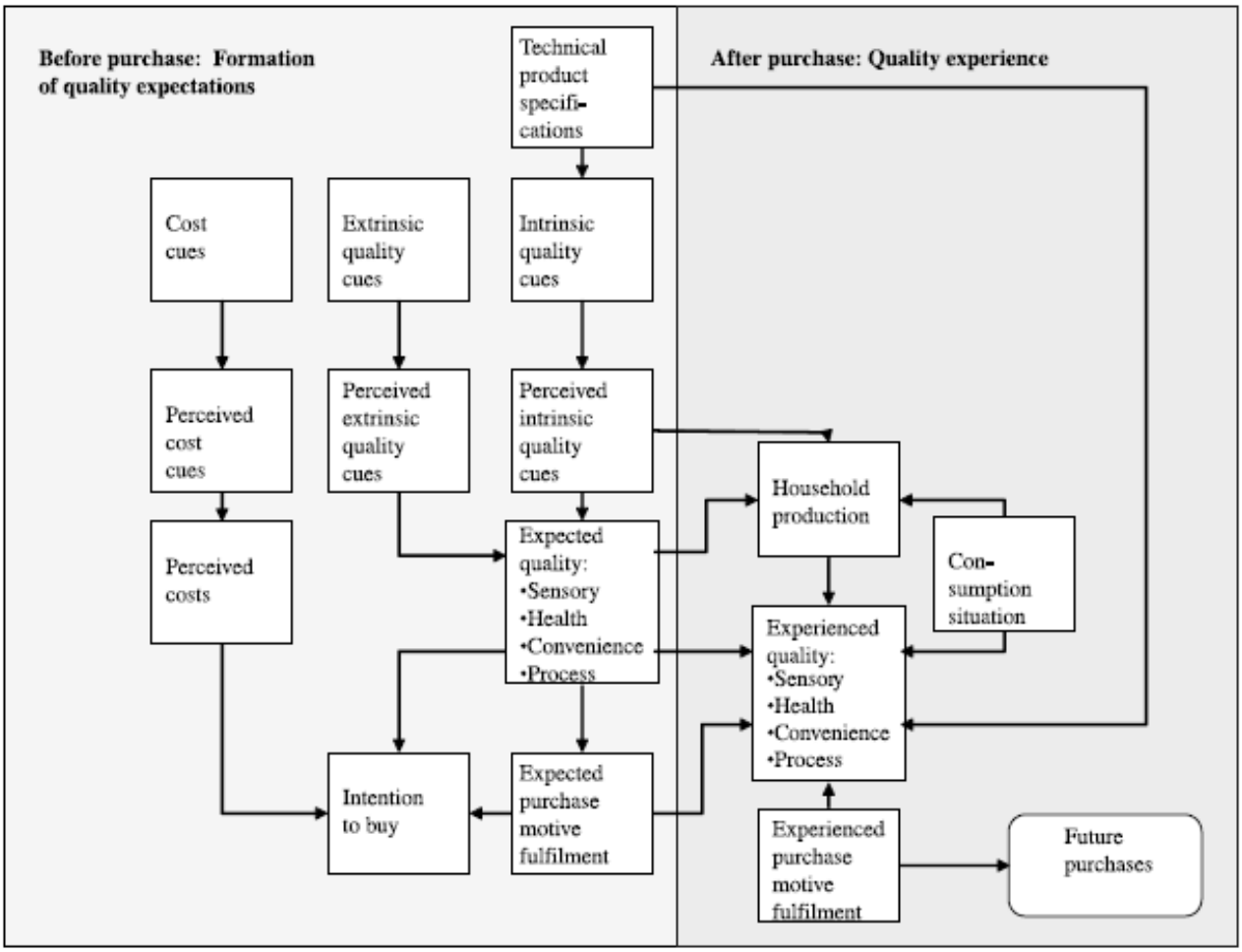


Fig.1: The Total Food Quality Model

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## 2 AIM OF THE STUDY

In Italy about 47000 and 3600 ha are produced annually for globe artichoke and strawberry, respectively. Besides, Italy is the European leader in leafy vegetables, with about 3000 ha produced annually under protected cultivation and destined to fresh-cut products such as wild rocket, leaf lettuce, lamb's lettuce, spinach, and swiss chard. Globe artichoke, strawberry and baby leaf vegetables were selected as the experimental crops due to their high importance on national and international markets. Within the context of studies of perception of quality and epidemiological studies, emerged the increasing attention of consumers to the nutritional quality of fruits and vegetables. The research described in this doctoral thesis mainly concerned the influence of agronomic, genotypic and environmental factors on nutritional/nutraceutical profile of these vegetable crops. The agronomic factor was evaluated showing crop performance in terms of number of mean weight, mineral composition, antioxidant activity, phenolic profile and sesquiterpene lactones content of globe artichoke grown in five different locations with or without inoculation with AM fungi and other beneficial bacteria The agronomic and genotypic factors were evaluated showing a composite examination of quality configuration in strawberry fruit through a analysis of the relative effects of farming system, and postharvest storage (cold storage). Two strawberry cultivars '*Sabrina*' and '*Ventana*', grown under organic and conventional farming methods were stored for 6 months at -40 ºC. Quality evaluation entailed physical (firmness and color), chemical (juice pH, titratable acidity, soluble solid and total sugars), and phytochemical (antioxidant activity, vitamin C, total phenols and ellagic acid) components. Finally, since little information is available concerning the effect of light intensity at time of harvest on desirable and undesirable compounds in several baby leaf vegetables, especially under greenhouse conditions, the genotypic and environmental factors were evaluated showing the nutritional characterization of selected baby leaf vegetables destined to fresh-cut products belonging to the families of Chenopodiaceae (red chard, spinach, swiss chard), Asteraceae (chicory, green and red lettuce), Brassicaceae (mizuna, rocket, tatsoi), Valerianaceae (Lamb's lettuce), and to assess the variation in visual appearance, proteins content, minerals content, antioxidant activity, ascorbic acid and total phenols content of the selected leafy vegetables in relation to the light intensity (low and high Photosynthetically Active Radiation, PAR) at time of harvest.

# 3 RESULTS AND DISCUSSIONS

## 3.1 Topic 1: Effect of arbuscular mycorrhizal inoculation and field locations on mineral composition, antioxidant activity, phenolic profile, sesquiterpene lactones content of globe artichoke.

Artichoke (Cynara cardunculus L. var. scolymus F.) is a poliennal plant species (Asteraceae), widely distributed all over the world and mainly concentrated in the Mediterranean regions (FAO 2008). Italy is the leading producer (40% of the global production) with 46.954 ha and a total production of 4.866.413 q (ISTAT 2013). In Southern Italy, artichoke is mainly cultivated in Puglia (14.980 ha), Sicilia (14.540 ha) and then Sardegna (13.392 ha) that produce early varieties, while in Central-Sourthen Italy late varieties are cultivated in Campania (958 ha), Lazio (936 ha) and Toscana (602 ha) (ISTAT 2013).

The gene pool of artichoke is organised into four varietal types, 'Spinoso', 'Violetto', 'Romanesco', 'Catanese', and based on physical characteristics of the main flowering head (Porceddu et al. 1976; Vannella et al. 1981; Mauromicale and Ierna 2000). The lines denominated as PGI include the Italian 'Romanesco del Lazio' globe artichoke (CE Reg. no. 2066/2002 of the Commission, published by GUCE no. L 318 of 22/11/2002), 'Tondo di Paestum' (CE Reg no 2081/92, published Official Journal C 153, 01/07/2003) and the Spanish "Blanca de Tudela" (CE Reg no 1971/2001 of the Commission, 09/10/2001).

The 'Romanesco' type, with its spherical or sub-spherical, non-spiny green violet heads accounts for about 9% of Italy's production (ISTAT 2005).

Several studies elucidated the health-promoting potential of artichokes, especially their hepatoprotective (Adzet et al., 1987; Eberhardt, 1973) anticarcinogenic (Clifford, 2000), antioxidative (Brown et al. 1998; Gebhardt, 1997) and hypocholesterolemic activities (Clifford and Walker, 1987; Englisch, et al. 2000). The beneficial effects of artichoke plant on human health are mainly due to flavonoids and phenolic acids, particularly caffeic acid and its derivatives mono and dicaffeoylquinic acids. Antibacterial, anti-HIV, bile-expelling, and diuretic effects have been also reported by Kukić et al (2008) and Shen et al., (2010). Inhibition of cholesterol biosynthesis and a dose-dependent reduction of low-density lipoprotein oxidation were observed for luteolin and, to a lesser extent, for its 7-O-glucoside (Brown et al. 1998; Gebhardt, 1998). These mechanisms may contribute to the reduction of atherosclerosis (Wegener and Schmidt 1995) by preventing oxidative modification of blood lipoproteins and reducing blood cholesterol levels (Fintelmann 1996) through choleretically induced elimination and inhibition of hepatic cholesterol biosynthesis (Gebhardt 1995; Kirchhoff et al. 1994). The characteristic bitterness of globe artichoke is mainly due to the presence of sesquiterpene

lactones (STLs), of which the two major representatives are cynaropicrin and, at lower concentration, grosheimin and its derivatives (Cravotto et al. 2005; Schneider and Thiele, 1974). The SLs of the guaianolide group are of particular interest as anti-tumor agents because each chemical substitution to the guaianolide skeleton confers a particular biological activity to the resulting compound (Ghantous et al. 2010).

Arbuscular mycorrhizae (AM) are the most widespread form of symbiosis, ready to establish the associations with over 80% of land plant species (Smith and Read, 2008) and a group of fungi that belongs mainly to the recently described order Glomerales in the new phylum Glomeromycota (formerly Glomales within Zygomycota; Schüβeler et al., 2001; Koide and Mosse, 2004) The first evidence of the positive influence of the AM symbiosis on horticultural production was provided by Menge et al. (1977) on citrus plants. Manipulation of target compounds in plants such as phytochemicals and antioxidants by the management of AM fungi colonization has been recognized as a research area attracting applied scientists interest (Ceccarelli et al., 2010; Colonna et al., 2013; Lopez-Raez et al., 2010; Candido et al., 2013).

For instance Ceccarelli et al. (2010) reported that inoculation with *G. mosseae* and *G. intraradices* significantly increased the total phenolic content and antioxidative activity in the leaves and flower heads extracts of artichoke. Lopez-Raez et al. (2010) reported that inoculation with with *Glomus intraradices* or *Glomus mosseae* altered the phenolic acid profile in the roots of tomato, with decreasing levels of caffeic and chlorogenic acid and increasing levels of ferulic acid. Despite this results, recent studies on tomato fruits reported that quality parameters were not significantly affected by plant AM fungi inoculation (Candido et al., 2013). The primary effect of AM symbiosis is to increase the supply of mineral nutrients to the plant (Hodge et al., 2001; Smith and Read, 2008) particularly those whose ionic forms have a poor mobility rate, or those which are present in low concentration in the soil solution. This mainly concerns phosphate, ammonium, zinc and copper (Barea, 1991). Furthermore, other functions of AM fungi have been studied, such as increasing the resistance to pathogens (Pozo et al., 2010; Wehner et al., 2010), increasing plant growth and yield (Douds et al.,2007; Hamel and Plenchette, 2007; Larsen et al., 2007), increasing crop uniformity, earlier flowering and fruiting. Previous studies reported that Rhizobacteria, commonly designated by the acronym PGPR (Kloepper et al., 1991; Linderman, 1992) can interact with AM fungi, when introduced as also mixed inoculants, to benefit horticultural crops. These synergetic positive interactions between AM fungi and PGPR have been documented by many researchers (Galleguillos et al., 2000, Hameeda et al., 2007), although some neutral effects of AMF-PGPR interaction have also been reported (Andrade et al., 1997; Walley and Germida, 1997). Recently, Xavier and Germida (2003) found that some species of *Bacillus* stimulate the germination of *Glomus* spp. Starting

from the above considerations, the aim of the current study was to assess the effect of beneficial microorganisms incolucation with AM fungi and PGPR on the nutritional parameters of globe artichoke cv. 'Romanesco' coming from five field experiments. Inoculated and non inoculated globe artichocke plants were compared in terms of yield, yield components, mineral composistion, antioxidant activity, nitrate content, polyphenol metabolite profile and sesquiterpene lactones.

## 3.2 Materials and methods

### 3.2.1 Plant material, growth conditions and treatments

Plants of 'Romanesco' type cv. C3 were grown in five different fields. The experimental fields were located at Calvi (BN) (41° 4′ N; 14° 51 E) and at Pietrelcina (BN) (41°12' N, 14°50' E). At Pietrelcina (field 5) the soil was a silty sand (34% sand, 46% silt, 20% clay), pH of 6.23, organic matter of 1.6% (w/w), total N at 0.095%, available P at 12 mg kg-1, exchangeable K at 164 mg kg-1, exchangeable Ca and Mg at 860 mg kg-1 and 80 mg kg-1 respectively. At Calvi (average of field 1, 2, 3, 4) the soil was a sandy loam (35% sand, 37% silt, 28% clay), pH of 7.10, organic matter of 0.96% (w/w), total N at 0.067%, available P at 4 mg kg-1, exchangeable K at 213 mg kg-1, exchangeable Ca and Mg at 871 mg kg-1 and 93 mg kg-1 respectively. In all experimental fields, soil was previously cultivated with wheat. At the end of August, soils of different experimental fields were plowed and then disked twice to break up the soil more finely. Micropropagated artichoke plants were transplanted from September 12 to 20, 2011, at a plant density of 8000 plants/ha (1.25 m × 1 m). In all experimental fields, plot size was about 5000 m2. In experimental fields 3 and 4, part of the micropropagated artichoke plants of 'Romanesco' type cv. C3 were inoculated at transplanting with a commercial inoculum (Aegis Sym Irriga; Italpollina S.p.A., Rivoli Veronese, VE, Italy) containing 700 spores/g of Glomus intraradices and 700 spores/g of Glomus mossae at a dose of 2 kg $ha^{-1}$, whereas another part of the plants was inoculated with a commercial inoculum (Endospor Dry Mix; Tecnologiás Naturales Internacional, Celaya, Gto., Mexico) containing 132 spores $g^{-1}$ of G. intraradices and 2 × 109 CFU/g of beneficial bacteria (Azospirillum brasilense, Azotobacter chroococcum, Bacillus megaterium, and Pseudomonas fluorescens). For the above reasons, three treatments were compared in experimental fields 3 and 4: (1) noninoculated artichoke plants; (2) artichoke plants inoculated with Aegis Sym Irriga; and (3) artichoke plants inoculated with Endospor Dry Mix. Commercial inocula were applied as spore suspension in water directly to the root apparatus of artichoke plantlets at transplanting before the roots were covered with soil. In experimental fields

1, 2, 4 and 5 preplant mineral fertilizers were broadcast (kg/ha) and incorporated into the soil at an average rate of 94 kg/ha of N, 51 kg/ha of P, and 95 kg/ha of K, whereas in field 3 manure was broadcast and incorporated at a rate of 40 t/ha. Additional fertilizer (kg/ha; 100 N) was applied at the end of February in experimental fields 1, 2, 4 and 5 whereas 25 kg/ha of N was applied in artichoke field 3. Artichoke plants were sprinkle-irrigated when necessary for 3 weeks after transplantation and drip-irrigated throughout the rest of the season. Crop water needs were determined by a farm manager visually monitoring the soil and the plants. Weeds were controlled with mechanical cultivation and hand hoeing; no pesticide applications were required to control pests and pathogens. Artichokes were harvested at commercial maturity from the beginning of May until the middle of June 2012. First harvest at 10 May 2012, second harvest at 28 May 2012.

### 3.2.2 Dry matter and mineral analysis

Dry matter (DM) and protein contents, were determined according to the Association of Official Analytical Chemists (AOAC 1990). Leaf DM was determined after drying the plant tissue in a forced air oven at 70°C. Dried leaf tissues were ground separately in a Wiley Mill to pass through a 20-mesh screen, then 0.5 g portions of the dried tissues were analyzed for the following macronutrients: N, P and K. Nitrogen (Total N) concentration in the plant tissues was determined after mineralization with sulfuric acid by Kjeldahl method (Bremner 1965). P and K were extracted by nitropercloric digestion. P content in the leaf tissue was determined by colorimetry (e.g. measure of absorbance), whereas K was analyzed by atomic absorption spectrophotometry according to the method described by Walinga et al. (1995). Iron content ($Fe^{2+}$) was analysed according the *o*-Phenanthroline colorimetric method (Saywell and Cunningham 1937).

### 3.2.3 Nitrate content

Nitrate ($N\text{-}NO_3$) content was determined on water extract of the dried samples according to the cadmium reduction method (Sah; 1994), using the Hach DR 2000 (Hach Co., Loveland, Colorado, USA) spectrophotometer. Briefly, the dry matter samples (0.5 g) of leaf tissue were solved in deionized water, then treated with the nitrate reagent NitraVer 5, for a 5-min reaction time. Spectrophotometer lecture was performed at 500 nm wavelength.

### 3.2.4 Hydrophilic and lipophylic antioxidant activities

Two different radical cation assays were used to determine the antioxidant activity of the hydrophilic (HAA) and lipophylic (LAA) fractions, on 0.2 g of lyophilized samples, extracted by distilled water and methanol, respectively. The HAA (i.e. water-soluble) was assessed using the N,N-dimethyl-p-phenylenediamine (DMPD) method (Fogliano et al., 1999). The principle of the assay is that in the presence of a suitable oxidant solution DMPD can form a stable and colored radical cation ($DMPD^{\cdot+}$). Antioxidant compounds (AO) which are able to transfer a hydrogen atom to $DMPD^{\cdot+}$ quench the color and produce a discoloration of the solution which is proportional to their amount. The LAA (i.e. liposoluble) was measured using the 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method as described by Pellegrini et al.(1999). The principle of the assay is that the inhibitory response of the radical cation is proportional to the antioxidant concentration and the reaction is complete at the time point selected of 2.5 min. The HAA and LAA were determined by UV–Vis spectrophotometry. The absorbance of the solution was measured at 505 and 734 nm, respectively. HAA and LAA were expressed as mmol ascorbic acid (AA) and as mmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per 100 g of fresh weight, respectively (Fogliano et al., 1999; Re et al., 1999).

### 3.2.5 Total phenol content

The total phenolic content in the methanol extracts was determined using Folin-Ciocalteau procedure (Singleton et al., 1999) using gallic acid as a standard. A 100 µL aliquot of the supernatant was combined with 500 µL of Folin-Ciocalteau's reagent (Sigma Aldrich Inc, St Louis, MO, USA) and 400 µL of 7.5% sodium carbonate/water (w/v). The tubes were mixed for 15 s and then allowed to stand for 30 min at 20°C. Absorption was measured at 765 nm using a UV-Vis spectrophotometer, and the result was expressed as mg gallic acid (Sigma Aldrich Inc, St Louis, MO, USA) per 100 g dry weight.

### 3.2.6 Extraction of sesquiterpene lactones (SLs)

All reagents and solvents of HPLC grade were purchased from Merck (Darmstadt, Germany). Cynaropicrin standard were from Sigma (Milano, Italy).

0.5 g of freeze-dried sample was exactly weighed in a screw-cap plastic centrifuge tube. Extraction solvents were 2% (v/v) formic acid in methanol/water 4/1 (v/v) (MeOH/H2O + 2.0

FA). The sample was sonicated at room temperature for 10 min and then centrifuged at 2647g for 10 min. After collection of the supernatant fraction, the extraction procedure was repeated. The extracts were pooled, dried under reduced pressure at 35 °C, subjected to nitrogen flux for 5 min, and recovered with 2 mL of methanol. One millilitre of the extract, to which was added 7 mL of dichloromethane, was stored at -18 °C in a plastic centrifuge tube until further analyses. This portion was used to determine free SL.

3.2.7 Purification of SL by solid phase extraction (SPE)

Total SL containing fractions were purified from phenols and other interfering compounds by SPE, employing Silica cartridges from Phenomenex, Torrance, CA (Si-1 cartridges, 3 mL reservoir, 500 mg sorbent mass). Before SPE, the free SL fraction was centrifuged at 2647g for 10 min to remove solid particles. The cartridges were conditioned with 6 mL of dichloromethane/*i*-propanol 1/1 (v/v), equilibrated with 6 mL of dichloromethane and, after sample loading, eluted with 6 mL of diclоromethane/ethyl acetate 3/2 (v/v). Both the loading and elution fractions were collected, dried under reduced pressure at 35 °C, recovered with 1 mL of methanol/water 1/1 (v/v), filtered in a HPLC glass vial through a nylon syringe filter (diameter: 13 mm; pore dimension:0.45 lm) and stored at -18 °C until HPLC analyses.

3.2.8 Sesquiterpene lactones Metabolite Profile by Orbitrap High-Resolution Mass Spectrometry (HRMS): Qualitative analysis

An ultrahigh-performance chromatography (U-HPLC) was performed on a U-HPLC Accela system (Thermo Fisher Scientific, San Jose, CA, USA) consisting of a degasser, a quaternary pump, an autosampler, and a column oven. Chromatographic separation was carried out on a Gemini C18-110A column (150 mm × 2 mm× 5 μm) (Phenomenex, Torrance, CA, USA). The mobile phase composed of a combination of A (0.1% formic acid in water, v/v) and B (0.1% formic acid in acetonitrile, v/v) was used at a flow rate of 200 μL/min 20 °C.
The U-HPLC was directly interfaced to an Exactive Orbitrap mass spectrometer (MS) (Thermo Fisher Scientific). The Exactive Orbitrap MS equipped with a heated electrospray interface (HESI) was operated in the positive mode scanning the ions in the m/z range of 100−1000.

| Compound | Molecular formula | m/z | | Mass accuracy (ppm) |
|---|---|---|---|---|
| | | theorical | experimental | |
| Cynaratriol | $C_{15}H_{22}O_5$ | 283.15400 | 283.15396 | -0.14 |
| 8-deoxy-11-hydroxy-13-chlorogrosheimin | $C_{15}H_{19}ClO_4$ | 299.10446 | 299.10367 | -2.64 |
| 8-deoxy-11, 13-dihydroxygrosheimin | $C_{15}H_{20}O_5$ | 281.13835 | 281.13766 | -2.45 |
| Grosheimin | $C_{15}H_{18}O_4$ | 263.12779 | 263.12793 | 0.53 |
| Cynaropicrin | $C_{19}H_{22}O_6$ | 347.14891 | 347.14792 | -2.85 |
| dihydrocinaropicrin | $C_{19}H_{24}O_6$ | 349.16456 | 349.16476 | 0.57 |

Table 1. High-Resolution Mass Spectrometry Identification of Sesquiterpene lactones Achieved by Orbitrap MS in Artichoke Edible Part and Artichoke leaves

3.2.9 Quantitative Analysis of Main Sesquiterpene lactones Compounds by HPLC-UV

One gram of freeze-dried artichoke edible part was extracted as described above. Chromatographic separation was performed using an HPLC apparatus (Shimadzu LC 10, Shimadzu, Kyoto, Japan), a UV/Vis detector (mod. SPD-M10A 230 V) set between 200 and 400 nm, and a Prodigy ODS3 100 Å column (250 × 4.6 mm, particle size 5 μm) (Phenomenex). SL elution was carried out in gradient mode employing the following solvent system: mobile phase A: water; mobile phase B: aceto nitrile. The gradient program was as follows: 10% B (0 min), 10-42% B (30 min), 42-10% B (38 min). The flow rate was 0.8 mL/min, and the injection volume was 20 μL. Each chromatogram was recorded at 256 nm. SL quantification was done relative to cynaropicrin used as external standard.

