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Nutritional quality of ten leafy vegetables harvested at two light intensities

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ABSTRACT

The nutritional composition of ten leafy vegetables (chicory, green lettuce, lamb's lettuce, mizuna, red chard, red lettuce, rocket, spinach, Swiss chard, and tatsoi) and quality traits of the selected leafy vegetables in relation to the light intensity (low and high Photosynthetically Active Radiation; PAR) at time of harvest were determined. Irrespective of the light intensity at time of harvest, the highest leaf dry matter (DM), proteins, nitrate, P, K and Ca contents were observed in rocket followed by mizuna. The highest lipophilic antioxidant activity (LAA) was recorded in red lettuce and rocket, whereas ascorbic acid (AA) and total phenolic (TP) contents of red lettuce were higher compared to the other leafy vegetables. When leafy vegetables were harvested at low as opposed to high PAR, the leaf content was higher in DM, protein, K, Ca and Mg, hydrophilic antioxidant activity (HAA), and LAA by 12.5%, 10.0%, 12.6%, 23.7%, 14.1%, 11.9%, and 18.5%, respectively. The highest values in TP for chicory, green lettuce, lamb's lettuce, mizuna, red chard, and red lettuce, were observed under high PAR.

1. Introduction

In recent years, phytochemical compounds found in vegetables have generated significant interest among consumers and researchers owing to their health-promoting effects (Khanam, Oba, Yanase, & Murakami, 2012). Several *in vitro*, pre-clinical and clinical investigations have revealed an inverse relationship between high consumption of vegetables and the lower incidence of chronic ailments such as cardiovascular and neuro-degenerative diseases, ischemic stroke, arthritis, inflammatory bowel and some forms of cancers (Slavin & Lloyd, 2012). Fresh-cut or minimally processed baby leaf vegetables are gaining importance among consumers worldwide, as they represent a good source of minerals, vitamins, and phytochemicals of considerable antioxidant potential (Subhasree, Baskar, Keerthana, Susan, & Rajasekaran, 2009).

Italy is the European leader in leafy vegetable production, with 15,000 ha under protected cultivation and a yearly production of about 140 Kt (http://agri.istat.it). The major production areas are located in Campania, Lombardy and Veneto (Pimpini, Giannini, & Lazzarin, 2005, chap. 4). Leafy vegetables commonly destined for fresh-cut products include wild rocket, leaf lettuce, lamb's lettuce, spinach, and Swiss chard.

The biosynthesis, composition and concentration of health-promoting compounds vary widely among leafy vegetables, and carry the influence of genetic and environmental factors (light and temperature), growing conditions, harvest practices and postharvest handling conditions (Rouphael et al., 2012). Among these factors, light plays a crucial role in driving phytochemical photosynthetic activity (Bian, Yang, & Liu, 2015). It has been demonstrated that phytochemical biosynthesis and accumulation in plants are well correlated with the amount of photosynthates (Wu et al., 2007). Therefore, optimal light conditions are of vital importance for maximizing phytochemical accumulation (Bian et al., 2015). Lefsrud, Kopsell, Kopsell, and Curran-Celentano (2006) found that the highest accumulation of lutein and β-carotene in spinach occurred under light intensity of 335 μmol m^{-2} s^{-1}. Similarly, Li, Hikosaka, and Goto (2009) demonstrated that lutein and β-carotene concentrations were higher in spinach under 300 μmol m^{-2} s^{-1} than under light intensity of 100 μmol m^{-2} s^{-1}. Additionally, Zhou, Liu, Wen, and Yang (2011) demonstrated that concentrations of soluble sugars and ascorbic acid in lettuce increased with an increase in light intensity from 50 to 200 μmol m^{-2} s^{-1}. On the other hand, leafy vegetable species are noted for their high nitrate accumulation that contributes toward high intake of nitrates in the human diet (Amr & Hadidi, 2001). In healthy adults, 5–7% of the total nitrate intake undergoes enterosalivary circulation and is converted to nitrite by oral bacteria and salivary enzymes, whereas a considerably higher