3.2.10 Polyphenol Metabolite Profile by Orbitrap High-Resolution Mass Spectrometry (HRMS).

All reagents and solvents of HPLC grade were purchased from Merck (Darmstadt, Germany). 5-O-Caffeoylquinic acid and lutein standards were from Sigma (Milano, Italy); 1,3-di-O-caffeoylquinic acid standard was from Carl Roth GmbH & Co.

One gram of freeze-dried material was extracted with 20 mL of methanol/water (70:30, v/v) by sonication at room temperature for 30 min. The mixtures were centrifuged at 2800g for 10 min at room temperature, filtered through a 0.45 μm Whatman filter paper (Whatman International Ltd., Maidstone, UK), and then used for analysis.

An ultrahigh-performance chromatography (U-HPLC) was performed as explained for SLs procedure.

After 1 min at 10% B, the linear gradient was from 10 to 90% B at 8 min, held at 90% B to 10 min, back to 10% B at 11 min. The U-HPLC was directly interfaced to an Exactive Orbitrap mass spectrometer (MS) (Thermo Fisher Scientific). The Exactive Orbitrap MS equipped with a heated electrospray interface (HESI) was operated in the negative mode scanning the ions in the m/z range of 65−1000. The resolving power was set to 25000 full widths at half-maximum, resulting in a scan time of 1 s. An automatic gain control target was set into high dynamic range, and the maximum injection time was 100 ms. The interface parameters were as follows: the spray voltage was 3.0 kV, the tube lens was at 100 V, the capillary voltage was 15 V, the capillary temperature was 275 °C, and sheath and auxiliary gas flows of 30 and 15 arbitrary units were used, respectively. Three determinations were performed for each sample.

| Compound | Molecular formula | m/z | | Mass accuracy (ppm) |
|---|---|---|---|---|
| | | theorical | experimental | |
| | | (a) Phenolic acids | | |
| chlorogenic acid | $C_{16}H_{18}O_9$ | 353.08781 | 353.08783 | 0.06 |
| dicaffeoylquinic acid | $C_{25}H_{24}O_{12}$ | 515.11950 | 515.11945 | −0.10 |
| caffeic acid | $C_9H_8O_4$ | 179.03498 | 179.03455 | −2.40 |
| caffeic acid hexoside | $C_{15}H_{18}O_9$ | 341.08781 | 341.08807 | 0.76 |
| tricaffeoylquinic acid | $C_{34}H_{30}O_{15}$ | 677.15119 | 677.15125 | 0.09 |
| coumaroyl quinic acid | $C_{16}H_{18}0_8$ | 337.09289 | 337.09308 | 0.56 |
| coumaric acid | $C_9H_8O_3$ | 163.04007 | 163.03943 | −3.,93 |
| coumaric acid glucoside | $C_{15}H_{18}O_8$ | 325.09289 | 325.09329 | 1.23 |
| feruloylquinic acid | $C_{17}H_{20}O_9$ | 367.10346 | 367.10367 | 0.57 |
| feruloylquinic acid hexoside | $C_{23}H_{30}O_{14}$ | 529.15628 | 529.15625 | −0.06 |
| feruloyl-caffeoylquinic acid[a] | $C_{26}H_{26}O_{12}$ | 529.13515 | 529.13605 | 1.7 |
| ferulic acid | $C_{10}H_{10}O_4$ | 193.05063 | 193.05034 | −1.50 |
| diferuloylgentiobiose[a] | $C_{32}H_{38}O_{17}$ | 693.20362 | 693.20343 | −0.27 |
| dimethoxybenzoic acid[a] | $C_9H_{10}O_4$ | 181.05063 | 181.05005 | −3.20 |
| gentisic acid glucoside[a] | $C_{15}H_{20}O_9$ | 343.10346 | 343.10403 | 1.66 |
| hydroxybenzoic acid glucoside[a] | $C_{13}H_{16}O_8$ | 299.07724 | 299.07730 | 0.2 |
| protocatechuic acid glucoside[a] | $C_{13}H_{16}O_9$ | 315.07216 | 315.07162 | −1.71 |
| syringic acid[b] | $C_9H_{10}O_5$ | 197.04555 | 197.04518 | −1.88 |
| digalloyl methyl glucose[a] | $C_{21}H_{22}O_{14}$ | 497.09368 | 497.09235 | −2.68 |
| gallic acid galloyl glucoside[a,b] | $C_{20}H_{20}O_{14}$ | 483.07803 | 483.07593 | −4.35 |
| gallic acid gallate[a,b] | $C_{14}H_{10}O_9$ | 321.02521 | 321.02469 | −1.62 |
| sinapic acid[a] | $C_{11}H_{12}O_5$ | 223.06120 | 223.06102 | −0.81 |

| | | | | |
|---|---|---|---|---|
| rosamarinic acid | $C_{18}H_{16}O_8$ | 359.07724 | 359.07733 | 0.25 |
| trihydroxycinnamic acid[a] | $C_9H_8O_5$ | 195.02990 | 195.02982 | −0.41 |
| | | (b) Flavonoids | | |
| luteolin | $C_{15}H_{10}O_6$ | 285.04446 | 285.04449 | 0.11 |
| luteolin hexoside | $C_{21}H_{20}O_{11}$ | 447.09328 | 447.09372 | 0.98 |
| luteolin glucuronide | $C_{21}H_{18}O_{12}$ | 461.07255 | 461.07291 | 0.78 |
| luteolin rutinoside | $C_{27}H_{30}O_{15}$ | 593.15119 | 593.15125 | 0.1 |
| luteolin malonyl glucoside | $C_{24}H_{22}O_{14}$ | 533.09368 | 533.09387 | 0.36 |
| luteolin glucuronide glucoside[a] | $C_{27}H_{28}O_{17}$ | 623.12537 | 623.12537 | 0 |
| luteolin diglucoside | $C_{27}H_{30}O_{16}$ | 609.14611 | 609.14606 | −0.08 |
| luteolin apiosyl malonyl glucoside[a,b] | $C_{29}H_{30}O_{18}$ | 665.13594 | 665.13782 | 2.83 |
| luteolin methyl ether diglucoside[a] | $C_{28}H_{32}O_{16}$ | 623.16176 | 623.16205 | 0.47 |
| apigenin | $C_{15}H_{10}O_5$ | 269.04555 | 269.04587 | 1.19 |
| apigenin hexoside | $C_{21}H_{20}O_{10}$ | 431.09837 | 431.09857 | 0.46 |
| apigenin glucuronide | $C_{21}H_{18}O_{11}$ | 445.07763 | 445.07797 | 0.76 |
| apigenin xyloside[a] | $C_{20}H_{18}O_9$ | 401.08781 | 401.08786 | 0.12 |
| apigenin rhamnoside[a] | $C_{21}H_{20}O_9$ | 415.10346 | 415.10519 | 4.17 |
| apigenin diglucoside[a] | $C_{26}H_{28}O_{14}$ | 563.14063 | 563.14087 | 0.43 |
| apigenin methylglucuronide | $C_{22}H_{20}O_{11}$ | 459.09328 | 459.09402 | 1.61 |
| apigenin rhamnoside rutinoside[a,b] | $C_{33}H_{40}O_{18}$ | 723.21419 | 723.21472 | 0.73 |
| apigenin rutinoside caffeate[a,c] | $C_{36}H_{36}O_{17}$ | 739.18797 | 739.18768 | −0.39 |
| apigenin acetylglucoside[a] | $C_{23}H_{22}O_{11}$ | 473.10893 | 473.10919 | 0.55 |
| apigenin glucosyllactate[a,b] | $C_{24}H_{24}O_{12}$ | 503.11950 | 503.11963 | 0.26 |
| methylapigenin[a] | $C_{16}H_{12}O_5$ | 283.06120 | 283.06158 | 1.34 |
| quercetin hexoside | $C_{21}H_{20}O_{12}$ | 463.08820 | 463.08868 | 1.04 |
| quercetin glucuronide | $C_{21}H_{18}O_{13}$ | 477.06746 | 477.06772 | 0.54 |
| quercetin glucoside glucuronide[a] | $C_{27}H_{28}O_{18}$ | 639.12029 | 639.12054 | 0.39 |
| quercetin diglucuronide [a] | $C_{27}H_{26}O_{19}$ | 653.09955 | 653.09943 | −0.18 |
| quercetin galloylglucoside[a] | $C_{28}H_{24}O_{16}$ | 615.09916 | 615.10028 | 1.82 |
| quercetin acetylglucoside | $C_{23}H_{22}O_{13}$ | 505.09876 | 505.09937 | 1.21 |
| quercetin galloylrutinoside[a] | $C_{34}H_{34}O_{20}$ | 761.15707 | 761.15729 | 0.29 |
| quercetin malonylgalactoside[a] | $C_{24}H22O15$ | 549.08859 | 549.08881 | 0.4 |
| dihydroquercetin rhamnoside[a] | $C_{21}H_{22}O_{11}$ | 449.10893 | 449.10956 | 1.4 |
| naringenin hesperioside | $C_{27}H_{32}O_{14}$ | 579.17193 | 579.17212 | 0.33 |
| naringenin hexoside | $C_{21}H_{22}O_{10}$ | 433.11402 | 433.11398 | −0.09 |
| naringenin rhamnoside[a,b] | $C_{21}H_{22}O_9$ | 417.11911 | 417.12018 | 2.57 |

| | | | | |
|---|---|---|---|---|
| naringenin diglucoside[a] | $C_{27}H_{32}O_{15}$ | 595.16684 | 595.16528 | −2.62 |
| eriodictyol | $C_{15}H_{12}O_6$ | 287.05611 | 287.05643 | 1.11 |
| eriodictyol diglucoside | $C_{27}H_{32}O_{16}$ | 611.16176 | 611.16193 | 0.28 |
| chrysoeriol glucoside | $C_{22}H_{22}O_{11}$ | 461.10893 | 461.10968 | 1.63 |
| myricetin arabinoside[a] | $C_{20}H_{18}O_{12}$ | 449.07255 | 449.07257 | 0.04 |
| myricetin hexoside | $C_{21}H_{20}O_{13}$ | 479.08311 | 479.08310 | −0.02 |

Table 2. High-Resolution Mass Spectrometry Identification of Phenolic Acids and Flavonoids Achieved by Orbitrap MS in Artichoke Edible Part

[a]Not previously reported in artichoke. [b]Found only in samples from plants inoculated with beneficial microorganisms. [c]Found only in samples from plants not inoculated with beneficial microorganisms.

3.2.11 Quantitative Analysis of Main Phenolic Compounds by HPLC-UV and HPLC-MS/MS.

One gram of freeze-dried artichoke edible part was extracted as described above. Chromatographic separation was performed using an HPLC apparatus equipped with two micropumps series 200 (Perkin-Elmer, Shelton, CT, USA), a UV−vis series 200 (Perkin-Elmer) detector set at 330 and 280 nm, and a Prodigy ODS3 100 Å column (250 × 4.6 mm, particle size 5 μm) (Phenomenex). The eluents were (A) 0.2% formic acid in water and (B) acetonitrile/methanol (60:40 v/v). The gradient program was as follows: 20−30% B (6 min), 30−40% B (10 min), 40−50% B (8 min), 50−90%B (8 min), 90−90% B (3 min), and 90−20% B (3 min), at a constant flow of 0.8 mL/min. The LC flow was split, and 0.2 mL/min was sent to the mass spectrometer. The injection volume was 20 μL.

MS and MS/MS analyses were performed on an API 3000 triplequadrupole mass spectrometer (Applied Biosystems, Canada) equipped with a TurboIonspray source working in the negative ion mode. The analyses were performed using the following settings: drying gas (air) was heated to 400 °C, capillary voltage (IS) was set at 4000 V, nebulizer gas (air) was set at 12 (arbitrary units), curtain gas (N2) was set at 14 (arbitrary units), and collision gas (N2) was set at 4 (arbitrary units).

After peak identification, the phenolics quantification was performed by HPLC as follows: filtered extract (20 μL) was injected into an HPLC (Shimadzu LC 10, Shimadzu, Kyoto, Japan) with a photodiode array detector. Separations were achieved on the same column with the same gradient program. The flow rate was 0.8 mL/min, and chromatograms were recorded at 330 and 280 nm. Monocaffeoylquinicacids were quantified as chlorogenic acid, dicaffeoylquinic acids were quantified as cynarine, and luteolin derivates were quantified as luteolin. Three injections were performed for each sample.

3.2.12 Statistical analysis

All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 21 for Windows). To separate treatment means within each measured parameter, Duncan's multiple range test was performed at $P < 0.05$

**3.3 Results and discussion 1**

3.3.1 Growth response and dry matter

Head numbers was significantly affected by field locations and the mycorrhizal treatments affected this parameter (Table 3). Artichoke plants grown in the control fields 1 and 5 and in the experimental field 4 showed the highest flower head numbers. The treatment with Endospor Dry Mix and Aegis Sym Irriga presented the higher head numbers than the control results. As reported by Marin et al., (2002) in a study on *Cynara cardunculus*, the applications of mycorrhizal inoculations to other species of the cardoon family such as the artichoke (*Cynara scolymus*) should be evaluated to asses its effects on commercial production. The results reported by field 4 showed that both mineral fertilization and mycorrhizal inoculation resulted in a higher head numbers than the field 3. Previous studies on different vegetable species reported the improvement in plant growth and yield (mainly due to a significantly higher number of fruits per plant) through AMF inoculation (Douds *et al*., 2007; Regvar *et al*., 2003; Salvioli *et al*., 2012).
There were no significant difference in flower head's mean weight for both field locations and mycorrhizal inoculation variables (Table 3). The second harvest showed higher mean weight value than the first one (144 g and 138 g respectively) (data not shown). Physiological and genetic adaptation of AM fungi to environmental conditions is important to associate the effectiveness in stimulating plant growth of different AM fungi (Barea et al., 1993; Smith and Read 2008).
Higher mean weight value was reported in treatment containing *Glomus* intraradices and mycorrhizal helper bacteria. Von Alten (1998) emphasized the use of mycorrhizae-helping bacteria (MHB) for enhancement of growth of the plants. He reported that rhizosphere strains of *Bacillus mycoides* and *Pseudomonas fluorescens* promoted AMF formation in various crop plants by improving susceptibility of roots to AMF. Garbaye and Duponnois (1992) found that some organic acids (predominantly malic and citric) excreted by the MHBs represented a carbon source as good as glucose for the fungal growth. Leaves mean weight (Table 3) was significantly influenced by field locations and mycorrhizal treatments. The two mycorrhizal treatments

showed a different trend and the Endospor Dry mix inoculum's performance was similar to the control one and better respect to Aegis Sym irriga performance. Control field 1 and field 3 presented the highest leaves mean weight.

A recent study on *Artemisia annua* L. (*Asteraceae*) conducted by Rapparini et al., (2008), reported that mycorrhizal plants had more leaves than control plants, although significant differences were observed only for mycorrhizal plants inoculated with the mixture of AM and bacteria, respect to the inoculum with *Glomus* without bacteria.

In according with Candido et al., (2013) in a study on tomato, our results showed that leaves and heads DM wasn't significantly influenced by the mycorrhizal treatments.

| | Head n°(/plant) | Head mean wt (g/head) | Leaves mean wt (kg/leaf) | Leaves DM (%) | Heads DM (%) |
|---|---|---|---|---|---|
| Fields | | | | | |
| 1 | 7.0 | 153.0 | 2.3 | 13.2 | 12.4 |
| 2 | 4.6 | 137.1 | 1.1 | 11.9 | 13.2 |
| 3 | 6.2 | 133.6 | 2.5 | 13.0 | 15.0 |
| 4 | 7.5 | 151.8 | 1.4 | 14.3 | 14.8 |
| 5 | 7.3 | 121.1 | 1.1 | 13.5 | 14.6 |
| Significance | * | n.s. | * | n.s. | n.s. |
| Treatment | | | | | |
| Control | 6.2 | 137.6 | 2.0 | 13.2 | 14.0 |
| Aegis | 7.2 | 136.3 | 1.3 | 13.8 | 14.6 |
| Endospor | 7.3 | 150.8 | 2.3 | 13.2 | 15.3 |
| Significance | * | n.s. | * | n.s. | n.s. |

Table 3. Head Number, Head Mean Weight, Leaves mean Weight , Leaves Dry matter (DM) and Heads Dry matter (DM) of Artichoke in different Field locations grown without Inoculation with Beneficial Microorganisms (Control), Inoculated with Aegis Sym Irriga (Aegis), and Inoculated with Endospor Dry Mix (Endospor). Means within columns separated using Duncan's multiple range test, $p = 0.05$. ns, *, **, = non-significant or significant at $p < 0.05$ and 0.01, respectively.

### 3.3.2 Mineral content

N and P content in flower heads showed a space-temporal variability (Table 4). It was significantly different among the field locations and harvest times, without influence due to mycorrhizal treatments. The highest values for both the parameters were reported for the flower heads grown in the control field 1 in the first collection data. Potassium content was not affected by the space-temporal-mycorrhizal variables in the flower heads, whereas in the leaves, field locations influenced this parameter with the lowest value in the samples from field 4 (Table 4). Foliar N and P content (Table 4) was significantly affected by space variable, with the highest value in samples from the control fields 5 (2,9% N) and field 1 (22,3 mg 100 $g^{-1}$ F.W. P). Mycorrhizal treatments did not influence the mineral content in leaves, on the contrary, data

showed higher K value in control samples than in treated ones (Table 4). These result are in line with Rapparini et al.,(2008) on *Artemisia annua* L. foliar K content. Most benefit from AM symbiosis to plant was the improvement to access to limited soil resources (i.e., nutrients—especially phosphorus—and water). Our results showed that, probably, the level of soil fertility and the adequate water availability made mycorrhizal symbiosis superfluous. AM symbiosis is important for N acquisition and N fertilizer utilization but this beneficial mycorrhizal effect on N nutrition is reduced under large quantities of N fertilizer (Azcón et al., 2008). Recently, Saia et al., (2014) showed that under water stress conditions, arbuscular mycorrhizal symbiosis resulted in increases in total biomass, N content, and N fixation, whereas no effect of crop mycorrhization was observed in the well-watered treatment. In general, the ability of AM-colonized roots to increase the utilization of nitrogen has been attributed, in most cases, to an indirect effect associated with phosphorus nutrition because this enzyme requires phosphate, which in turn may form a complex with molybdenum and thereby facilitate nitrate reduction (Hageman and Reed, 1980). High soil P content usually results in low AMF colonization (Smith and Read 2008). For instance, Duan et al. (2010) found low colonization levels in maize, soybean, and wheat grown on fertilized soils. Previously, as reported by Lekberg and Koide (2005), conditions of soil rich in phosphorus can strongly reduce the mycorrhizal benefit in terms of plant growth and phosphorus uptake. At a high rate of P, onset of both entry points and vesicles in leeks (*Allium porrum* L.) is reduced; these are essential for colonization by *G. mosseae* (Amijee et al., 1989). Previous studies on chile pepper (*Capsicum annum* L.), cilantro (*Coriandrum sativum* L.), tomato (*Solanum lycopersicum* L.), and corn (*Zea mays* L.) plants, also exposed to high P application, had significant decreases in AMF colonization (Schroeder and Janos, 2004). The analysis of the iron content did not show significant differences among field locations and mycorrhizal treatments. The inoculum Aegis, on average, showed the highest value (0.94 mg $Fe^{2+}$ 100 $g^{-1}$ F.W.) followed by the control samples with 0.90 mg $Fe^{2+}$/100 g F.W. and samples inoculated with Endospor Dry mix with 0.86 mg $Fe^{2+}$ 100 $g^{-1}$ F.W. The flower heads from the field 4, followed by the field 1, confirmed the best value in total iron content (data not shown). Previous studies showed that the AM symbiosis enhance the acquisition of $Fe^{2+}$ in alkaline soils, where the availability is low, but not in acidic soils, where the iron is much more available (Clark and Zethos 1996).

| | Head | | | Leaves | | |
|---|---|---|---|---|---|---|
| | N | P | K | N | P | K |
| | g/100 g d.w. | mg/100 g f.w. | mg/100 g f.w. | g/100 g d.w. | mg/100 g f.w. | mg/100 g f.w. |
| Harvest | | | | | | |
| I | 2.9 a | 53.7 a | 444.5 | | | |
| II | 2.3 b | 44.6 b | 467.0 | | | |
| Significance | * | * | *n.s.* | | | |
| Fields | | | | | | |
| 1 | 3.4 a | 59.5 a | 460.5 | 2.6 ab | 22.3 a | 746.1 a |
| 2 | 2.7 bc | 42.9 b | 450.6 | 2.8 ab | 20.1 ab | 804.6 a |
| 3 | 2.3 c | 47.7 b | 486.2 | 2.2 c | 14.2 b | 607.4 a |
| 4 | 2.5 bc | 49.8 ab | 427.9 | 2.4 bc | 16.6 b | 582.7 b |
| 5 | 2.9 ab | 47.4 b | 448.3 | 2.9 a | 14.6 b | 766 a |
| Significance | * | * | *n.s.* | * | ** | * |
| Treatment | | | | | | |
| Control | 2.8 | 49.4 | 458.5 | 2.6 | 18.3 | 727.8 a |
| Aegis | 2.5 | 47.5 | 431.3 | 2.4 | 18.0 | 610.6 b |
| Endospor | 2.4 | 50.3 | 473.4 | 2.3 | 14.6 | 515.7 b |
| Significance | *n.s.* | *n.s.* | *n.s.* | *n.s.* | *n.s.* | * |

Table 4. Mineral content (mg 100 $g^{-1}$ f.w.) of flower heads and artichoke leaves in different harvest time, Field locations and grown without Inoculation with Beneficial Microorganisms (Control), Inoculated with Aegis Sym Irriga (Aegis), and Inoculated with Endospor Dry Mix (Endospor). Means within columns separated using Duncan's multiple range test, p = 0.05. ns, *, **, = non-significant or significant at $p < 0.05$ and 0.01, respectively.

### 3.3.3 Nitrate content

Nitrate content was significantly influenced by field locations and mycorrhizal treatment (Table 5). The highest value was observed in flower heads from experimental field 3 with 642,9 mg $kg^{-1}$ F.W., while experimental field 4, where, instead, mineral fertilizers were broadcast, presented 531,5 mg $kg^{-1}$ F.W. Conversely, samples from control field 1 showed the lowest value (383,6 mg $Kg^{-1}$). Aegis Sym Irriga inoculum reported the highest nitrate value in flower heads respect to the control and to Endospor Dry mix inoculum (636,9 mg $kg^{-1}$ F.W., 518,5 mg $kg^{-1}$ F.W. and 528,8 mg $kg^{-1}$ F.W. respectively). In a study conducted by Jia et al., (2004) on *Vicia faba,* the indirect effects of AMF colonization on plant nitrate uptake was probably due to an increase in the plant's total transpirational surface area that would tend to increase the flux of water through the soil–plant–atmosphere continuum and increase the flux of nitrate to the plant root system, supposed that plant N productivity in terms of leaf area production would have been enhanced by

an increase in P accumulation.The nitrate mobilized from soil by AM fungal mycelia might be transferred directly as an anion to the root cells where reduction could take place. According to Kaldorf et al., (1994), AM fungi have the gene-set for assimilatory nitrate reduction and so nitrate can also be reduced inside the AM fungal cells by the assimilatory reduction pathway. A mycorrhizal gene (LjAMT2; 2) that was upregulated in arbuculated cells of *Lotus japonicus* (Regel) K. Larsen colonized by *G. margarita* transported only the NO3 form (Guether et al., 2009).

### 3.3.4 Antioxidant activity

Compared to other vegetables, the artichoke is a source of antioxidants and contains high levels of total phenols (Brat et al., 2006), which can be absorbed directly by the small intestine or hydrolysed by the microflora of the large intestine (Nardini et al., 2002; Stalmach 2010).
The hydrophilic (HAA) was significantly affected by field locations and by the portional division of the bracts, without significant differences among the control samples and the inoculated ones (Table 5). Lipophylic (LAA) antioxidant activity showed no difference related to field locations and mycorrhizal treatments, with significant difference only for the portional division of the bracts (Table 5). HAA was higher in flower heads harvested in field 4 and control field 5 than in those from field 1, 2 and 3. It is well known that antioxidant activity and the phenolic content are influenced by biological factors (genotype , organ and ontogeny), as well as soil and environmental conditions (temperature, salinity, water stress and light intensity) (Emmons and Peterson, 2001; Howard et al. 2002; Mpofu et al., 2006).
The analysis of HAA and LAA was conducted by separating the inner from the outer bracts (Table 5). External bracts had higher HAA value than internal ones (0,97 mmol ascorbic acid 100 $g^{-1}$ FW and 0,73 mmol ascorbic acid 100 $g^{-1}$ FW respectively). LAA reported the highest value in internal bracts respect to the external ones (1,03 mmol trolox 100 $g^{-1}$ FW and 0,38 mmol trolox 100 $g^{-1}$ FW respectively). External bracts contain woody tissue, protect the reproductive organs to biotic and abiotic stresses and so show more difficulties to the extraction of these compounds. In line with our results, a study conducted by Negro (2012), where it was assumed that the reduced content of these compounds in the outer bracts could be due to the fact that the metabolic flux is directed toward the synthesis of lignin.
Our results showed no influence of the mycorrhization on HAA and LAA content. Ceccarelli et al., (2010) studied the effect of different mycorrizal inoculations on antioxidant activity and total

phenol content in artichoke bracts and leaves. He reported a large increase in both parameters, but this phenomenon was strongly dependent on the mycorrizal fungal species used.

| | $NO_3$-N | HAA | LAA |
|---|---|---|---|
| | mg $kg^{-1}$ f.w. | mmol ascorbic ac. eq 100 $g^{-1}$f.w. | mmol Trolox eq.100 $g^{-1}$ f.w. |
| Fields | | | |
| 1 | 383.6 c | 0.51 b | 0.96 |
| 2 | 494.3 b | 0.54 b | 0.80 |
| 3 | 642.9 a | 0.63 b | 0.61 |
| 4 | 531.5 b | 1.18 a | 0.60 |
| 5 | 630.7 a | 1.19 a | 0.85 |
| Significance | ** | ** | n.s. |
| Treatment | | | |
| Control | 518.5 c | 0.80 | 0.79 |
| Aegis | 636.9 a | 0.87 | 0.48 |
| Endospor | 582.8 b | 0.97 | 0.66 |
| Significance | ** | n.s. | n.s. |
| Bracts | | | |
| Internal | _ | 0.73 b | 1.03 a |
| external | _ | 0.97 a | 0.38 b |
| Significance | | * | ** |

Table 5. Nitrate content (mg $kg^{-1}$ f.w.) hydrophilic (HAA, mmol AA 100 $g^{-1}$ FW) and lipophylic (LAA, mmol Trolox 100 $g^{-1}$ FW) antioxidant activity of flower heads in different Field locations grown without Inoculation with Beneficial Microorganisms (Control), Inoculated with Aegis Sym Irriga (Aegis), and Inoculated with Endospor Dry Mix (Endospor) and Internal and External bracts. Means within columns separated using Duncan's multiple range test, p = 0.05. ns, *, **, = non-significant or significant at $p < 0.05$ and 0.01, respectively.

### 3.3.5 Sesquiterpene lactones content

The sesquiterpene lactones (SLs) are a group constituted by more than 500 compounds, characteristic of *Asteraceae* that occasionally are found in other plant families (Seaman, 1982)
The SLs are responsable of the peculiar bitter taste of both globe artichoke heads and cultivated cardoon stems (Ishida et al., 2010), and have been found to represent the major family of lipophilic components in cultivated cardoon leaves (95 g kg−1dry weight) (Ramos et al., 2013). Cynaropicrin, the major guaianolide found in the extracts, contributes approximately 80% to the bitter taste (Fritsche et al., 2002); at lower concentration grosheimin and its derivatives (Menin et al., 2012; Schneider and Thiele, 1974).

SLs are interesting compounds from the chemical and chemotassonomic point of view, and show anti-tumor, anti-leukemic, cytotoxic, antimicrobial activities and allergenic (Malarz et al. 2002; Price 1990; Tamaki 1995). Other properties are linked to the appetite and to the promotion of the digestion in humans (Kisiel and Zielińska, 2001).

Recently, Tanaka et al., (2013) reported that oral administration of cynaropicrin could protect the morphological changes of skin induced by UVB, such as epidermal hyperproliferation and melanocyte proliferation.

Artichoke leaves presented higher SLs content than flower heads (Table 6). SLs in edible part and leaves showed great space variability, exept for cynaratriol. Field 5 showed the highest total SLs content. Cynaratriol and cynaropicrin were the most abundant compounds detected in edible part (avg. 149,9 mg 100 $g^{-1}$ D.W. and 144,4 mg 100 $g^{-1}$ d.w. respectively). Cynaratriol has not been detected in artichoke leaves, whereas cynaropicrin was the most abundant SL observed in leaves and ranged from 509,7 mg 100 $g^{-1}$ d.w. to 841 mg 100 $g^{-1}$ d.w. (in field 4 and in control field 1, respectively), on an average value of 686,2 mg 100 $g^{-1}$ d.w. in a ratio about 1:5 in comparison to its edible part content. In edible part, this main SL ranged from 73 mg 100 $g^{-1}$ d.w. to 184 mg 100 $g^{-1}$ d.w. (in control field 1 and 2 respectively). Dyhidrocinaropicrin was present only in the edible part (avg.17,9 mg 100 $g^{-1}$ d.w.) and not in the leaves. Grosheimin, 8-deoxy-11-hydroxy-13-chlorogrosheimin and 8-deoxy-11, 13-dihydroxygrosheimin in leaves showed an average value of 223,2 mg 100 $g^{-1}$ d.w., 118 mg 100 $g^{-1}$ d.w. and 94,5 mg 100 $g^{-1}$ d.w. respectively; whereas in edible part grosheimin, 8-deoxy-11-hydroxy-13-chlorogrosheimin and 8-deoxy-11, 13-dihydroxygrosheimin reported an average value of 16,3 mg 100 $g^{-1}$ d.w., 22,1 mg 100 $g^{-1}$ d.w. and 15,2 mg 100 $g^{-1}$ d.w.

SLs content was not significantly influenced by mycorrhizal treatments, except for dyhidrocinaropicrin in edible part (45,2 mg 100 $g^{-1}$ d.w.) and 8-deoxy-11-hydroxy-13-chlorogrosheimin in leaves (505,5 mg 100 $g^{-1}$ d.w.) in which Aegis Sym Irriga affected positively their content respect to the control and to Endospor Dry Mix inoculated samples (Table 6).

Previous studies on *Artemisia annua* L. (Rapparini et al., 2008) and on *Plantago lanceolata* L. (Fontana et al., 2009) demonstrate the absence of the positive effects of mycorrhization (*Glomus* spp.) on the total content of SLs.

In contrast, Teiten et al., (2013) and Jurkiewicz et al., (2010) affirmed that mycorrhizal plants of *Arnica montana* L. accumulated active sesquiterpene lactones.

Cynaratriol, cynaropicrin, grosheimin and its derivates recorded, on average value in edible part, higher content in Endospor Dry Mix treatment's samples than Aegis Sym Irriga ones. This result is partially in line with a study on a synergistic effect of *Glomus* fungi and *Bacillus subtilis*

recorded in an increasing of artemisinin content in Artemisia annua L. (Awasthi et al., 2011). The methylerythritol phosphate (MEP) pathway was considered the principal route to monoterpene production, and the mevalonate (MVA) pathway, thought to be responsible for the production of sesqui- and triterpenes. There are no reports in the literature on how the mevalonate pathway is altered by AMF infection. However, evidence of a MEP pathway involvement in the production of sesquiterpene biosynthesis precursors in the Asteraceae (Adam et al., 1999; Arimura et al., 2004; Bertea et al., 2006; Steliopoulos et al., 2002) suggests that differences in the activity levels of related synthases could be less evident on total emission or production of terpenes, as also found in basil (*Ocimum basilicum* L., *Lamiaceae*; Iijima et al., 2004).

| Compound | | Field 1 | Field 2 | Field 3 | Field 4 | Field 5 | Mean value | Control | Aegis Sym Irriga | Endospor Dry mix | Mean value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg 100 $g^{-1}$ d.w. | | | | | | | | | |
| Cynaratriol | Edible parts | 190.4 a | 153.8 a | 173.2 a | 132.6 a | 99.3 a | 149.9 | 149.1 ab | 95.2 b | 212.4 a | 152.2 |
| | Leaves | nd | nd | nd | nd | nd | - | nd | nd | nd | - |
| Cynaropicrin | Edible parts | 73.0 c | 184.0 a | 130.2 b | 168.2 a | 167.5 a | 144.6 | 161.9 a | 81.6 b | 173.5 a | 139.0 |
| | Leaves | 841.0 a | 652.0 b | 712.5 a | 509.7 b | 715.7 a | 686.2 | 696.3 a | 617.2 a | 579.7 a | 631.1 |
| 8-deoxy-11-hydroxy-13-chlorogrosheimin | Edible parts | 36.6 a | 14.7 b | 20.3 b | 18.8 b | 20.3 b | 22.1 | 23.7 a | 13.9 b | 21.4 a | 19.7 |
| | Leaves | 61.3 b | 285.4 a | 78.5 b | 54.3 b | 110.5 b | 118.0 | 245.3 c | 505.5 a | 375.3 b | 375.3 |
| 8-deoxy-11, 13-dihydroxygrosheimin | Edible parts | 13.5 b | 11.1 b | 8.7 b | 30.6 a | 12.2 b | 15.2 | 21.9 a | 9.2 b | 13.3 ab | 14,8 |
| | Leaves | 118.3 a | 81.1 b | 115.6 a | 60.7 b | 96.6 a | 94.5 | 92.2 ab | 64.6 b | 119.4 a | 92.0 |
| Grosheimin | Edible parts | 8.3 c | 24.9 a | 15.7 bc | 11.5 c | 21.2 ab | 16.3 | 16.6 a | 7.9 b | 18.4 a | 14.3 |
| | Leaves | 228.6 ab | 159.2 bc | 291.6 a | 119.4 c | 317.9 a | 223.2 | 215.7 a | 165.7 b | 237.3 a | 206.2 |
| dihydrocinaropicrin | Edible parts | nd | 8.7 b | 39.0 a | 12.3 b | 11.4 b | 17.9 | 11.6 b | 45.2 a | 18.7 b | 25.2 |
| | Leaves | nd | nd | nd | nd | nd | - | nd | nd | nd | - |

Table 6. Sesquiterpene lactones content (mg per 100 $g^{-1}$ Dry Matter) in different Field locations and Parts of Artichoke Plants grown without Inoculation with Beneficial Microorganisms, Inoculated with Aegis Sym Irriga, and Inoculated with Endospor Dry Mix. Means within columns separated using Duncan's multiple range test, p = 0.05. ns, *, **, = non-significant or significant at $p < 0.05$ and 0.01, respectively.

### 3.3.6 Polyphenols metabolite profile

The beneficial properties of artichoke are mainly related to the phenolic compounds present in the flower heads (Lattanzio et al., 1994; Schütz et al., 2004) and in the leaves (Llorach et al., 2002; Wang et al., 2003).

Total phenols (TP) content showed no temporal difference, with similar values between the first and the second harvest and it was affected by field locations (highest TP value was recorded in flower heads from control field 1 followed by flower heads from field 5-data not shown-) and by the portional division of the crop. As shown in Table 7 the edible part showed the highest TP content (3.09 g $100^{-1}$ g), followed by the external bracts (1.84 g $100^{-1}$ g) and leaves (0.91 g $100^{-1}$ g).

Recent studies conducted by Lombardo et al. (2010) on Romanesco cv C3 on the influence of the parts of the plant on the phenolic composition, confirmed highest polyphenol content in the receptacle, since to define C3, the variety most suitable for fresh consumption. Fratianni et al. (2007) observed that irrespective of the genotype, the highest content of total polyphenols was found in the internal bracts and receptacle. Negro et al., (2012) instead confirmed that the TP content mainly depends by the parts of the plant as well as by the genotype.

Although it is well-known that AM fungi can stimulate synthesis of phenolic compounds (phenolic acids, flavonoids) and activate the carotenoid pathway in roots (Harrison and Dixon 1993; Schliemann et al. 2008; Strack and Fester 2006).Only few analyses have targeted final crop products (leaves, roots or fruits) of mycorrhizal plants used in food or in medicinal remedies. Literature indicate that activation of plant secondary metabolism in response to AM fungi can result in increases in essential oil concentration of plant tissues or in the content of individual molecules.

As partially shown in Table 2, inoculation with beneficial microorganisms influenced artichoke metabolite profile. In particular, among phenolic acids, the greatest variations were observed in gallic acid derivatives with a decrease in digalloyl methyl glucose and the appearance of gallic galloyl glucoside and gallic gallate not found in control samples.

Also, the flavonoid metabolite profile of artichoke was significantly modified by inoculation with beneficial microorganisms. It has been suggested that modification of the flavonoid profile in response to biotic stress such as mycorrhizal colonization may be the consequence of a general plant defense response, which is later suppressed (Volpin et al., 1994). Generally, inoculation of plants results in an overall increase in the production of some new phenolic compounds during the progression of the infection (Devi and Reddy, 2002). This evidence was confirmed in

artichoke: five flavonoids (namely, luteolin apiosyl malonyl glucoside, apigenin glucosyl lactate, apigenin rhamnoside rutinoside, naringenin rhamnoside, and myricetin arabinoside) were found in inoculated samples and not in control. Among quercetin derivatives, mycorrhization caused increases in glucoside glucuronide and galloyl rutinoside concentrations. On the other hand, corresponding decreases in glucuronide and diglucuronide levels was observed. Similarly, a reduction in luteolin glucuronide glucoside and an increase in apigenin acetylglucoside were also observed in the treated plants. Quantitative data of the main phenolic compounds showed a different distribution within head parts caused by the two different types of inoculum tested (Table 7). Results of the quantitative analysis performed by HPLC-UV and MS/MS are summarized in Table 7. In the edible parts of the samples, a percentage decrease in cynarin and a corresponding percentage increase in chlorogenic acid was observed as a result of artichoke plant inoculation with Endospor Dry Mix. On the other hand, an inverse trend was observed in outer bracts: data showed a percentage increase in cynarin and a corresponding percentage decrease in chlorogenic acid using both types of inoculum. Leaves of the inoculated plant also showed a different phenolic profile with respect to the control ones. According to previous works (Colla et al., 2012) the most abundant compound was 5-O caffeoylquinic acid, and it increased (in percentage) in samples with inoculations. 1,5-Di-O-caffeoylquinic acid was less abundant in leaves than in heads; however, it increased upon inoculation. Luteolin derivatives are the most typical flavonoids of artichoke leaves (Negro et al., 2012), and they showed a percentage decrease in samples with inoculations. These variations were most evident when the inoculation was performed with Endospor Dry Mix, indicating that besides mycorrhizal fungi (Glomus spp.) also beneficial bacteria played an active role in changing the phenolic profile of artichoke plants.

| | Total phenols | 1.5-di-*O*-caffeoylquinic acid | 5-*O*-caffeoylquinic acid | 3-*O*-caffeoylquinic acid | 4-*O*-caffeoylquinic acid | 1-*O*-caffeoylquinic acid | 4.5-di-*O*-caffeoylquinic acid | 3.5-di-*O*-caffeoylquinic acid | luteolin 7-*O*-Glucoside | luteolin 7-*O*-Rutinoside | luteolin-acetyl hexoside | coumaroyl quinic acid | feruloyl quinic acid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | g/100g d.w. | mg/100 g d.w. | | | | | | | | | | | |
| | | | | | | Leaves | | | | | | | |
| Control | 1.01 b | 8.3 b | 52.8 c | nd | nd | 0.9 a | nd | nd | 10.4 a | 16.0 a | 9.0 a | 1.6 a | 0.8 b |
| Aegis | 0.47 c | 16.3 a | 59.1 b | nd | nd | 0.6 c | nd | nd | 7.5 b | 8.4 b | 6.3 b | 1.0 b | 0.9 a |
| Endospor | 1.24 a | 16.6 a | 68.4 a | nd | nd | 0.7 b | nd | nd | 4.3 c | 5.4 b | 3.5 c | 0.5 c | 0.6 c |
| Mean value | 0.91 | 13.7 | 60.1 | - | - | 0.7 | - | - | 7.4 | 9.9 | 6.3 | 1.0 | 0.7 |
| | | | | | | External bracts | | | | | | | |
| Control | 1.67 a | 52.9 b | 41.2 a | 1.1 a | 0.6 b | 0.1 b | 0.9 a | 2.1 a | 0.4 b | nd | nd | 0.4 a | 0.2 a |
| Aegis | 1.78 a | 66.4 a | 28.5 b | 0.6 ab | 0.9 a | 0.3 a | 0.3 b | 1.3 a | 1.1 a | nd | nd | 0.4 a | 0.2 a |
| Endospor | 2.08 a | 68.7 a | 27.1 b | 0.5 b | 0.5 b | 0.1 b | 0.6 ab | 1.7 a | 0.7 b | nd | nd | 0.3 a | 0.2 a |
| Mean value | 1.84 | 62.7 | 32.3 | 0.7 | 0.7 | 0.2 | 0.6 | 1.7 | 0.7 | - | - | 0.4 | 0.2 |
| | | | | | | Edible part | | | | | | | |
| Control | 2.32 b | 79.4 a | 19.2 b | 0.2 a | 0.3 a | nd | 0.3 a | nd | 0.2 a | nd | nd | 0.1 a | 0.2 a |
| Aegis | 2.41 b | 77.8 a | 20.9 b | 0.3 a | 0.3 a | nd | 0.2 b | nd | 0.2 a | nd | nd | 0.1 a | 0.1 a |
| Endospor | 4.55 a | 72.2 b | 26.5 a | 0.3 a | 0.3 a | nd | 0.2 b | nd | 0.3 a | nd | nd | 0.1 a | 0.2 a |
| Mean value | 3.09 | 76.5 | 22.2 | 0.3 | 0.3 | - | 0.2 | - | 0.2 | - | - | 0.1 | 0.2 |

Table 7. Phenolic Total Content (Grams per 100 g Dry Matter) and Phenolic Profile (Each Component Expressed as Percent of Total) in Parts of Artichoke Plants Grown without Inoculation with Beneficial Microorganisms (Ctr), Inoculated with Aegis Sym Irriga (Aeg) and Inoculated with Endospor Dry Mix (End). Means within columns separated using Duncan's multiple range test, p = 0.05. ns, *, **, = non-significant or significant at $p < 0.05$ and 0.01, respectively.

### 3.4 Conclusions 1

It can be concluded that head number, leaves mean weight, minerals content, nitrate, antioxidant activity, total phenols and sesquiterpene lactones content varied widely among field locations. Control field 1 showed the highest N, P and total phenols content in flower heads. Control field 5 showed the highest N and K content in artichoke leaves and the highest HAA and sesquiterpene lactones content in flower heads. Field 5 and 3 showed the highest nitrate content. Our results also demonstrated that mycorrhizal treatments couldn't significantly affect the mineral content of the crop, showing that the high level of soil fertility and the adequate water availability made mycorrhizal symbiosis superfluous. Treatments with *Glomus mossea* and *Glomus intraradices* without helper bacteria, increased the nitrate and dihydrocinaropicrin content. The activation of plant secondary metabolism in response to AM fungi resulted in increases of the content of individual molecules, with a general but not significant positive influence due to the synergistic effect of *Glomus* spp and mycorrhizal helper bacteria. The portional division of the plant and head parts showed the highest SLs content in the leaves and the highest TP content in the edible part, without univocal result if related to inoculation.

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### 3.6 Topic 2: Effect of farming system and cold storage on quality attributes in strawberry (Fragaria × ananassa Duch.) cultivars *Sabrina* and *Ventana*.

Organic agriculture is a holistic production system that promotes health and the sustainable development of agroecosystems by obeying biodiversity, biological cycles and soil biological activity using fertilizers of organic origin and renewable energy sources" (FAO/WHO Codex Alimentarius Commission, 2007). The demand for organic products has increased rapidly in the last two decades, partially due to the general perception among consumers that organic foods are healthier, and more nutritive than conventionally produced products (Bourn and Prescott, 2002); even if scientific evidence is still insufficient to confirm or reject this assumption (Magkos et al., 2006).

Strawberry is a major crop in Italy with about 6,000 ha produced annually under both open field and protected cultivation (ISTAT, 2013). The major production areas of strawberry are located in Campania (1539 ha), Veneto (817 ha) and Emilia Romagna (592 ha) (ISTAT 2013). The Italian strawberry production is almost obtained from conventional farming; however , in the last decade the area devoted to organic strawberry production has doubled from 15 to 34 ha (CSO, 2013). Strawberries are among the most important fruit in the Mediterranean diet due to of high content of phytochemical compounds in particular antioxidant which may have relevant biological activity in human health (Giampieri et al., 2012). For instance, Sun et al. (2002) indicated that strawberries are considered a relatively potent antiproliferative activity on the growth of human liver cancer cells HepG2. Moreover, strawberries are also appreciated by consumers because of their relatively high content of ellagic acid, an antioxidant that has been proposed to exert anticarcinogenic and antimutagenic effects (Conney, 2003, Maas et al., 1991). Strawberries also contain other antioxidants with health promoting properties, such as vitamin C, anthocyanins, and other flavonoids such as quercetin and kaempferol (Häkkinen and Törrönen, 2000; Olsson et al., 2004). Concerning the quality attributed of organic strawberry, Olsson et al. (2006) demonstrated that strawberries grown organically had higher ratio of ascorbate to dehydroascorbate, total phenolics, ellagic acid and flavonols, indicating that organically grown strawberries might have a higher content of secondary metabolites than the conventional strawberries. However, other studies (Hakkinen and Torronen, 2000; Hargreaves et al., 2008) reported that organic cultivation had no consistent effect on the sugars, antioxidant levels, macro and micronutrients in strawberries when compared to conventional farming. Therefore, it is premature to conclude that either organic or conventional fruits are better in terms of nutritional value. Thus, additional researches comparing these two farming management systems are needed to aid in further evaluations (Jin et al., 2011).

Strawberries are generally consumed as fresh product and in processed forms in particular as frozen fruits (Oszmiański et al., 2007; 2009). The large scale of frozen strawberry production could be associated to the short shelf-life of the fruit and to the very broad scale of its use as a frozen product. Frozen berries are the most usual raw material used in the industrial manufacturing of strawberry jam and juices (Oszmiański et al., 2007; 2009). However, during the frozen food chain (freezing, subsequent frozen storage and thawing) strawberry could be subject to quality changes such as the reduction in the antioxidant compound content (Lindley, 1998). Similarly, Garrote and Bertone (1989) demonstrated that strawberry phenolics (i.e. ellagic acid, p-coumaric acid, quercetin and keampferol) are unstable and undergo deterioration during fruit transformation in frozen products particularly in the thawing process. An implicit hypothesis in characterizing the effects of farming system on physicochemical components of strawberry, is that these effects may actually occur in both pre and postharvest (e.g. storage).

Based on the above considerations, the overall objective of the current work was a composite examination of quality configuration in strawberry fruit through a factorial analysis of the relative effects of farming system, and postharvest storage (cold storage). Two strawberry cultivars, grown under organic and conventional farming methods were stored for 6 months at -40 ºC. Quality evaluation entailed physical (firmness and color), chemical (juice pH, titratable acidity, soluble solid and total sugars), and phytochemical (antioxidant activity, vitamin C, total phenols and ellagic acid) components.

## 3.7 Materials and methods 2

### 3.7.1. Plant material and sampling

Strawberries (*Fragaria* × *ananassa* Duch. cvs. '*Sabrina*' and '*Ventana*') grown under conventional and organic management systems at Vitulazio-Caserta (41°09′46″ N, 14°12′48″ E) and Sezze-Latina (41°30′33″ N, 13°04′37″ E)., were picked at commercial maturity (> 80% of the surface showing red color). Fruits were selected, based on uniformity of size, the absence of physical damage and fungal infection.

The organic farming for both strawberry cultivars was conducted following the EU regulations (EC 834/2007), whereas the conventional farming system was done according to the standard farming management practices. All of the fruits originated from plants grown in clay soils in outdoor plantations with drip irrigation. The plantations were divided into plots, separated by paths 50–60 cm. Samples of both cultivars were collected in three replications early in the morning and were immediately transferred to the laboratory for further analyses. After

harvesting, the fruits were cleaned with water and each repetition was subdivided into two parts for sample preparation. One half was used for analysis of firmness, colorimetry, soluble solids, titratable acidity, total sugars, antioxidant activity, and bioactive content. The other half was stored at – 40 ºC in a climatic chamber for 6 months. Afterwards, the strawberries samples were analyzed.

In both experiments, treatments were arranged in a randomized complete block design with three replicates. Treatments were defined by a factorial combination of two farming systems (conventional or organic), and two postharvest storage treatments (with or without cold storage).

### 3.7.2 Color measurements

For both strawberry cultivars, external color was measured on opposite sides of 15 fruits per experimental unit using a Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd, Osaka, Japan). The measuring aperture diameter was 8 mm, and the instrument was calibrated with Minolta standard white reflector plate before sampling the berries. Samples were placed on a white background and single readings were taken with the hand-held unit on the upper surface of each strawberry midway between the apical and basal ends. $L^*$ (lightness ranging from 0 = black to 100 = white), $a^*$ (ranging from green [-] to red [+]), $b^*$ (ranging from blue [-] to yellow [+]) readings were transformed to those of the $L^*$, $a^*$, $b^*$ color space. Numerical values of a* and b* were converted into hue angle (h° = arctan b*/a*) and chroma [chroma = $(a^2+ b^2)1/2$] (Francis, 1980). The h° is an angle in a color wheel of 360°, with 0, 90, 180 and 270° representing the hues red-purple, yellow, bluish-green and blue, respectively, while Chroma is the intensity or purity of the hue.

### 3.7.3 Physicochemical analysis

Fruit firmness (kg $cm^{-2}$) was determined using a penetrometer (Ametek, Hunter division, Hatfield, PA) fitted with an 6 mm-diameter round-head probe. From the liquid extract obtained by liquefying and filtering strawberry fruit, the total soluble solids (TSS) contents of the juice were determined using an Atago N1 refractometer (Atago Co. Ltd., Tokyo, Japan) and expressed as ºBrix at 20ºC. Acidity was determined by potentiometric titration with 0.1 M NaOH up to pH 8.1 using 10 ml of juice. Results were expressed as percentage of citric acid in the juice. Reducing sugars were determined by reaction of a water soluble portion of the sample with an excess of standard copper sulfate in alkaline tartrate (Fehling's) solution under controlled

conditions of time, temperature, reagent concentration and composition, so that the amount of copper reduced is proportional to the amount of reducing sugars in the sample analyzed. Fruit juice pH was also determined. Fruits were dried in a forced-air oven at 80°C for 72 h and weighed to determine the fruit dry matter (DM).

### 3.7.4 Hydrophilic and lipophylic antioxidant activities

Two different radical cation assays were used to determine the antioxidant activity of the hydrophilic (HAA) and lipophylic (LAA) fractions, on 0.2 g of lyophilized samples, extracted by distilled water and methanol, respectively. The HAA (i.e. water-soluble) was assessed using the N,N-dimethyl-p-phenylenediamine (DMPD) method (Fogliano et al., 1999). The principle of the assay is that in the presence of a suitable oxidant solution DMPD can form a stable and colored radical cation ($DMPD^{\cdot+}$). Antioxidant compounds (AO) which are able to transfer a hydrogen atom to $DMPD^{\cdot+}$ quench the color and produce a discoloration of the solution which is proportional to their amount. The LAA (i.e. liposoluble) was measured using the 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method as described by Pellegrini et al.(1999). The principle of the assay is that the inhibitory response of the radical cation is proportional to the antioxidant concentration and the reaction is complete at the time point selected of 2.5 min. The HAA and LAA were determined by UV–Vis spectrophotometry. The absorbance of the solution was measured at 505 and 734 nm, respectively. HAA and LAA were expressed as mmol ascorbic acid (AA) and as mmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per 100 g of fresh weight, respectively (Fogliano et al., 1999; Re et al., 1999).

### 3.7.5 Total ascorbic acid and phenol contents

The total ascorbic acid defined as ascorbic acid (AsA) and dehydroascorbate (DHA) acid was assessed by spectrophotometric detection on fresh plant tissues. The assay is based on the reduction of $Fe^{3+}$ to $Fe^{2+}$ by AsA and the spectrophotometric detection of $Fe^{2+}$ complexes with 2,2'-dipyridyl (Kampfenkel et al., 1995). DHA is recued to ASA by pre-incubation of the sample with dithiothreitol (DTT). The absorbance of the solution was measured at 525 nm, and data were expressed as mg ascorbic acid on 100 g fresh weight.

The total phenolic content was determined in methanol extracts (1 g of freeze-dried strawberries were extracted in 20 mL of 60% methanol) according to the Folin-Ciocalteau procedure (Singleton et al., 1999) using gallic acid as a standard. A 100 µL aliquot of the supernatant was combined with 500 µL of Folin-Ciocalteau's reagent (Sigma Aldrich Inc., St Louis, MO, USA)

and 400 µL of 7.5% sodium carbonate/water (w/v). The tubes were mixed for 15 s and then allowed to stand for 30 min at 20°C. Absorption was measured at 765 nm using a UV-Vis spectrophotometer, and the result was expressed as mg gallic acid per 100 g dry weight.

### 3.7.6 Ellagic acid content

To determine the total amount of ellagic acid, the berry samples (5 g) were extracted and hydrolysed in 50% (v/v) aqueous methanol according to the procedure described by Hakkinen et al. (1998). The samples were heated to 85 °C for 20 h in 0.6 M HCl and 0.15% ascorbic acid. Samples were then centrifuged at *10000g* for 5 min. The supernatant of each sample was diluted with distilled water to twice the volume, placed on a Seppak C18 column and eluted with 1.4 mL of methanol before analysis on an HPLC apparatus (Shimadzu LC 10, Shimadzu, Kyoto, Japan) equipped with a UV/Vis detector (mod. SPD-M10A 230 V) and a Prodigy ODS3 100 Å column (250 × 4.6 mm, particle size 5 µm) (Phenomenex, Torrance, CA, USA). The mobile phase was a linear binary gradient with solvent A (50 mM acetic acid and 5% (v/v) acetonitrile) and solvent B (acetonitrile/5% (v/v) MeOH) at a flow rate of 1 mL min-1. Each chromatogram was recorded at 256 nm.

### 3.7.7 Statistical analysis

All data were statistically analyzed by ANOVA using the SPSS 20 software package (www.ibm.com/ software/analytics/spss). To separate treatment means within each measured parameter, Duncan's multiple range test was performed at $P < 0.05$. Principal component analysis (PCA) using correlation matrix was performed on quality attributes to assess relationships among variables and treatments, and also to determine which traits were the most effective in discriminating between farming system and storage. PCA outputs include treatment component scores and variable loading to each selected component. The first two components PC1 and PC2 were selected for the ordination analysis, and the correlation between the original traits and the respective PC was calculated. Loadings > |0.6| indicate significant correlations between the original variables and the extracted components (Matus et al., 1996).

## 3.8 Results and discussion 2

### 3.8.1 Colorimetry

Strawberry surface color has been conventionally regarded by consumers as indicative of the overall fruit quality, and the intensity of red hue in particular has been associated arbitrarily with sweetness. In both cultivars, the changes in hunter color parameters were mainly due to farming system and to a lesser degree to the effect of cold storage. For 'Sabrina' cultivar no significant differences were observed between the fresh and frozen fruits, whereas for '*Ventana*' cultivar, the brightness (L*) of fresh strawberry fruit (avg. 21.3) was higher by 12.2% when compared to the frozen ones (avg. 19.0), while an opposite trend was observed for the redness (a*) parameter (Table 1).

When averaged over the cold storage, organic farming significantly increased the L*, a*, b*, ho, and C* in 'Sabrina' by 20.1%, 4.8%, 20.5%, 11.4%, and 9.7%, respectively, in comparison to those recorded in the conventional farming system (Table 1). The increasing is the hunter color parameters was more pronounced in '*Ventana*' since the L*, a*, b*, ho, and C* values of strawberry fruit coming from organic farming were significantly higher by 77.9%, 26.1%, 58.0%, 19.0%, and 33.7%, respectively when compared to those observed in the conventional farming (Table 1).

Overall, in both cultivars the color of organic fruits was brighter and more vivid (higher L* and C* values). The results reported in the current experiment, are in contrast with those recorded by Crecente-Campo et al. (2012), who observed that the color of organic strawberry fruits (cv. Selva) was darker, less vivid and tended to be redder (lower L*, C*, ho, values) in comparison to the conventional farming system. Explanations for this disagreement could be attributed to the genetic, weather and agronomic factors between the two experiments. Moreover, the color of '*Ventana*' fruit became darker and was more red with cold-storage. This was in agreement with the findings of Koyuncu (2004), who reported similar color changes on three strawberry cultivars '*Camarosa*', '*Cavendish*' and '*Chandler*', subjected to cold storage at 0 ºC.

| Farming system | Storage | L* | a* | b* | h° | C* |
|---|---|---|---|---|---|---|
| | | | | *Sabrina* | | |
| Organic | Fresh | 19.3 | 42.2 a | 33.1 | 38 | 53.7 a |
| | Frozen | 18.8 | 44.3 a | 32.4 | 36.1 | 54.9 a |
| | *Mean* | *19.1 a* | *43.3* | *32.8 a* | *37.1 a* | *54.3* |
| Conventional | Fresh | 17.6 | 44.5 a | 30.1 | 34.1 | 53.8 a |
| | Frozen | 14.1 | 38.1 b | 24.2 | 32.4 | 45.2 b |
| | *Mean* | *15.9 b* | *41.3* | *27.2 b* | *33.3 b* | *49.5* |
| Significance | | | | | | |
| Farming (F) | | ** | NS | ** | ** | ** |
| Storage (S) | | NS | NS | NS | NS | NS |
| F × S | | NS | * | NS | NS | ** |
| | | | | *Ventana* | | |
| Organic | Fresh | 27.6 | 37.7 | 25.2 | 33.8 | 45.4 |
| | Frozen | 24.0 | 40.6 | 26.0 | 32.4 | 48.3 |
| | *Mean* | *25.8 a* | *39.1 a* | *25.6 a* | *33.1 a* | *46.8 a* |
| Conventional | Fresh | 15.0 | 28.1 | 16.3 | 30.2 | 32.5 |
| | Frozen | 14.0 | 33.9 | 16.1 | 25.4 | 37.5 |
| | *Mean* | *14.5 b* | *31.0 b* | *16.2 b* | *27.8 b* | *35.0 b* |
| Significance | | | | | | |
| Farming (F) | | ** | ** | ** | * | ** |
| Storage (S) | | * | * | NS | NS | NS |
| F × S | | NS | NS | NS | NS | NS |

Table 1. Effects of farming system and cold storage on hunter color parameters L* (brightness), a* (+a* = red; -a* = green) and b* (+b* = yellow; -b = blue), hue (h°), Chroma (C*) of two strawberry cultivars.

Means for two farming systems over storage treatments are reported in italic font. Means with a common letter within columns are not significantly different according to Duncan's test ($P$= 0.05). NS, *, ** are nonsignificant or significant at $P < 0.05$ or 0.01, respectively.

3.8.2 Mean weight, Firmness and flavor compounds

The fruit mean weight in both strawberry cultivars was significantly affected by storage and farming system. For instance, the fruit mean weight was negatively affected by cold storage since weight loss increased as a function of storage in '*Sabrina'* and '*Ventana*' (Table 2). In the present study, organic strawberries, had a lower mean weight as previously reported by Abu-Zahra and Tahboub (2009) using the cultivar '*Camarosa*', on which cultivar, the same results were reported by Barbieri (2014). Although this may not be always true according to Baruzzi et al. (2009) and Conti et al. (2014) using different Italian strawberry cultivars.

No significant differences among treatments were recorded for reducing sugars in both cultivars 'Sabrina' (avg. 4.6%) and '*Ventana*' (3.9%), whereas the fruit dry matter was only affected by farming system in '*Ventana*' with the highest values recorded under organic farming management (Table 2). The fruit firmness in both cultivars was significantly affected by the cold storage with the highest values recorded on fresh fruits in comparison the frozen ones (Table 2). Firmness is a critical quality parameter in the consumer acceptability of fresh fruits. Strawberry is a soft fruit that suffers a rapid loss of firmness during ripening which contributes greatly to its short postharvest life (Goulas and Manganaris, 2011). The biochemical basis of strawberry softening is still unclear. However, strawberry softening has been correlated with the deterioration of the middle lamella of cortical parenchyma cells, leading to a sharp increase in pectin solubilisation, with small changes in pectin molecular weight and small decreases in the hemicelluloses content (Koh and Melton, 2002). Our results concerning cold storage are in line with Nunes et al. (2006) who demonstrated that strawberry firmness decreases during storage, due to loss of cell wall material (Koh and Melton, 2002).

The juice pH in both cultivars was significantly affected by the farming system with the highest values recorded in conventional (avg. 3.7) than in organic farming (avg. 3.5). The values of the juice pH recorded in the current experiment were consistent with the findings of Koyuncu (2004) on several strawberry cultivars (pH values ranged between 3.4 and 4.2). Contrarily to juice pH the highest titratable acidity (expressed as citric acid) in cultivar '*Ventana*' was recorded in organic in comparison to conventional farming. This negative correlation between pH and titratable acidity could be expected, considering that pH is determined primarily by organic acids (Sartono et al., 2003). Moreover, the titratable acidity recorded in '*Ventana*' fruits subjected to cold storage was significantly higher by 45.4% in comparison to those recorded at harvest (Table 2).

The total soluble solids (TSS) content of the juice, which comprises mainly soluble carbohydrates, has been regarded by convention as a measure of sweetness in most fruits including strawberry. In the current experiment, the TSS in both cultivars, was significantly affected by farming system, cold storage and their interaction, with the lowest valued recorded in frozen fruits coming from the conventional system (Table 2). Both cultivars were found to be significantly different in their TSS contents, with the highest values recorded in 'Sabrina' (avg. 6.6 ºBrix) when compared to '*Ventana*'(avg. 6.0 ºBrix). When averaged over farming system, the TSS decreased sharply in both cultivars after cold storage for 6 months. Finally, irrespective of storage, the organic management resulted in higher values of soluble solids in both cultivars (Table 2). These results are in agreement with several published studies (Abu-Zahra et al., 2006;

Abu-Zahra and Tahboub, 2009; Conti et al., 2014; Hargreaves et al., 2008) who founds that organic berries have a higher TSS content than conventionally grown fruit.

| Farming system | Storage | MW (g fruit$^{-1}$) | DM (%) | Firmness (kg cm$^{-2}$) | pH | TSS (ºBrix) | TA (%) | RS (%) |
|---|---|---|---|---|---|---|---|---|
| | | | | *Sabrina* | | | | |
| Organic | Fresh | 11.0 | 9.1 | 1.3 a | 3.5 | 7.3 ab | 0.9 | 4.2 |
| | Frozen | 10.4 | 8.7 | 0.3 c | 3.5 | 6.4 b | 0.8 | 5.2 |
| | *Mean* | *10.7 b* | *8.9* | *0.8* | *3.5 b* | *6.8* | *0.8* | *4.7* |
| Conventional | Fresh | 16.2 | 8.8 | 1.1 a | 3.7 | 7.7 a | 0.7 | 4.9 |
| | Frozen | 10.4 | 8.0 | 0.6 b | 3.7 | 5.3 c | 0.7 | 4.0 |
| | *Mean* | *13.3 a* | *8.4* | *0.8* | *3.7 a* | *6.5* | *0.7* | *4.5* |
| Significance | | | | | | | | |
| Farming (F) | | ** | NS | NS | ** | * | NS | NS |
| Storage (S) | | ** | NS | ** | NS | ** | NS | NS |
| F × S | | NS | NS | * | NS | ** | NS | NS |
| | | | | *Ventana* | | | | |
| Organic | Fresh | 11.9 | 7.5 | 1.3 a | 3.6 | 6.3 a | 0.6 | 3.5 |
| | Frozen | 9.1 | 8.7 | 0.2 b | 3.6 | 6.4 a | 0.9 | 4.2 |
| | *Mean* | *10.5 b* | *8.1 a* | *0.7* | *3.6 b* | *6.3* | *0.8 a* | *3.9* |
| Conventional | Fresh | 17.8 | 6.2 | 1.3 a | 3.7 | 6.5 a | 0.5 | 4.4 |
| | Frozen | 12.8 | 6.2 | 0.3 b | 3.7 | 5.0 b | 0.7 | 3.7 |
| | *Mean* | *15.3 a* | *6.2 b* | *0.8* | *3.7 a* | *5.8* | *0.6 b* | *4.0* |
| Significance | | | | | | | | |
| Farming (F) | | ** | ** | NS | * | * | * | NS |
| Storage (S) | | ** | NS | ** | NS | ** | ** | NS |
| F × S | | NS | NS | NS | NS | ** | NS | NS |

Table 2. Effects of farming system and cold storage on fruit mean weight (MW), dry matter (DM), firmness, juice pH, total soluble solids (TSS) content, titratable acidity (TA), and reducing sugars (RS) of two strawberry cultivars

Means for two farming systems over storage treatments are reported in italic font. Means with a common letter within columns are not significantly different according to Duncan's test ($P$= 0.05). NS, *, ** are nonsignificant or significant at $P < 0.05$ or 0.01, respectively.

3.8.3 Antioxidant activity and bioactive compounds

The effects of farming system and cold storage on the hydrophilic (HAA) and lipophylic (LAA) antioxidant activities were more pronounced in '*Ventana*' than in '*Sabrina*'. The HAA values of the frozen '*Sabrina*' fruits (avg. 18.9 mmol ascorbic acid 100 g-1 FW) were significantly higher by 29.4% than those recorded on fresh berries (avg. 14.6 mmol 100 g-1 ascorbic acid FW) (Table 3). The HAA in '*Ventana*' was significantly higher in organic in comparison to

conventional farming, and in frozen than in fresh fruits, whereas an opposite trend was observed for LAA (Table 3).

Total ascorbic acid has long be considered an important nutritional compound of strawberries (Scalzo et al., 2005), since it offers potential benefits to human health for protection against diseases (Halliwel et al., 2005). The total ascorbic acid in '*Sabrina*' was significantly affected by cold storage with the highest values recorded in fresh compared to the frozen berries. Similar results were found by Perez et al. (1998) and Koyuncu (2004) in two studies related to the storage of different strawberry cultivars at 2 and 0 ºC, respectively. Similarly, Lisiewska and Kmiecik (1996) observed that freezing resulted in little change of the vitamin C content, which was reduced by 15–18% in broccoli and by 6–13% in cauliflower after 12 months of frozen-storage at -40°C. Our results also demonstrated that the cultivation system did not affect significantly the total ascorbic acid content in both cultivars. The literature on this topic is inconclusive. For instance, Asami et al. (2003) studied the 'Northwest Totem' strawberry variety and demonstrated that the AA contents in sustainably grown samples were significantly higher than the contents for conventionally grown crops. Similarly, Jin et al. (2011) indicated that the AA content was higher in organically cultivated versus conventionally cultivated 'Earliglow' and 'Allstar' strawberries. However, Hakala et al. (2003) indicated that organic cultivation had no effect on the vitamin C content of the 'Honeoye', 'Jonsok' and 'Polka', varieties, whereas Cardoso et al. (2011) showed that the AA content was significantly lower in organic strawberries (30.74 mg 100 g-1) than conventional strawberries (42.45 mg 100 g-1).

No significant differences among treatments were recorded for total phenols content in '*Sabrina*' (avg. 106.4 mg gallic acid 100 g-1 dw), whereas a significant effect of farming system was observed in '*Ventana*', since an increasing of 13.4% in total phenols content was observed on fruits harvested from the conventional system (Table 3). Similarly to the total phenols, the ellagic acid a naturally occurring polyphenolic secondary metabolite that accumulates in strawberry fruits (Vattem and Shetty, 2005), was affected by the cultivation management system. The ellagic acid content in '*Sabrina*', recorded in the conventional farming was significantly higher by 7.2% in comparison to the content of this phenolic acid in organic strawberry (Table 3). Finally, our results demonstrated that the ellagic acid content significantly decreased by 11.9% and 18.6% in '*Sabrina*' and '*Ventana*', respectively when fruits were frozen at -40 ºC for 6 months. This is in agreement with the findings of Häkkinen and Törrönen (2000) who reported a 40% decrease in ellagic acid content in strawberries during 9 months of storage in a freezer.

| Farming system | Storage | HAA | LAA | AA | TP | Ellagic acid |
|---|---|---|---|---|---|---|
| | | | | *Sabrina* | | |
| Organic | Fresh | 15.7 | 19.7 | 72.8 | 106.8 | 55.0 |
| | Frozen | 19.6 | 17.7 | 41.8 | 104.2 | 49.8 |
| | *Mean* | *17.7* | *18.7* | *57.3* | *105.5* | *52.4 b* |
| Conventional | Fresh | 13.6 | 18.1 | 58.0 | 100.5 | 60.4 |
| | Frozen | 18.2 | 18.1 | 49.3 | 114.2 | 51.9 |
| | *Mean* | *15.9* | *18.1* | *53.7* | *107.3* | *56.2 a* |
| Significance | | | | | | |
| Farming (F) | | NS | NS | NS | NS | * |
| Storage (S) | | ** | NS | ** | NS | ** |
| F × S | | NS | NS | NS | NS | NS |
| | | | | *Ventana* | | |
| Organic | Fresh | 24.9 a | 15.2 | 23.7 | 96.7 | 55.6 |
| | Frozen | 19.7 b | 12.6 | 21.8 | 88.4 | 44.9 |
| | *Mean* | *22.3* | *13.9 b* | *21.8* | *92.6 b* | *50.3* |
| Conventional | Fresh | 12.0 c | 21.7 | 38.9 | 111.6 | 57.0 |
| | Frozen | 24.7 a | 17.6 | 40.5 | 98.5 | 46.7 |
| | *Mean* | *18.4* | *19.7 a* | *39.7* | *105.0 a* | *51.9* |
| Significance | | | | | | |
| Farming (F) | | ** | ** | NS | * | NS |
| Storage (S) | | ** | * | NS | NS | ** |
| F × S | | ** | NS | NS | NS | NS |

Table 3. Effect of farming system and cold storage on hydrophilic (HAA, mmol AA 100 $g^{-1}$ FW) and lipophilic (LAA, mmol Trolox 100 $g^{-1}$ FW) antioxidant activities, total ascorbic acid (AA, mg 100 $g^{-1}$ FW), total phenols (TP, mg gallic acid 100 $g^{-1}$ DW), and ellagic acid (mg 100 $g^{-1}$ DW) contents of two strawberry cultivars.
Means for two farming systems over storage treatments are reported in italic font. Means with a common letter within columns are not significantly different according to Duncan's test (P= 0.05). NS, *, ** are nonsignificant or significant at $P < 0.05$ or 0.01, respectively.

### 3.8.4 Principal component analysis

As defined by Lawless and Heymann (2010), "the PCA is a multivariate technique used to summarize and extract trends when many variables are used". PCA formulates new variables called principal components (PC), which summarize better the variability of the original variables and are correlated to them. The first two PCs were associated with Eigen values > 1, and explained 74.3% of the total variance, with PC1 accounting for 44.3% and PC2 for 30.0% (Table 4). PC1 was positively and significantly correlated with C*, ho, a*, b*, fruit dry matter, total soluble solids and titratable acidity (Table 4). PC2 was positively correlated with lipophylic antioxidant activity, total ascorbic acid, total phenols, ellagic acid content and negatively

correlated with hydrophilic antioxidant activity. Moreover PC3, which explained 10.1% of the variance, was negatively correlated with L* (data not shown).

Generally, the first two PCs account for meaningful variance; therefore only PC1 and PC2 were retained and interpreted (Fig. 1 A, B). The loading plot (Fig. 1 A) illustrates the relationships among variables (i.e. quality attributes), where two vectors with an angle less than 90° are positively correlated and two vectors with an angle > 90° are negatively correlated (Sartono et al., 2003). For instance, ellagic acid content was positively correlated with total ascorbic acid, and to a lesser degree tototal soluble solids and ho, whereas total soluble solids content was highly correlated to reducing sugars. Similarly total phenols were strongly correlated to lipophylic antioxidant activity, than to ellagic acid content. Moreover, a negative correlation between total phenols and titratable acidity was also observed (Fig. 1 A).

Components 1 and 2 score plot (Fig. 2 B) discriminates treatments into four groups. The positive side of PC1 (quadrants 2 and 4) included fruit of '*Sabrina*' and '*Ventana*' with and without cold storage coming organic farming management, but also conventional '*Sabrina*' fruit without storage. The treatments of the upper right quadrant, in particular organic and conventional '*Sabrina*' fruit at harvest were characterized by high total soluble solids, ascorbic acid and ho, whereas the cluster in the lower right quadrant represents fruit characterized by high brightness (L*) (Fig. 1 B). The negative side of PC1 (quadrants 1 and 3) corresponded to conventional fruit of '*Ventana*' with and without cold storage, but also to conventional '*Sabrina*' frozen at -40 ºC for 6 months. The treatments of the upper left quadrant (1), were characterized by high lipophylic antioxidant activity and total phenols, whereas those of the lower left quadrant (3) had relatively the lowest fruit quality among treatments (Fig. 2 B). The PCA carried out in the current experiment demonstrated the ability of this analysis to track the effects of farming management and postharvest storage on two strawberries fruit quality and could constitute the basis of future strategies to optimize quality.

| Trait | principal component (PC) | |
|---|---|---|
| | PC1 | PC2 |
| $L^*$ | 0.473 | -0.556 |
| $C^*$ | **0.981** | 0.034 |
| $h^\circ$ | **0.821** | 0.310 |
| $a^*$ | **0.968** | -0.109 |
| $b^*$ | **0.968** | 0.143 |
| Fruit dry matter | **0.963** | -0.049 |
| Total soluble solids | **0.652** | 0.471 |
| Reducing sugars | 0.587 | 0.279 |
| Titratable acidity | **0.661** | -0.489 |
| Hydrophilic antioxidant activity | -0.039 | **-0.852** |
| Lipophylic antioxidant activity | -0.362 | **0.882** |
| Ascorbic acid | 0.324 | **0.764** |
| Total phenols | -0.290 | **0.781** |
| Ellagic acid | 0.148 | **0.725** |
| Eigenvalue | 6.21 | 4.20 |
| Percentage of variance | 44.3 | 30.0 |
| Cumulative variance | 44.3 | 74.3 |

Table 4. Correlation coefficient for each quality attributes with respect to the first two principal components PC1 and PC2, eigenvalues, relative and cumulative proportion of total variance. The bold values indicate the most relevant characters for each principal component.

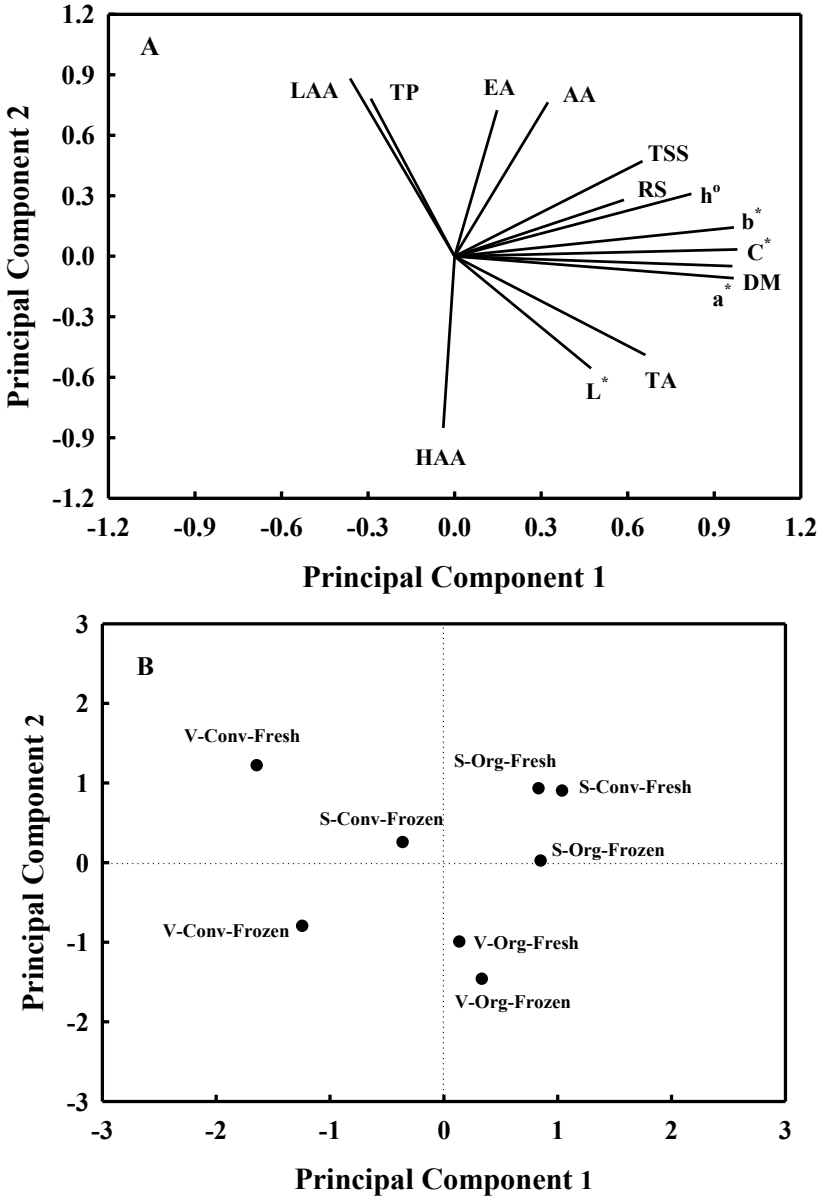


Figure 1. (A) Principal component loading plot and (B) scores of principal component analysis of physicochemical fruit traits of two strawberry cultivars (S, *Sabrina*; V, *Ventana*) as a function of farming system management (Org, organic; Conv, conventional), and postaharvest storage (Fresh, at harvest; Frozen, cold storage at -40ºC for 6 months). $L^*$, lightness; $C^*$, chroma; $h^o$, hue angle; $a^*$, redness; $b^*$, yellowness; DM, fruit dry matter; TSS, total soluble solids content; RS, reducing sugars; TA, titratable acidity; HAA, hydrophilic antioxidant activity; LAA, lipophylic antioxidant activity; AA, ascorbic acid; TP, total phenols; EA, ellagic acid.

### 3.9 Conclusions 2

As a summary, the results of the two trials designed to determine cold storage effects on the stability of quality traits and bioactive compounds of ripe strawberry fruit indicate that the skin lightness, firmness, total soluble solids content, and ellagic acid scored higher at harvest (fresh fruit without storage) than after storage at -40 ºC for 6 months. Of the flavor and health-

promoting compounds, storage affected only hydrophilic antioxidant activity, leaving reducing sugars and total phenols without significant alterations. Our results also demonstrated that the type of farming management may have important consequences in terms of physical, flavor and health-related compounds. In both cultivars the color of organic fruits was brighter and more vivid (higher L* and C* values). Strawberries grown under organic farming were found to contain higher HAA and LAA in '*Ventana*' and higher ellagic acid content in '*Sabrina*' indicating that these characteristics of cultivars should be taken into consideration in choosing a cultivar for a specific farming system.

## 3.10 References


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### 3.11 Topic 3: Nutritional quality of ten leafy vegetables harvested at two light intensities

In recent years, phytochemical compounds found in vegetables have generated significant interest among consumers and researchers due to their health-benefit effects (Khanam et al., 2012; Bian et al., 2015). Several *in vitro*, pre-clinical and clinical investigations have revealed an inverse association between a high consumption of vegetables and a lower incidence of many chronic diseases such as cardiovascular and neuro-degenerative diseases, ischemic stroke, arthritis, inflammatory bowel and also some cancers (Jainet al., 1999; Slavin and Lloyd, 2012). Therefore, fresh-cut or minimally processed baby leaf vegetables are gaining importance among consumers worldwide, since they represent a good source of minerals, vitamins, phytochemicals, especially antioxidants (Altundag and Tuzen, 2011; Grusak and DellaPenna 1999).

Italy is the European leader in leafy vegetables, with about 3000 ha produced annually under protected cultivation and destined to fresh-cut products such as wild rocket, leaf lettuce, lamb's lettuce, spinach, and swiss chard. The major production areas are located in Campania (in the Sele Plain in the Province of Salerno), Lombardy and Veneto (Pimpini et al., 2005).

The biosynthesis, composition and concentration of health-promoting compounds vary widely among leafy vegetables, a phenomenon that is influenced by genetic and environmental factors, cultural practices, harvesting and postharvest handling conditions (Rouphael et al., 2012; Samuolienė et al., 2013). On the other hand, leafy vegetables are among the vegetable species most sensible to nitrate accumulation, thus contributing greatly to the total intake of nitrates in the diet (Amr and Hadidi 2001; Lucarini et al., 2012). A high level of nitrate in leafy vegetables is considered undesirable because ofthe possible implications on health (Parks et al., 2008). Therefore, the World Health Organization (WHO) has set an acceptable daily intake (ADI) for nitrate of 3.7 mg $kg^{-1}$ body weight (Speijers and Van Den Brandt, 2003). However, due to the lack of substantial epidemiological studies (Milkowski et al., 2010), many researches did not find a direct correlation between nitrate concentration in food and incidence of cancer (Speijers and Van Den Brandt, 2003). Despite the debate about the positive or negative effects of nitrate on human health, the production and commercialization of some leafy vegetables in Europe is subjected to limitations (Commission Regulation (EU) No 1258/2011 of 2 December 2011 Cavaiuolo and Ferrante, 2014). This is because some groups of human population (e.g. vegetarians and babies) could be at higher risk to develop cancer when subjected to elevated levels of dietary nitrate intakes (Cavaiuolo and Ferrante, 2014).

Genetic, agronomic (e.g. amount, timing, and form of nitrogen fertilizer) and environmental factors (e.g. temperature, light intensity and photoperiod, carbon dioxide concentration) can also significantly influence the level of nitrate in raw leafy vegetables (Maynard et al., 1976; Santamaria, 2006). Among these, the genetic background, nitrate supply, harvesting time and light intensity during the growing season are predominant (Amr and Hadidi 2001; Santamaria, 1997; Burns et al., 2010) For instance, the relationship between light levels during the growing cycle and nitrate concentration has been demonstrated in a number of studies (Amr and Hadidi 2001; Proietti et al., 2004Walters 1991;). Recently, Chang et al. (2013a; 2013b) carried out a field and a growth chamber studies on changes in nitrate concentrations over a 24 h period for spinach, sweet basil and scallions. The authors demonstrated that the nitrate content fluctuated over the 24 h period, and these variations were strongly correlated to the changes in light intensity over the same period, and were also species dependent. The authors concluded that the reduction of nitrate content could be possible, by harvesting the vegetables at the right time during the day. Nevertheless, little information is available concerning the effect of light intensity at time of harvest on desirable and undesired compounds in several baby leaf vegetables, especially under greenhouse conditions.

Therefore, the objectives of this study were 1) to evaluate the nutritional composition of selected baby leaf vegetables belonging to the families of *Chenopodiaceae* (red chard, spinach, swiss chard), *Asteraceae* (chicory, green and red lettuce), *Brassicaceae* (mizuna, rocket, tatsoi), *Valerianaceae* (Lamb's lettuce), and 2) to assess the variation in visual appearance, proteins content, minerals content, antioxidant activity, ascorbic acid and total phenols content of the selected leafy vegetables in relation to the light intensity (low and high Photosynthetically Active Radiation, PAR) at time of harvest. These findings will allow a better understanding of the nutritional values of selected baby leaf vegetables for both food nutritionists and consumers and will help producers in identifying the best species-specific time of harvest in order to achieve higher nutritional value for the candidate baby leaf species in a sustainable way.

## 3.12 Materials and methods 3

### 3.12.1 Plant material, growth conditions and treatments

The experiment was carried out in spring 2014, in a greenhouse covered by plastic film in ethyl vinyl acetate (EVA) 0.2 mm, situated at the experimental farm of Pontinatura-Latina in central Italy (41° 24’ N, 13° 03’ E, altitude 4 m a.s.l.). Plants were grown under natural solar radiation.

The air temperature and the day/night relative humidity inside the greenhouse were maintained between 12/28 °C and 55/80%, respectively. The soil was a sandy loam (73% sand, 11% silt, 16% clay), with a bulk density of 1.1 g $cm^{-3}$, pH of 7.5, electrical conductivity of 0.4 dS $m^{-1}$, organic matter of 1.2% (w/w), total N at 0.08%, available P at 81 mg $kg^{-1}$, exchangeable K at 641 mg $kg^{-1}$.

Ten species of leafy vegetables, as fresh and ready-to-use baby leaf vegetables, were used in this study. Common and scientific name, botanical family, cultivar and source seeds are given in Table 1.

Baby leaf species seeds were sown on March, and plants were harvested on April 24. Preplant organic fertilizer (BIOREX, Italpollina, S.p.A., Verona, Italy) containing 2.8% N, 2.5% $P_2O_5$, 3% $K_2O$, 38% organic carbon, and 65% organic matter was broadcast (1000 kg $ha^{-1}$) and incorporated into the soil. An additional fertilizer (10.5 kg N $ha^{-1}$; 11.5 kg $P_2O_5$ $ha^{-1}$ and 8.0 kg $K_2O$ $ha^{-1}$) was also applied throughout the irrigation system. Irrigation was applied by movable boom spray system (e.g. overhead irrigation). Plants were kept free from weeds, insects, and diseases using greenhouse standard management procedures. Randomized complete block design was used in the current experiment, with treatments replicated three times. Treatments were defined by a factorial combination of two light intensities at time of harvest (low and high Photosynthetically Active Radiation, PAR) and ten baby leaf vegetables (chicory, green lettuce, lamb's lettuce, mizuna, red chard, red lettuce, rocket, spinach, swiss chard, and tatsoi). Each experimental unit consists of a 8 $m^2$ plot area.

| Leafy vegetables | Scientific name | Family | Cultivar | Source |
|---|---|---|---|---|
| Chicory | *Cichorium intybus* L. | *Asteraceae* | Catalonia | L'ortolano, Cesena, Italy |
| Green Lettuce | *Lactuca sativa L.* | *Asteraceae* | Bataser | Ortis, Cesena, Italy |
| Lamb's lettuce | *Valerianella locusta L.* | *Valerianaceae* | Juvert | Enza Zaden, Viterbo, Italy |
| Mizuna | *Brassica rapa var. nipposinica* L.H. Bailey | *Brassicaceae* | Japonica | Ortis, Cesena, Italy |
| Red chard | *Beta vulgaris var. cicla L* | *Chenopodiaceae* | Pluton | Ortis, Cesena, Italy |
| Red lettuce | *Lactuca sativa L.* | *Asteraceae* | MS 151 | Ortis, Cesena, Italy |
| Rocket | *Eruca sativa* Miller | *Brassicaceae* | - | Enza Zaden, Viterbo, Italy |
| Spinach | *Spinacea oleracea* L. | *Chenopodiaceae* | Antelope | Rijk Zwaan, Bologna, Italy |
| Swiss chard | *Beta vulgaris var. cicla L* | *Chenopodiaceae* | Agila | Olter, Milano, Italy |
| Tatsoi | *Brassica rapa var. narinosa* L.H. Bailey | *Brassicaceae* | - | Olter, Milano, Italy |

Table 1. Common and scientific name, botanical family, cultivar and source of the ten leafy vegetables considered in this study

### 3.12.2 Collection of samples

On April 24, the plant tissue were harvested at two light intensities: low (200-400 µmol m-2 s-1) and high PAR (800-1200 µmol m-2 s-1) using scissors and particles such as soil, were brushed off of the samples. The light intensity at time of harvest was measured with a LI-COR Model LI-190 quantum sensor (Lincoln, NE, USA), which was connected to light logger meter Model LI-1500 (Lincoln, NE, USA). The sensor was placed close and at the same level to the plants being sampled. For the determination of mineral composition, nitrate content, antioxidant activity, ascorbic acid and total phenols contents, fresh leaves were frozen in liquid nitrogen and stored at - 80°C until used. Yield was determined in sampling areas of 1 $m^2$ from the center of each experimental unit. Marketable yield of the selected baby leaf vegetables was considered after eliminating decayed leaves, and was expressed in kg $m^{-2}$.

### 3.12.3 SPAD and color measurements

A chlorophyll meter SPAD-502 (Konica-Minolta corporation, Ltd., Osaka, Japan) was used to take greenness readings (i.e. light transmittance) from the fully expanded leaves. Fifteen leaves per experimental unit were randomly measured and averaged to a single SPAD value for each treatment. Moreover, at both harvest time the leaf color was measured as reflected in the CIELAB ($L^*$ $a^*$ $b^*$) color space using a Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd, Osaka, Japan). The observations were made on fifteen randomly selected leaves per plot. The measuring aperture diameter was 8 mm, and the instrument was calibrated with Minolta standard white reflector plate before sampling baby leaves. Whole leaf samples were placed on a white background and single readings were taken with the hand-held unit on the upper surface of each leaf midway between the apical and basal ends. Individual leaves were then positioned between two paper towels moistened with distilled water in a plastic bag and held on ice until all readings were completed. $L^*$ (lightness ranging from 0 = black to 100 = white), $a^*$ (ranging from green [-] to red [+]), $b^*$ (ranging from blue [-] to yellow [+]) readings were transformed to those of the L, a, b color space (Fallovo et al., 2012). Chroma, C* represents color saturation which varies from dull (low value) to vivid color (high value) and was calculated using the following formula $(a^2 + b^2)^{1/2}$.

### 3.12.4 Dry matter, protein and mineral analysis

Dry matter (DM) and protein contents, were determined according to the Association of Official Analytical Chemists (AOAC 1990). Leaf DM was determined after drying the plant tissue in a forced air oven at 70°C. Dried leaf tissues were ground separately in a Wiley Mill to pass through a 20-mesh screen, then 0.5 g portions of the dried tissues were analyzed for the following macronutrients: N, P, K, Ca, and Mg. Nitrogen (Total N) concentration in the plant tissues was determined after mineralization with sulfuric acid by Kjeldahl method (Bremner, 1965). The total protein content was calculated by multiplying the evaluated nitrogen by a conversion factor of 6.25 (AOAC 2001.11). P, K, Ca, and Mg were extracted by nitropercloric digestion. P content in the leaf tissue was determined by colorimetry (e.g. measure of absorbance; Walinga et al., 1995), whereas K, Ca, and Mg contents were analyzed by atomic absorption spectrophotometry according to the method described by Walinga et al (1995).

### 3.12.5 Nitrate content

Nitrate ($N\text{-}NO_3$) content was determined on water extract of the dried leaf samples according to the cadmium reduction method (Sah, 1994), using the Hach DR 2000 (Hach Co., Loveland, Colorado, USA) spectrophotometer. Briefly, the dry matter samples (0.5 g) of leaf tissue were solved in deionized water, then treated with the nitrate reagent NitraVer 5, for a 5-min reaction time. Spectrophotometer lecture was performed at 500 nm wavelength.

### 3.12.6 Hydrophilic and lipophylic antioxidant activities

Two different radical cation assays were used to determine the antioxidant activity of the hydrophilic (HAA) and lipophylic (LAA) fractions, on 0.2 g of lyophilized leaf samples, extracted by distilled water and methanol, respectively. The HAA (i.e. water-soluble) was assessed using the N,N-dimethyl-p-phenylenediamine (DMPD) method (Fogliano et al., 1999). The principle of the assay is that in the presence of a suitable oxidant solution DMPD can form a stable and colored radical cation ($DMPD^{\cdot+}$). Antioxidant compounds (AO) which are able to transfer a hydrogen atom to $DMPD^{\cdot+}$ quench the color and produce a discoloration of the solution which is proportional to their amount. The LAA (i.e. liposoluble) was measured using the 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method as described by Pellegrini et al. (1999). The principle of the assay is that the inhibitory response of the radical cation is proportional to the antioxidant concentration and the reaction is complete at the time point selected of 2.5 min. The HAA and LAA were determined by UV–Vis spectrophotometry.

The absorbance of the solution was measured at 505 and 734 nm, respectively. HAA and LAA were expressed as mmol ascorbic acid (AA) and as mmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per 100 g of fresh weight, respectively (Fogliano et al., 1999; Re et al., 1999).

### 3.12.7 Total ascorbic acid and phenol contents

The total ascorbic acid defined as ascorbic acid (AsA) and dehydroascorbate (DHA) acid was assessed by spectrophotometric detection on fresh plant tissues. The assay is based on the reduction of $Fe^{3+}$ to $Fe^{2+}$ by AsA and the spectrophotometric detection of $Fe^{2+}$ complexes with 2,2'-dipyridyl (Kampfenkel et al., 1995). DHA is recued to ASA by pre-incubation of the sample with dithiothreitol (DTT). The absorbance of the solution was measured at 525 nm, and data were expressed as mg ascorbic acid on 100 g fresh weight.

The total phenolic content in the methanol extracts was determined using Folin-Ciocalteau procedure (Singleton et al., 1999) using gallic acid as a standard. A 100 µL aliquot of the supernatant was combined with 500 µL of Folin-Ciocalteau's reagent (Sigma Aldrich Inc, St Louis, MO, USA) and 400 µL of 7.5% sodium carbonate/water (w/v). The tubes were mixed for 15 s and then allowed to stand for 30 min at 20°C. Absorption was measured at 765 nm using a UV-Vis spectrophotometer, and the result was expressed as mg gallic acid (Sigma Aldrich Inc, St Louis, MO, USA) per 100 g dry weight.

### 3.12.8 Statistical analysis

All data were statistically analyzed by analysis of variance (ANOVA) using the SPSS 10 software package for Windows 2001 (www.ibm.com/ software/analytics/spss). Duncan's multiple range test was performed on each of the significant ($P < 0.05$) variables measured. Nutritional traits were subjected to principal component analysis (PCA), to explore relationships among variables and treatments, and also to determine which traits were the most effective in discriminating between species and light intensity at time of harvest. PCA outputs include variable loading to each selected component and treatment component scores. The first three

components (PC1, PC2, and PC3) were selected for the ordination analysis, and the correlation between the original traits and the respective principal component was calculated. Loadings > |0.6| indicate significant correlations between the original variables and the extracted components (Matus et al., 1996). All calculations and analyses were performed using the appropriate options within SPSS 10, and SigmaPlot version 10 (Systat Software, Inc., Chicago, IL, USA).

## 3.13 Results and discussion 3

### 3.13.1 Marketable fresh yield

The marketable fresh yield was significantly different among the baby leaf species. The highest yield was observed for lamb's lettuce (1.35 kg $m^{-2}$), while the two oriental leafy vegetables tatsoi and mizuna exhibited the lowest leaf fresh production (avg. 0.51 kg $m^{-2}$; Fig. 1). The marketable yield of the remaining vegetables was in the following order: swiss chard ≥ chicory = green lettuce > red chard = red lettuce > rocket = spinach (Fig. 1). The leaf fresh production of chicory (1.08 kg $m^{-2}$), green lettuce (0.98 kg $m^{-2}$), lamb's lettuce (1.35 kg $m^{-2}$), rocket (0.65 kg $m^{-2}$) and swiss chard (1.20 kg $m^{-2}$) were consistent with the findings of Pimpini et al. (2005). In contrast, the fresh production of lettuce and lamb's lettuce recorded in the current study was lower than those reported by Fallovo et al. (2009) and Manzocco et al. (2011) (2.2 and 1.6 kg $m^{-2}$, for lettuce and lamb's lettuce, respectively). An explanation for this difference could be the different growing systems (soil *vs.* soilless), in which the plants were grown. An important advantage of soilless over soil based culture is a more precise control of nutrition and a more efficient use of water and fertilizers leading to a better crop performance (Rouphael et al., 2004). This was also confirmed by Fontana and Nicola (2009), who observed that corn salad and rocket plants grown in soilless culture produced more than two-fold and four-fold, respectively in terms of leaf fresh production in comparison with the same crops cultivated in soil.

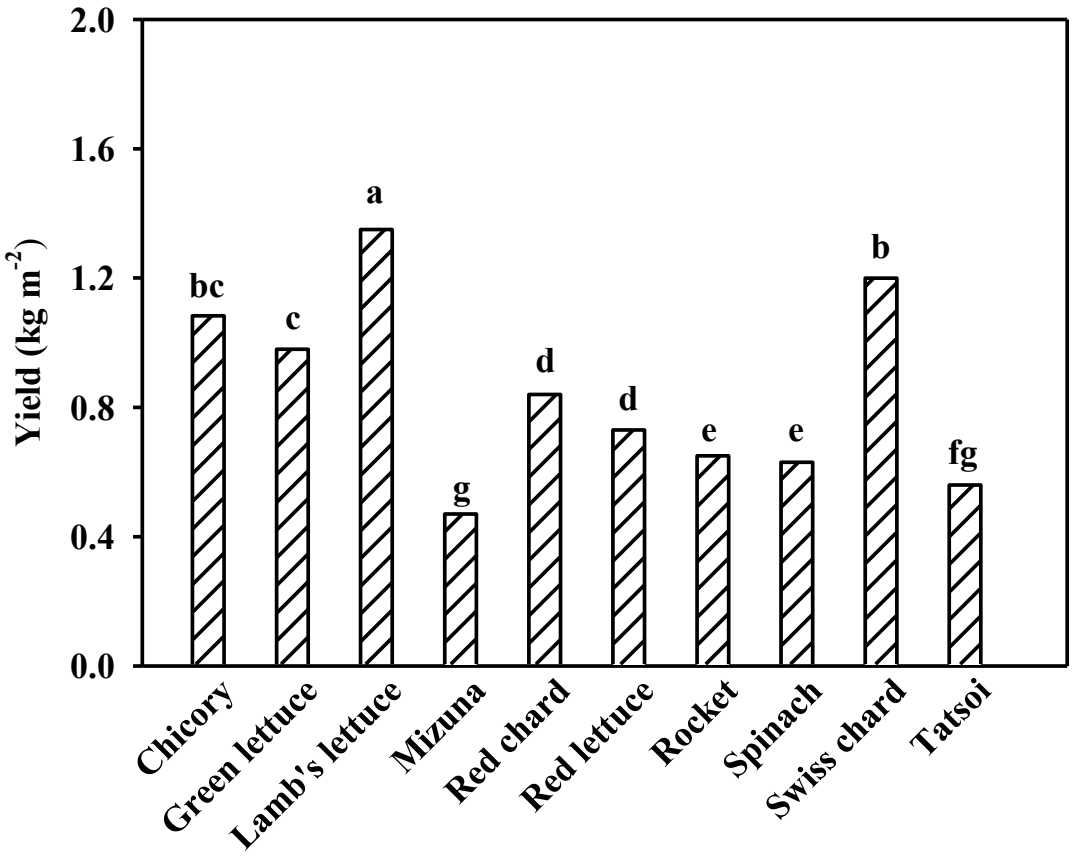


Figure 1. Marketable fresh yield of the selected leafy vegetables. Data are means of three replicates. Columns marked with the same lowercase letters are not significantly different based on Duncan's test ($p$ = 0.05).

3.13.2 Color parameters

Color is one of the most important trait for consumers, playing a crucial role in the choice, preference and acceptability of the product, and may also be considered as an indicator for estimating the antioxidant properties of the baby leaf vegetables (Ali et al., 2009). The SPAD index and the Hunter color values ($L^*$, $a^*$, $b^*$, and $C^*$) were significantly different among the species, and light intensity at time of harvest affects the color response (Table 2). The chlorophyll content (SPAD index) decreased with species in the following order: tatsoi > chicory = swiss chard > lamb's lettuce > mizuna = rocket = spinach > red lettuce > red chard > green lettuce (Table 2). The deepest color [highest redness ($a^*$ = 3.5) and lowest yellowness ($b^*$ = 10.0) value] with lowest lightness ($L^*$ = 32.3) was recorded in red-fleshed leafy vegetables (i.e. red lettuce), whereas the brightest color [lowest redness ($a^*$ = -11.0) and highest yellowness ($b^*$ = 40.5) value] with highest lightness ($L^*$ = 59.5) was observed in green lettuce (Table 2). The highest redness and yellowness values recorded in red and green lettuce could be expected since these two varieties are characterized by the high presence of coloring pigments (anthocyanins

and carotenoids/chlorophyll, respectively) involved in leaf coloration (Neocleous et al., 2014). When averaged over baby leaf vegetable species, the SPAD index and $L^*$ were significantly higher by 10.2% and 13.2%, respectively at low light intensity in comparison to the high light intensity at time of harvest (Table 2). Finally, negative values of $a^*$ (green intensity), moved toward zero (reduced green intensity) and the degree of yellowness ($b^*$) was more pronounced when leafy vegetables were harvested at high light intensity (Table 2).

| Treatment | **SPAD index** | **L** | **HUE** | **CHROMA** |
|---|---|---|---|---|
| **Species** | | | | |
| Chicory | 39.5 b | 46.5 cd | 112,1 a | 28.9 c |
| Green lettuce | 19.3 g | **59.5** a | 96,1 bc | **42.6** a |
| Lamb's lettuce | 36.5 c | 52.5 b | 112,8 a | 28.3 c |
| Mizuna | 32.3 d | 43.3 d | 105,7 ab | 23.5 d |
| Red chard | 25.3 f | 51.5 bc | 102,8 ab | 31.4 bc |
| Red lettuce | 30.1 e | 32.3 e | **60,4 d** | 11.9 e |
| Rocket | 32.0 d | 48.0 bcd | 91,6 c | 28.9 c |
| Spinach | 32.2 d | 49.7 bc | 97,8 bc | 29.6 bc |
| Swiss chard | 40.0 b | 46.8 cd | 109 a | 34.5 b |
| Tatsoi | **41.9** a | 44.2 d | 114,4 a | 23.6 d |
| **PAR** | | | | |
| Low | **34.5** a | **50.4** a | **108,5 a** | 24.3 b |
| High | 31.3 b | 44.5 b | 92,0 b | **32.3** a |
| Significance | | | | |
| Species (S) | ** | ** | ** | ** |
| PAR | ** | ** | ** | ** |
| S × PAR | ns | ns | ns | * |

Table 2. Effect of leafy vegetable species (S) and light intensity at time of harvest (Photosynthetically Active Radiation, PAR) on SPAD index, and Hunter color parameters L* (brightness), a* (redness), b* (yellowness), and chroma (C*). Means within columns separated using Duncan's multiple range test, p = 0.05. ns, *, **, = non-significant or significant at $p < 0.05$ and 0.01, respectively.

3.13.3 Dry matter, protein content and mineral composition

The DM percentage, the protein content and the mineral composition (K, Ca, and Mg) were significantly influenced by species (S) and light intensity at time of harvest (PAR), with no S × PAR interaction (Table 3). The DM percentage and the protein content ranged from 5.3 to 9.4% and from 1.5 to 3.6 g 100 $g^{-1}$ FW, respectively (Table 3). The highest leaf DM and protein contents were recorded in *Brassicaceae*, particularly rocket and mizuna. Similar results on the protein content of chicory, green lettuce, red chard, and swiss chard were also reported by the

USDA database. Concerning the light intensity at time of harvest, our results showed that the leaf DM and protein contents were significantly higher by 12.5% and 10%, respectively when leafy vegetables were harvested at low in comparison to high PAR (Table 3). It is well known that the most diffused nutritional disorders are generated by diets lacking in vitamins and minerals. Vegetables contribute normally by 11%, 35%, 7%, and 24% to the dietary intake of total P, K, Ca and Mg, respectively (Levander, 1990). Differences among the concentrations of leaf mineral elements were mainly due to species and to a lesser degree to the effect of light intensity at time of harvest. Among species, K was the predominant macronutrient present (global mean content of 460.1 mg 100 $g^{-1}$ FW; Table 3). All the leafy vegetables contain high levels of minerals particularly rocket, spinach, mizuna, and green lettuce. For instance, the highest P and K contents were recorded in rocket, spinach and green lettuce, whereas the highest Ca content was observed in both *Brassicaceae* species rocket and mizuna (Table 3). Data on the mineral content of chicory, green lettuce, red chard, rocket, red lettuce, spinach, swiss chard and tatsoi have been reported earlier by the USDA database, whereas mineral composition of lamb's lettuce and mizuna is still missing in the literature. The values reported (USDA) were within the same order of magnitude for some of the minerals, whereas for others the macronutrient contents differ from the values recorded in the current study. Differences between the mineral composition in the published literature could be attributed to the different farming practices, environmental conditions and cultivars, thus indicating the need for locally prepared food composition tables. Moreover, when averaged over all vegetables, the contents of K, Ca, and Mg were significantly higher by 12.6%, 23.7%, and 14.1%, respectively, in plants harvested at low in comparison to high PAR (Table 3). Our results are in line with those reported by Mills and Jones (1996), and Lefsrud et al. (2006), who observed that low light resulted in increases in K and Ca contents in soybean and kale, respectively. Light intensity can indirectly affect the mineral composition of plants by first impacting enzymatic activity (Lefsrud et al. 2006).

| Treatment | DM | Protein | P | K | Ca | Mg |
|---|---|---|---|---|---|---|
| Species | | | | | | |
| Chicory | 7.5 b[z] | 2.1 cd | 16.0 e | 446.5 bc | 29.9 c | 19.7 d |
| Green lettuce | 5.8 c | 1.7 de | 29.5 ab | 528.5 ab | 22.9 c | 27.4 c |
| Lamb's lettuce | 8.0 b | 1.9 cd | 23.8 bc | 420.0 c | 21.6 c | 28.8 c |
| Mizuna | 8.4 ab | 3.1 b | 26.6 ab | 467.1 bc | 167.9 a | 27.9 c |
| Red chard | 5.8 c | 1.9 cd | 28.4 ab | 402.0 c | 16.4 c | 38.9 b |
| Red lettuce | 6.3 c | 1.9 cd | 18.9 d | 464.7 bc | 26.6 c | 19.1 d |
| Rocket | 9.4 a | 3.6 a | 34.9 a | 584.9 a | 162.2 a | 27.8 c |
| Spinach | 5.3 c | 1.6 de | 26.4 ab | 626.1 a | 25.4 c | 52.0 a |
| Swiss chard | 5.4 c | 1.5 e | 19.1 d | 243.5 d | 12.1 c | 14.9 e |
| Tatsoi | 6.2 c | 2.2 c | 31.0 ab | 417.4 c | 58.9 b | 25.1 c |
| PAR | | | | | | |
| Low | 7.2 a | 2.2 a | 26.8 | 487.4 a | 60.1 a | 30.0 a |
| High | 6.4 b | 2.0 b | 24.2 | 432.8 b | 48.6 b | 26.3 b |
| Significance | | | | | | |
| Species | *** | *** | *** | *** | *** | *** |
| PAR | ** | * | ns | * | * | * |
| S × PAR | ns | ns | ns | ns | ns | ns |

Table 3. Effect of leafy vegetable species and light intensity at time of harvest (Photosynthetically Active Radiation, PAR), on dry matter (DM, %), protein content (g 100 $g^{-1}$ fw), and macronutrient composition (mg 100 $g^{-1}$ fw) of leaves.

Means within columns separated using Duncan's multiple range test, p = 0.05. ns, *, **, = non-significant or significant at $p < 0.05$ and 0.01, respectively.

### 3.13.4 Nitrate content

The nitrate content was significantly influenced by species and light intensity at time of harvest, with no interaction (Table 4). The highest nitrate accumulating baby leaf was rocket (avg. 3583 mg $kg^{-1}$ FW), a hyper-accumulator species (Santamaria et al., 2002) belonging to the family of *Brassicaceae*. The nitrate content of the remaining leafy vegetables decreased in the following order: mizuna = chicory = tatsoi > green lettuce = lamb's lettuce = red chard = red lettuce = spinach = swiss chard (Table 4). The different capacity to accumulate nitrate could be correlated to the genetic factors, to the different location of the nitrate reductase activity, as well as the different degree of nitrate absorption and transfer in the plant (Santamaria, 2006; Andrews, 1986; Blom-Zandstra, 1989). According to the "Opinion of the Scientific Panel on Contaminants in the Food Chain" (2008) and also to the results of two surveys on leafy vegetables (Parks et al., 2008; Santamaria et al., 1999), the concentration of nitrates in chicory, green lettuce, lamb's lettuce, rocket, spinach, swiss chard and tatsoi ranged between 446-2284, 36-3660, 121-3833, 1528-7340, 64-3048, 12-1078, and 105-1197 mg $kg^{-1}$ FW, respectively. Thus, the nitrate values of the

selected leafy vegetables recorded in the current experiment were in the range reported by the former studies, and were never as high as the limit values imposed by the European Community for lettuce, spinach and rocket. Moreover, irrespective of baby leaf vegetable species the nitrate content was significantly higher by 36.5% in plants harvested under low light compared to the high PAR (Table 4). Low light levels are associated with nitrate accumulation in some leafy vegetables. Nitrate is favored as an osmoticum to maintain turgor pressure in lettuce at low light levels, replacing energy-expensive carbohydrates (Blom-Zandstra and Lampe; 1985). For instance, Chadjaa et al. (1999) and Fallovo et al. (2009) reported that a reduction in light level was associated with reduced nitrate reductase activity and increased nitrate accumulation in lamb's lettuce, spinach, and *L. sativa* L. *var. acephala* cv. 'Green Salad Bowl'. Cantliffe et al. (1972) reported that the nitrate concentration in leaf was higher by 46-48% at the lowest irradiance level (160 µmol $m^{-2}$ $s^{-1}$) compared with higher irradiance levels. In a previously mentioned study, Chang et al. (2013a) observed that nitrate concentration in spinach varied significantly over a 24 h period and was highly related to changes in light intensity. The authors indicated that the nitrate concentrations peaked 3 h prior to any significant increase in light levels, whereas a sharp decrease in the nitrate concentration was recorded under high light level. A similar trend was also observed by Makus and Lester (2004) who reported that the nitrate levels of leafy mustard greens, were significantly affected by time of sampling with the highest values recorded when plants were harvested under low light intensity.

### 3.13.5 Antioxidant activity, vitamin C and total phenols

The hydrophilic (HAA) and lipophylic (LAA) antioxidant activity were significantly affected by species (S) and light intensity at time of harvest (PAR), with no S × PAR interaction, whereas the total ascorbic acid content showed differences only among the species (Table 4). Furthermore, the total phenols content was significantly affected by S and PAR with a significant interaction (Table 4). The antioxidant activity of leafy vegetables is an important parameter in assessing the quality of fresh products, since antioxidant molecules have a fundamental role in inhibiting the production of free radicals in both plants and humans (Khanam et al., 2012). In the current experiment, the HAA and LAA of the selected baby leaf vegetables ranged from 0.70 to 1.54 mmol ascorbic acid 100 $g^{-1}$ FW and from 0.45 to 0.78 mmol trolox 100 $g^{-1}$ FW, respectively. The highest level of HAA was observed in mizuna followed by rocket, and tatsoi, which implies that these baby leaf are a potential sources of natural antioxidants. Besides, the highest values of LAA, were recorded in rocket, red lettuce and mizuna, followed by tatsoi. Since cruciferous vegetables have higher content of ascorbic acid than non-cruciferous

vegetables (Lee and Kader, 2000) the higher HAA recorded in mizuna, rocket and tatsoi was likely associated to a higher concentration of this compound. Concerning the light intensity at time of harvest, our results showed that the HAA and LAA were significantly higher by 11.9% and 18.5%, respectively when leafy vegetables were harvested at low in comparison to high PAR (Table 4). Many leafy vegetables are regarded as a good source of vitamin C. The total ascorbic acid (AA) content, including ascorbic and dehydroascorbic acid, of the ten baby leaf vegetables tested varied widely (Table 4). Red lettuce had very high total AA content (avg. 96.6 mg 100 $g^{-1}$ FW), whereas the lowest value was recorded in red chard (avg. 8.2 mg 100 $g^{-1}$ FW). The total AA of the remaining baby leaf vegetables decreased in the following order: rocket > spinach = mizuna = tatsoi > swiss chard > chicory = green lettuce = lamb's lettuce (Table 4). However, the total AA was not affected by the light intensity time of harvest. These results are consistent with the findings of Makus and Lester (2004), who reported similar total ascorbate content when the leaves of mustard greens were sampled at low and high light intensity. In contrast, Chang et al. (2013a) observed a decrease in ascorbic acid concentration, when spinach plants were harvested after the light intensity peaked. Explanations for this disagreement could be the different environments (greenhouse, growth chamber and open-field, respectively) in which the plants were grown.

Total phenols (TP), as plant secondary metabolites, known to possess a wide range of therapeutic uses (Subhasree et al., 2009) ranged from 21.7 to 82.3 mg gallic acid 100 $g^{-1}$ DW, with the highest value recorded in red lettuce harvested at high light intensity (Fig. 2). Among species, the red-fleshed leafy vegetable was richer in TP than green colored leafy vegetables due to the presence of anthocyanins (Llorach et al., 2008). Our results also indicated that TP content fluctuated over the two harvests, and these variations were highly correlated to the changes in light intensity, and were also species dependent (Fig. 2). In fact, the TP content in chicory, green lettuce, lamb's lettuce, mizuna, red chard, and red lettuce were significantly higher by 48.8%, 46.9%, 96.3%, 12.7%, 100.5%, and 68.0%, respectively when plants were harvested at high light intensity, whereas no significant differences among treatments were recorded in rocket, spinach, swiss chard, and tatsoi (Fig. 2). Our results are in line with those reported by Oh et al. (2009), who observed an increase of phenolic compounds, when lettuce plants grown in growth chamber were exposed to high light intensity (800 μmol $m^{-2}$ $s^{-1}$ for 24 hours).

| Treatment | $NO_3$-N | HAA | LAA | AA | TP |
|---|---|---|---|---|---|
| Species | | | | | |
| Chicory | 1990 b | 0.76 d | 0.54 de | 17.1 e | 34.9 f |
| Green lettuce | 1127 c | 0.81 cd | 0.47 e | 22.3 e | 21.7 g |
| Lamb's lettuce | 618 c | 0.70 e | 0.52 de | 17.2 e | 71.5 b |
| Mizuna | 2405 b | 1.54 a | 0.71 ab | 51.0 c | 64.6 c |
| Red chard | 919 c | 0.74 d | 0.46 e | 8.2 f | 53.6 d |
| Red lettuce | 1072 c | 0.84 c | 0.75 a | 96.9 a | 82.3 a |
| Rocket | 3583 a | 1.01 b | 0.78 a | 86.2 b | 47.6 e |
| Spinach | 1122 c | 0.86 c | 0.63 bc | 51.9 c | 49.3 e |
| Swiss chard | 1022 c | 0.71 de | 0.45 e | 39.0 d | 33.3 f |
| Tatsoi | 1991 b | 0.98 b | 0.60 c | 46.1 c | 49.8 e |
| PAR | | | | | |
| Low | 1831 a | 0.94 a | 0.64 a | 44.4 | 42.6 b |
| High | 1341 b | 0.84 b | 0.54 b | 42.8 | 59.2 a |
| Significance | | | | | |
| Species | *** | *** | *** | *** | *** |
| PAR | *** | ** | *** | ns | *** |
| S × PAR | ns | ns | ns | ns | *** |

Table 4. Effect of leafy vegetable species and light intensity at time of harvest (Photosynthetically Active Radiation, PAR), on nitrate content (mg $kg^{-1}$ FW), hydrophilic (HAA, mmol AA 100 $g^{-1}$ FW) and lipophylic (LAA, mmol Trolox 100 $g^{-1}$ FW) antioxidant activity, total ascorbic acid (AA, mg 100 $g^{-1}$ FW), and total phenols (TP, mg gallic acid 100 $g^{-1}$ DW) contents.

Means within columns separated using Duncan's multiple range test, p = 0.05. ns, *, **, = non-significant or significant at $p < 0.05$ and 0.01, respectively.

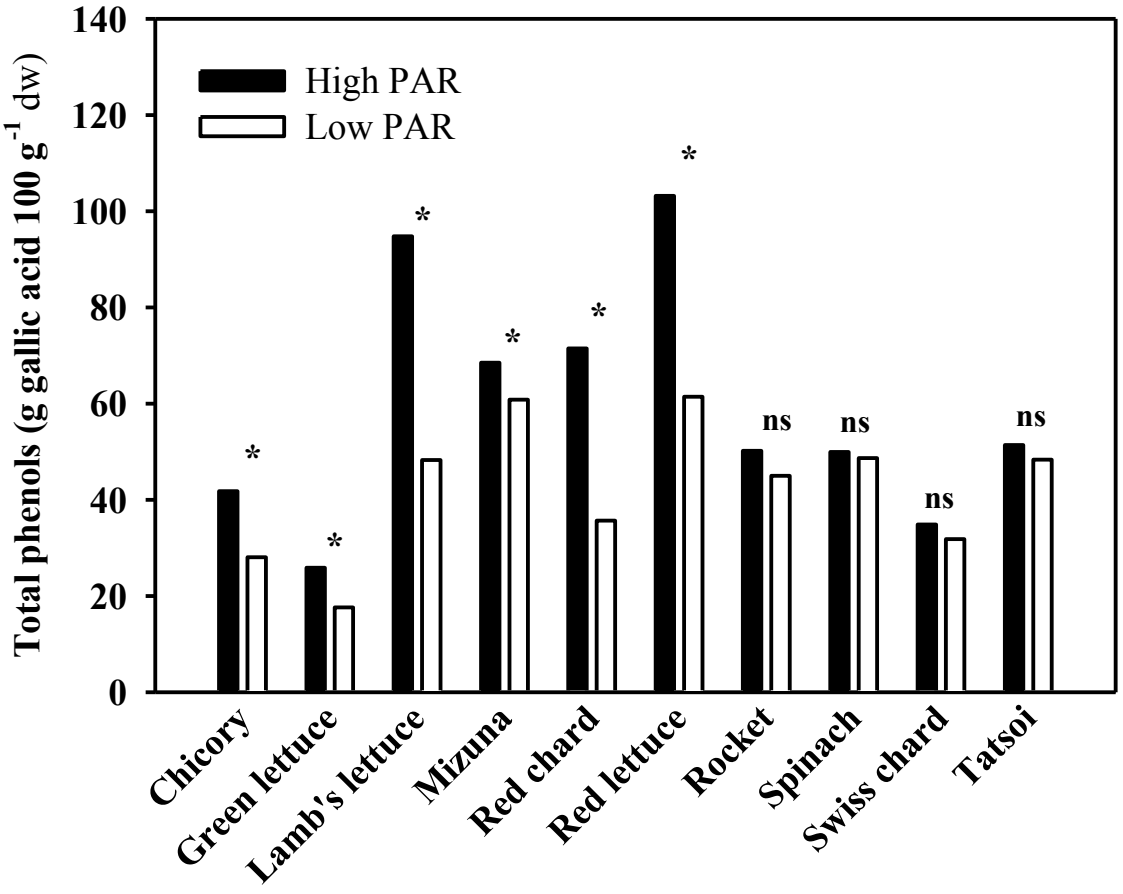


Figure 2**.** Effect of leafy vegetable species and light intensity at time of harvest (Photosynthetically Active Radiation, PAR), on total phenols content.* = significant differences between the high and the low PAR at time of harvest; ns = non-significant differences at $p < 0.05$.

3.13.6 Principal component analysis

The high number of nutritional traits contributing to the quality perception of leafy vegetables makes it difficult to discriminate the effects of species and light intensity at time of harvest on the base of the overall quality. A principal component analysis (PCA) approach was undertaken in order to reduce the number of variables to only the relevant ones, to better visualize the quality performances of the ten baby leaf species harvested at the two light intensities, and to allow grouping factors that could not possibly come up by analyzing each factor separately(Table 5). As defined by Lawless and Heymann (2010), "the PCA is a multivariate technique used to summarize and extract trends when many variables are used". PCA formulates new variables (i.e. principal components), which summarize better the variability of the original

variables and are correlated to them. The first three principal components (PC) were associated with an eigenvalues > 1, and together accounted for more than 75% of the cumulative variance (Table 5). PC1, which explained 47.8% of the variance, was positively and significantly correlated with protein, and Ca contents, nitrate, DM percentage, HAA, LAA, and to lesser extent with P, and K contents. Moreover PC2 and PC3, which explained 16.8% and 11.9% respectively, of the variance were positively and strongly correlated with Mg and total phenols contents, respectively (Table 5). Figure 3 illustrates the PCA for the first two components (PC1 and PC2). The loading plot illustrates the relationships among variables (i.e. nutritional traits), where two vectors with an angle less than 90° are positively correlated and two vectors with an angle > 90° are negatively correlated (Sartono et al., 2003). For instance, HAA was positively correlated with ascorbic acid (AA), protein, and Ca contents and DM percentage. Similarly LAA was more strongly correlated to P and K contents than to HAA and total AA content. Mg content and LAA were not well correlated with TP content (Fig. 3). Components 1 and 2 score plot (Fig. 3) discriminates treatments into four groups. The positive side of PC1 included mizuna and rocket harvested at low and high light intensity, and were distinguished by their high HAA, AA, Ca and protein contents, DM percentage, but also high nitrate content. The positive side of PC2 included red lettuce, and was characterized from the remaining green baby leaf by its relatively high TP content. Spinach harvested at low light intensity was distinguished by its high minerals content in particular Mg, and to a lesser degree P and K (Fig. 3). The unique distribution of nutritional traits in these baby leaf vegetables make them candidates for a further in-depth nutritional profile.

| Principal components | PC1 | PC2 | PC3 |
|---|---|---|---|
| Eigen value | 5.2 | 1.8 | 1.3 |
| Percentage of variance | 47.8 | 16.8 | 11.9 |
| Cumulative variance | 47.8 | 64.7 | 76.4 |
| | | | |
| Eigen vectors [a] | | | |
| DM[b] | **0.790** | -0.257 | -0.177 |
| N-$NO_3$ | **0.854** | -0.157 | -0.313 |
| Proteins | **0.947** | -0.199 | 0.157 |
| P | **0.600** | 0.548 | -0.193 |
| K | **0.654** | 0.575 | 0.174 |
| Ca | **0.925** | -0.193 | -0.153 |
| Mg | 0.117 | **0.897** | 0.117 |
| HAA[c] | **0.781** | -0.074 | -0.008 |
| LAA[d] | **0.751** | 0.121 | -0.399 |
| TP[e] | 0.109 | -0.284 | **0.733** |
| AA[f] | 0.543 | -0.180 | 0.591 |

Table 5. Eigen values, relative and cumulative proportion of total variance, and correlation coefficients for each nutritional trait with respect to the three principal components

[a] Boldface factor loadings are considered highly weighed

[b] DM, dry matter; cHAA, hydrophilic antioxidant activity; dLAA, lipophylic antioxidant activity; eTP, total phenols content, fAA, ascorbic acid content.

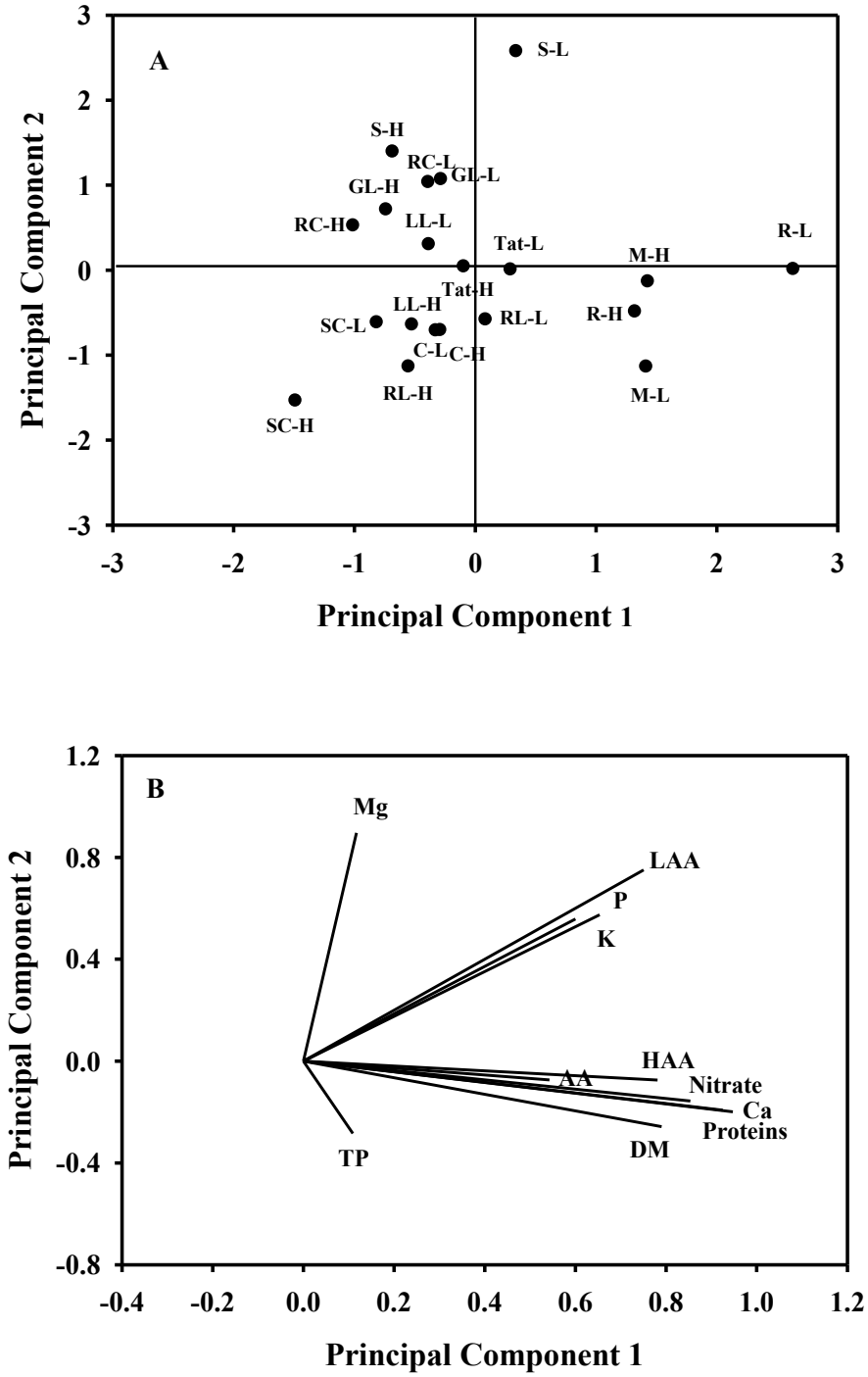


Figure 3. (A) Principal component scores and (B) loading plot of Principal Component Analysis of nutritional traits in the ten leafy vegetables. C, chicory; GL, green lettuce; LL, lamb's lettuce; M, mizuna; RC, red chard; R, rocket; RL, red lettuce; S, spinach; SC, swiss chard; Tat, tatsoi. L and H correspond to the low and high light intensity at time of harvest (Photosynthetically Active Radiation, PAR).

## 3.14 Conclusions 3

It can be concluded that, the color parameters, dry matter percentage, protein content, minerals content, nitrate, antioxidant activity, ascorbic acid and total phenols contents varied widely among the ten selected baby leaf vegetables. Rocket and mizuna showed the highest dry matter percentage, protein content and HAA; spinach exhibited the highest values of P, K, and Mg contents, while the LAA, total AA and TP contents of red lettuce were significantly higher than in the other leafy vegetables, which implies that these baby leaf can be considered potential sources of health-promoting phytochemical compounds. Our results also demonstrated that the light intensity at time of harvest could significantly affect the concentration of some of these compounds. The highest nitrate concentration, protein content, minerals content, HAA, LAA occurred at low light intensity. The total phenols contents in some leafy vegetables were higher when plants were harvested under high light intensity conditions.

## 3.15 References


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# APPENDIX

## Nutritional quality of ten leafy vegetables harvested at two light intensities

***Running title*:** Nutritional quality of baby leaf at two light intensities

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### Abstract

**BACKGROUND**: There is an increase in consumer interest in food products, including leafy vegetables, with health promoting properties . The aim of this study is to evaluate the nutritional composition of ten baby leaf vegetables: chicory, green lettuce, Lamb's lettuce, mizuna, red chard, red lettuce, rocket, spinach, swiss chard, and tatsoi; and to assess the variation in the quality traits of the selected leafy vegetables in relation to the light intensity (low and high Photosynthetically Active Radiation, PAR) at time of harvest.

**RESULTS**: Rocket showed the highest dry matter (DM), proteins, P, K and Ca contents, followed by mizuna, however these two *Brassicaceae* accumulated the highest nitrate content. Spinach exhibited high values of P, K and Mg contents. The highest lipophylic antioxidant activity (LAA) were recorded in red lettuce and rocket, whereas ascorbic acid (AA) and total phenols (TP) contents of red lettuce were higher than in the other leafy vegetables. The deepest and brightest leaf color was observed in red and green lettuce respectively. Light intensity at time of harvest affects the concentration of several nutritional traits. Leaf DM, proteins, K, Ca and Mg contents, hydrophilic antioxidant activity (HAA), and LAA were higher by 12.5%, 10%, 12.6%, 23.7%, 14.1%, 11.9%, and 18.5%, respectively when leafy vegetables were harvested at low in comparison to high PAR. The highest values of TP in Chicory, green lettuce, lamb's lettuce, mizuna, red chard, and red lettuce, were observed at high PAR.

**CONCLUSIONS**: These findings are helpful for consumers, food nutritionists, and producers to identify the most suitable baby leaf candidates for food security and for a healthy sustainable diet and to understand the nutritional changes in relation to the light intensity at time of harvest.

**Keywords**: antioxidant activity; Ascorbic acid; baby leaf; mineral composition; nitrate; total phenols

# Progetto (Pro.Bio.Ca)

***Tecnologie innovative per la produzione di biomassa di carciofo e cardo da destinare all'estrazione di composti nutraceutici***

**Convegno di presentazione dei risultati**
**Martedì 17 dicembre 2014 ore 10:00**
Federazione Provinciale Coldiretti Benevento, Via Mario Vetrone

**Ore 10,00**- Registrazione dei partecipanti

**Ore 10,20**- Apertura dei lavori e moderazione degli interventi
***Angela Maria Diodato***
NTR24

**Ore 10,25**- Saluti di benvenuto
***Giuseppe Brillante***
Direttore Coldiretti Benevento

**Ore 10,30**- Presentazione generale del progetto
***Giuseppe Colla***
Coordinatore del progetto, Università della Tuscia, Viterbo

**Ore 10,40** - Presentazione attività ATI
***Giancarlo Pepe***
Coordinatore delle attività per la Coldiretti Benevento

**Ore 10,50**- Interventi genetici per valorizzare il carciofo e il cardo
***Francesco Saccardo***
Università della Tuscia, Viterbo

**Ore 11,10** - Coffee break

**Ore 11,30**- Vivaismo e micropropagazione
***Mariateresa Cardarelli***
CRA-RPS, Roma

**Ore 11,50**- Tecniche agronomiche per la produzione di biomassa
***Giuseppe Colla, Antonio Fiorillo***
Università della Tuscia, Viterbo

**Ore 12,10**- Fattori agronomici e qualità nutrizionale del carciofo
***Emma Colonna, Mariantonella Palermo, Vincenzo Fogliano, Giancarlo Barbieri***
Università Federico II, Napoli

**Ore 12,30**- Aspetti economici e di mercato della coltivazione del carciofo
***Antonio Pizzi***
Responsabile tecnico delle sperimentazioni in campo, Benevento

**Ore 12,50**- Conclusioni
***Gennaro Masiello***
Presidente Coldiretti Benevento

***Con il patrocinio della***

***Progetto finanziato dal***

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DIPARTIMENTO AGRARIA

INNOVAZIONI IN AGRICOLTURA

# Qualità della fragola: Confronto tra sistema di produzione biologico e convenzionale

Emma Colonna, Giancarlo Barbieri

Dipartimento di Agraria, Università degli Studi di Napoli Federico II

## PREMESSA

Negli ultimi anni si è assistito alla rapida affermazione del concetto di agricoltura sostenibile, a cui ha contribuito una maggiore consapevolezza dei consumatori in materia di ambiente e sicurezza alimentare. In tale contesto si inserisce l'agricoltura biologica e pertanto tale studio si propone di confrontare il metodo di produzione biologico con quello convenzionale su fragola. Inoltre, si è valutato l'effetto del trattamento di surgelazione e stoccaggio a -40°C per sei mesi.

## MATERIALI E METODI

Cv. Sabrina
Provincia di Caserta
Raccolta: 26 marzo 2012
Strumentazione: HPLC, Spettrofotometro

**PAROLE CHIAVE**: *Fragaria vesca L., attività antiossidante, vitamina C, acido ellagico, polifenoli, sistema colturale.*

## RISULTATI

| | Peso medio | Sostanza secca | Consistenza | pH | Zuccheri riduttori | TSS | L | HUE |
|---|---|---|---|---|---|---|---|---|
| | g | (%) | kg | | %p.f. | ° Brix | | |
| Conv. | 13,3±0,8 | 8,4±0,3 | 0,52±0,03 | 3,74±0 | 4,71±0,2 | 6,8±0,2 | 15,8±0,3 | 33,3±0,4 |
| Bio | 10,7±0,2 | 8,9±0,2 | 0,53±0,02 | 3,49±0,05 | 4,45±0,15 | 6,5±0,4 | 19±0,3 | 37,1±0,3 |
| Int. | ** | *n.s.* | *n.s.* | ** | *n.s.* | *n.s.* | ** | *n.s.* |

*Tab.1: Effetto del sistema colturale sulle caratteristiche chimico-fisiche (valori medi ± ES)*

| | Acido ascorbico | Acido ellagico | Fenoli totali |
|---|---|---|---|
| | mg/100 g p.f. | mg/100 g p.f. | mg ac. Gallico eq./100 g p.s. |
| Conv. | 53,7±5,4 | 56,2±1,6 | 107,3±3,4 |
| Bio. | 57,3±4,5 | 52,4±,11 | 105,5±1,8 |
| | *n.s.* | *n.s.* | *n.s.* |
| Fresco | 65,4±7,2 | 57,7±1,1 | 103,6±6,1 |
| Surgelato | 45,6±9,2 | 50,8±1,6 | 109,2±4 |
| | ** | ** | *n.s.* |
| *Interazione* | *n.s.* | *n.s.* | *n.s.* |

*Tab.2: Effetto del sistema colturale e del trattamento di surgelazione sul profilo qualitativo (valori medi ± ES)*

Le fragole convenzionali presentano peso medio dei frutti maggiore rispetto a quelle biologiche, che di contro mostrano maggiore consistenza e sostanza secca percentuale. L'effetto della conservazione sui parametri di peso e consistenza premia il prodotto convenzionale surgelato rispetto al biologico. L'analisi indica valori superiori nei frutti convenzionali per il pH, solidi solubili e zuccheri riduttori, che si mantengono una volta subito il trattamento di conservazione. In merito alla tonalità (HUE) non si segnala differenza tra i frutti dei due sistemi mentre la luminosità (L) si presenta più elevata nei frutti biologici.

Il contenuto in polifenoli non mostra differenze tra i frutti provenienti dai due diversi sistemi colturali, né tantomeno in seguito al trattamento termico. Si sottolinea una riduzione del contenuto in acido ellagico ed acido ascorbico successivamente al trattamento di surgelazione.

## CONCLUSIONI

Il profilo qualitativo ottenuto non mostra differenze significative tra i due sistemi colturali, sia per le caratteristiche chimico-fisiche che per la componente nutrizionale. L'effetto della surgelazione risulta significativo per il contenuto in acido ellagico e acido ascorbico

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Aissa 2014
XII CONVEGNO
INNOVAZIONI IN AGRICOLTURA

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DIPARTIMENTO AGRARIA

# Qualità di ortaggi da foglia (*Brassicaceae*) raccolti in due differenti momenti della giornata

Emma Colonna. Youssef Rouphael. Stefania De Pascale. Giancarlo Barbieri

Dipartimento di Agraria, Università degli Studi di Napoli Federico II

**PREMESSA** Il presente lavoro si propone di valutare l'aspetto qualitativo delle *Brassicaceae* il cui rapido ciclo di crescita e l'alto contenuto di fitonutrienti, spingono a considerarle specie di elevato interesse per le crescenti esigenze di mercato, sempre volte a nuove strategie e nuove specie per la preparazione dei prodotti ready-to-eat.

**PAROLE CHIAVE**: *Eruca sativa* M.; *Brassica juncea* Bailey; *Brassica narinosa* Bailey; Nitrati; Vitamina C; PAR

**MATERIALI E METODI**: Rucola (*Eruca sativa* Miller), Mizuna (*Brassica juncea*, var japonica Bailey) e Tatsoi (*Brassica narinosa* Bailey) sono state allevate su suolo a Pontinia (LT) in coltura protetta con una concimazione organica di fondo e fertirrigazioni in copertura, in due diversi momenti della giornata con differenti valori di PAR.

## RISULTATI

| | Fenoli totali mg ac.gallico eq./100 g p.s. | AAI mmol ac.ascorbico eq./100 g p.f. | AAL mmol Trolox eq./100 g p.f. | Sostanza secca % | Nitrati (g kg$^{-1}$ p.f.) | Ca (mg 100 g$^{-1}$ p.f.) | L |
|---|---|---|---|---|---|---|---|
| **Specie** | | | | | | | |
| Mizuna | 64,6 a | 1,54 a | 0,71 a | 8,4 ab | 2405 b | 168 a | 43,3 b |
| Rucola | 47,6 b | 1,00 b | 0,78 a | 9,4 a | 3583 a | 162 a | 48,2 a |
| Tat soi | 49,9 b | 0,88 b | 0,60 b | 6,2 b | 1991 b | 59 b | 44,1 b |
| **PAR** | | | | | | | |
| Basso | 53,1 b | 1,17 a | 0,75 a | 8,5 a | 3152 a | 144 a | 47,1 a |
| Alto | 54,9 a | 1,11 a | 0,65 b | 7,5 a | 2167 b | 114 b | 43,3 b |
| **Significatività** | | | | | | | |
| Specie (S) | *** | *** | ** | * | * | * | * |
| PAR (P) | ** | NS | * | NS | * | * | * |
| S X P | NS | NS | NS | NS | NS | NS | NS |

*Tab.1: Effetto della specie e del livello di radiazione fotosinteticamente attiva (PAR) durante la raccolta sul contenuto in polifenoli (F.T), attività antiossidante idrofila (AAI), lipofila (AAL), sulla percentuale di sostanza secca, contenuto in nitrati, calcio e luminosità (L).*

Mizuna presenta il più alto contenuto di fenoli totali ad alto PAR, ed il più elevato valore in termini di attività antiossidante idrofila. La rucola presenta il più alto contenuto di nitrati, influenzato dalla raccolta a basso PAR. Per il contenuto in Ca, sia Rucola che Mizuna presentano i valori più elevati, subendo l'influenza dell'intensità luminosa a basso PAR. Per il parametro L (Luminosità) prevale la rucola influenzata positivamente da basso PAR

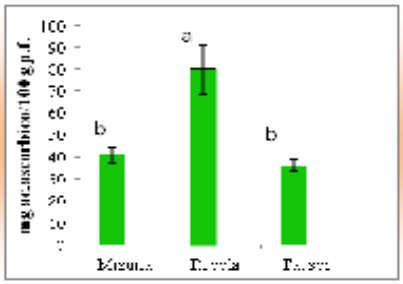


*Fig.1: Effetto della specie sul contenuto di acido ascorbico*

La determinazione dell'acido ascorbico vede prevalere la rucola sulle altre due specie, indipendentemente dall'intensità luminosa.

**CONCLUSIONI**
Rucola e Mizuna possono quindi essere considerate specie di elevato interesse dato l'elevato profilo nutrizionale. I risultati mostrano anche che raccogliendo tali specie durante condizioni di basso PAR, persiste l'alto valore nutrizionale legato però ad un'alta concentrazione di nitrati, comunque inferiore rispetto ai limiti fissati dalla UE.

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Santamaria P. 1999, J. Sci Food Agric 79:1882–1888

# Nutrizione azotata in floating system e qualità post-raccolta di lattuga di IV gamma

*Progetto PRIN 2008*

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*Dipartimento di Agraria, Università degli Studi di Napoli Federico II, via Università 100, 80055 Portici (NA)*

## Processo di lavorazione di lattuga in IV gamma

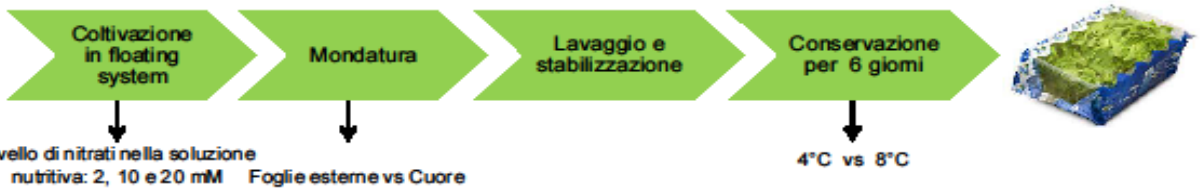


*Fig. 1: Studio della catena di produzione di "fresh cut vegetable": influenza del livello di concimazione azotata e dei fattori "post-harvest" sulla shelf life*

## RISULTATI

✓ **Soluzione nutritiva:** La concimazione azotata ha determinato un aumento significativo del peso medio dei cespi ed una diminuzione significativa della variabilità tra i cespi stessi, mentre non ha sostanzialmente modificato la frazione edibile. Una maggiore concentrazione di azoto nella soluzione nutritiva ha favorito l'accumulo di acqua con conseguente miglioramento delle performance di conservazione; inevitabilmente il maggiore apporto di azoto si è andato a ripercuotere negativamente sul contenuto di $NO_3$ ma ad un livello intermedio di concimazione azotata si è associato anche un minore tenore di $NO_2$.

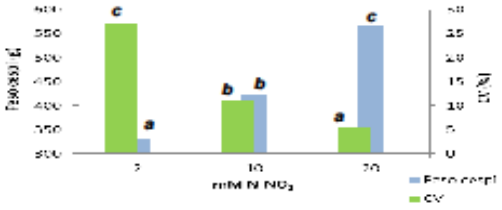


*Fig. 2 Effetto della nutrizione azotata sulla resa di lattuga*
*A lettere diverse corrispondono campioni statisticamente diversi*

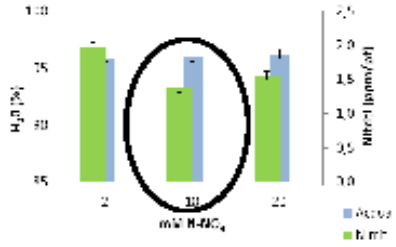


*Fig. 3: Effetto della nutrizione azotata sul contenuto di acqua e nitrati (valori medi±ES)*

✓ **Mondatura:** Nonostante il cuore di lattuga presenti un minor tenore in polifenoli totali (con conseguente riduzione dell'attività antiossidante idrofila) il maggior accumulo di nitrati nelle foglie esterne è tale da suggerire una mondatura più spinta.

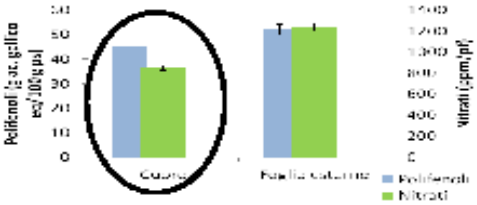


*Fig. 3: Effetto della mondatura sul contenuto di polifenoli e nitrati (valori medi±ES)*

✓ **Conservazione:** Nonostante il naturale scadimento qualitativo, tutti i parametri valutati hanno mostrato valori accettabili al termine del ciclo di conservazione

| | Calo peso | pH | Clorofilla | Chroma | Nitriti | Acido ascorbico | Polifenoli totali | AA lipofila |
|---|---|---|---|---|---|---|---|---|
| | % | | µg/cm² | | ppm | mg/100 g pf | mg GAE/100g ps | mmoli Trolox. eq/100 g ps |
| **Giorno 0** | | 6,13 | 15,2 | 33,4 | 1,37 | 28,4 | 53,3 | 3,4 |
| **Giorno 1** | -1,0 | 6,23 | 18,5 | 30,4 | 1,46 | 19,3 | 53,2 | 3,2 |
| **Giorno 3** | -0,7 | 6,24 | 11,9 | 26,8 | 1,62 | 12,6 | 47,8 | 2,0 |
| **Giorno 6** | -1,7 | 6,28 | 12,4 | 26,8 | 2,11 | 13,5 | 41,9 | 2,3 |
| *F sign.* | *0.012 ** | *0.000 *** | *0.024 ** | *0.000 *** | *0.001 *** | *0.001 *** | *0.000 *** | *0.000 *** |

✓ **Temperatura:** Influenza minima sulle caratteristiche qualitative con maggiore riduzione dei polifenoli totali a seguito della conservazione a 8°C

## CONCLUSIONI

Il floating system è un metodo efficace per produrre lattuga di alta qualità destinata al mercato della IV gamma. La scelta opportuna dei parametri di produzione primaria e trasformazione consente di minimizzare il contenuto di nitrati e ottimizzare le caratteristiche nutrizionali.

# INFLUENZA DELLA MICORRIZAZIONE SULLA QUALITÀ DEL CARCIOFO

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## PREMESSA

Il presente lavoro si inserisce nel progetto di ricerca PRO.BIO.CA con lo scopo di indagare sull'influenza dell'associazione micorrizica nel complemento della qualità del carciofo.

## MATERIALI E METODI

Cv. "Romanesco"
Cinque aziende a confronto
Provincia di Benevento
Impianto: settembre 2011 Raccolte: 10 e 28 maggio 2012
Trattamenti: 1) controllo; 2) Inoculo micorrizico Aegis Sym Irriga (*Glomus intraradices* e *Glomus mossae*); 3) Inoculo micorrizico Endospor Dry Mix (*Glomus intraradices, Azospirillum brasilense, Azotobacter chroococcum, Bacillus megaterium, Pseudomonas fluorescens)*
Strumentazione: HPLC, Spettrometro di massa

## RISULTATI

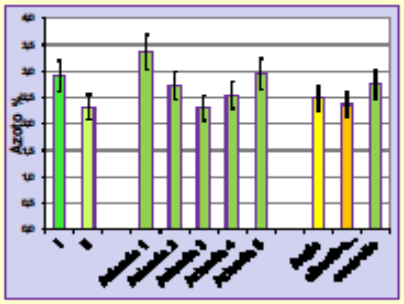


*Fig. 1 – % di azoto nei capolini in relazione alle epoche di raccolta (I e II), alle aziende e ai trattamenti micorrizici*

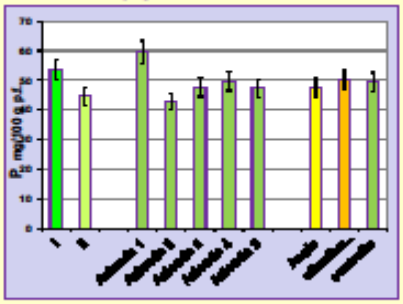


*Fig. 2 – Contenuto di P nei capolini in relazione alle epoche di raccolta (I e II), alle aziende e ai trattamenti micorrizici*

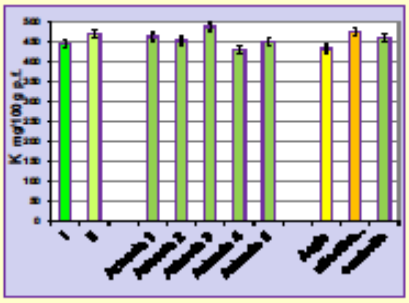


*Fig. 3 – Contenuto di K nei capolini in relazione alle epoche di raccolta (I e II), alle aziende e ai trattamenti micorrizici*

L'impiego degli inoculi micorrizici non ha influenzato il contenuto in N, P e K dei capolini mentre per N e P si nota una variabilità spaziale e temporale: valori più alti nell'Azienda 1 e nella prima raccolta.

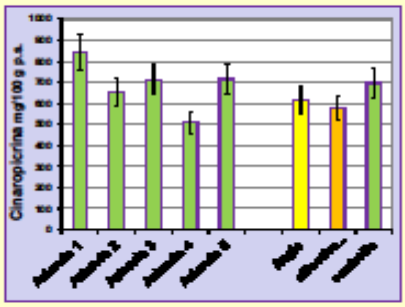


*Fig. 4 – Contenuto di cinaropicrina nelle foglie in relazione alle aziende e ai trattamenti micorrizici*

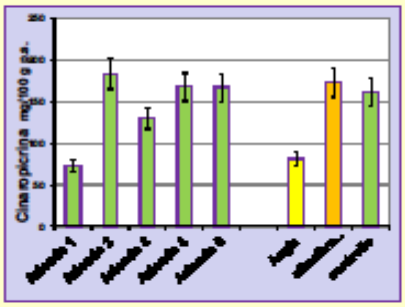


*Fig. 5 – Contenuto di cinaropicrina nelle brattee interne in relazione alle aziende e ai trattamenti micorrizici*

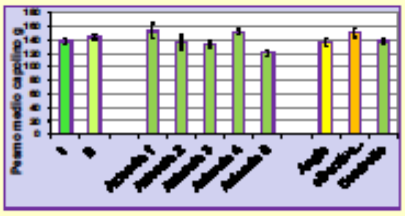


*Fig. 6 Peso medio dei capolini in relazione alle epoche di raccolta (I e II), alle aziende e ai trattamenti micorrizici*

Rispetto al controllo, il contenuto di molecole bioattive nelle foglie non è migliorato per effetto delle micorrize, come anche il peso medio dei capolini.
Si afferma un maggior contenuto di polifenoli nelle brattee interne (951 mg GAE/100 g p.s) piuttosto che nelle brattee esterne (370 m GAE/100 g p.s).

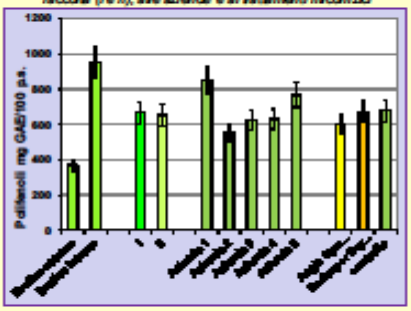


*Fig. 7 – Contenuto in polifenoli nelle brattee interne ed esterne in relazione alle epoche di raccolta (I e II) alle aziende e ai trattamenti micorrizici*

## CONCLUSIONI

Lo stato nutrizionale del carciofo non sembra presentare particolari benefici a seguito dell'inoculo micorrizico.

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*Pandino G. 2011, Journal of Food Composition and Analysis 24:148–153*

# Nutrizione azotata in floating system e qualità post-raccolta di rucola di IV gamma

*Progetto PRIN 2008*

**Palermo M., Colonna E., Barbieri G.**

*Dipartimento di Agraria, Università degli Studi di Napoli Federico II, via Università 100, 80055 Portici (NA)*

## Processo di lavorazione di rucola in IV gamma


*Fig. 1: Studio della catena di produzione di "fresh cut vegetable": influenza del livello di concimazione azotata e dei fattori "post-harvest" sulla shelf life*

## RISULTATI

✓ **Soluzione nutritiva:** La concimazione azotata ha determinato un aumento significativo della resa. La maggiore concimazione azotata ha consentito, inoltre, migliori performance in conservazione (calo dello 0.8% contro l'1% nelle due tesi a concentrazione minore) ma di contro ha comportato svantaggi nutrizionali legati ad maggiore accumulo di nitrati e minore tenore di Vitamina C.

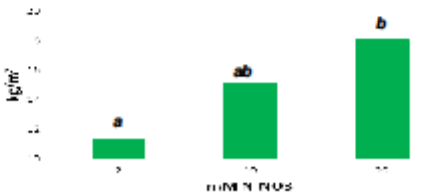


*Fig. 2 Effetto della nutrizione azotata sulla resa di rucola. A lettere diverse corrispondono campioni statisticamente diversi*

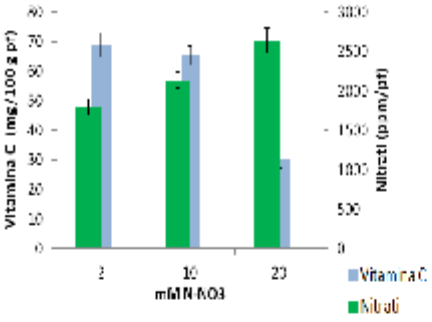


*Fig. 3: Effetto della nutrizione azotata sul contenuto di acido ascorbico e nitrati (valori medi±ES)*

✓ **Temperatura:** Gli svantaggi nutrizionali legati ad elevate somministrazioni azotate si sono minimizzati con più bassa temperatura di conservazione: a 4°C si è osservata una minore degradazione della Vitamina C ed un minore accumulo di nitrati.

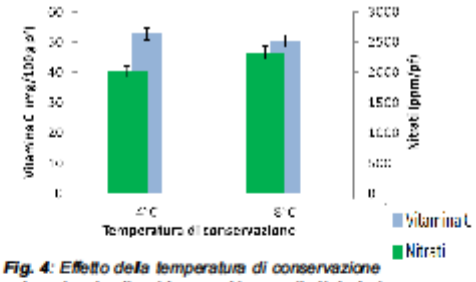


*Fig. 4: Effetto della temperatura di conservazione sul contenuto di acido ascorbico e nitrati (valori medi±ES)*

✓ **Conservazione:** Nonostante il naturale scadimento qualitativo, i tutti i parametri valutati hanno mostrato valori accettabili al termine del ciclo di conservazione

| | Sostanza secca | pH | L | HUE | Clorofilla | Acido ascorbico | Attività antiossidante idrofila |
|---|---|---|---|---|---|---|---|
| | % | | | | µg/cm² | mg/100 g pf | mmoli ac. ascorbico eq/100 g ps |
| **Giorno 0** | 9,40 | 6,36 | 45,4 | 114,6 | 54,2 | 143,9 | 18,3 |
| **Giorno 1** | 9,51 | 6,33 | 35,4 | 118,5 | 53,4 | 145,4 | 16,9 |
| **Giorno 3** | 9,96 | 6,19 | 40,3 | 123,6 | 52,5 | 124,7 | 16,8 |
| **Giorno 6** | 10,31 | 6,12 | 39,4 | 125,4 | 52,3 | 96,8 | 15,3 |
| *F sign.* | 0.020 * | 0.000 ** | 0.000 ** | 0.074 ns | 0.158 ns | 0.019 * | 0.001 ** |

✓ **Illuminazione:** Preservando il prodotto dall'esposizione alla luce non si è osservato alcun miglioramento significativo nei parametri qualitativi

## CONCLUSIONI

Il floating system è un metodo efficace per produrre rucola di alta qualità destinata al mercato della IV gamma. Una giusta combinazione dei parametri di produzione (opportuna nutrizione azotata) e post raccolta (mantenimento di 4°C su tutta la catena) consente di conciliare esigenze tecnologiche ed esigenze nutrizionali.