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conversion rate is exhibited by infants or patients with gastroenteritis, due to their higher gastric pH (Bruning-Fann & Kaneene, 1993). Nitrate *per se* is relatively non-toxic but its reaction products and metabolites such as nitrite, nitric oxide and N-nitroso compounds, have raised concern because of their implications for adverse health effects (Parks, Huett, Campbell, & Spohr, 2008), most notably methaemoglobinaemia or 'blue baby syndrome'. This disease in particularly hazardous for infants but can also affect children and adults leading to suffocation (Camp, 2007). More than three hundred nitrosamines and N-nitroso compounds were proven to be carcinogenic to more than 40 animal species (Gangolli et al., 1994). However, the significance on human health is still equivocal, since several epidemiological studies (Milkowski, Garg, Coughlin, & Bryan, 2010), did not confirm any direct correlation between nitrate concentration in food and the incidence of cancer (Speijers & Van Den Brandt, 2003). On the other hand, McKnight, Duncana, Leifert, and Golden (1999) demonstrated that nitrite, nitric oxide and N-nitrosamines exert an important antimicrobial role in the stomach against gastrointestinal pathogens. Nitrate metabolites were also shown to have important physiological and pharmacological function, such as vasoregulation, as well as tissue protective properties (EFSA, 2008). Despite the debate on the effects of nitrate on human health, the production and commercialization of some leafy vegetables is subject to regulatory limitations (Cavaiuolo & Ferrante, 2014), since some population groups (e.g. vegetarians and babies) could be at higher risk for developing cancer when consistently exposed to elevated dietary intake of nitrate (Cavaiuolo & Ferrante, 2014). Therefore, the World Health Organization (WHO) has set an acceptable daily intake (ADI) for nitrate of 3.7 mg kg^{-1} body weight (Speijers & Van Den Brandt, 2003). In line with the World Health Organization (WHO), the European Commission regulation N° 1881/2006 (EC, 2006) established the thresholds of nitrates in spinach (2500–3500 mg kg^{-1} FW), lettuce (3000–5000 mg kg^{-1} FW), and lettuce type 'Iceberg' (2000–2500 mg kg^{-1} FW), whereas the European Commission regulation N° 1258/2011 (EC, 2011) introduced the maximum limits for rocket (6000–7000 mg kg^{-1} FW).

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 influence significantly the level of nitrate in raw leafy vegetables (Santamaria, 2006). Among these, the genetic background, nitrate supply, harvest time and light intensity during the growing season are predominant (Amr & Hadidi, 2001; Santamaria, Elia, Gonnella, Parente, & Serio, 2001). For instance, the relationship between light levels during the growing cycle and nitrate concentration has been demonstrated in a number of studies (Amr & Hadidi, 2001; Dennis & Wilson, 2003). Recently, Chang, Yang, and Riskowski (2013a,b) carried out field and 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 of the day. Nevertheless, little information is available concerning the effect of light intensity at the time of harvest on desirable and undesirable compounds in 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 colour components [e.g. lightness (L^*), redness (a^*) and yellowness (b^*)], protein content, mineral content, antioxidant activity, ascorbic acid and total phenolic content of the selected leafy vegetables in relation to the light intensity (low and high Photosynthetically Active Radiation, PAR) at the time of harvest. These findings will allow for a better understanding of the variation in nutritional values of selected baby leaf vegetables, from a nutritional or consumer perspective, and will assist producers in identifying the optimum species-specific time of harvest for achieving high nutritional value for the candidate baby leaf species in a sustainable way.

2. Materials and methods

2.1. Plant materials, growth conditions and treatments

The experiment was carried out in spring 2014, in a greenhouse covered by ethyl vinyl acetate (EVA) 0.2 mm plastic film, situated at the experimental farm of Pontinatura-Latina in central Italy (latitude 41° 24′ N, longitude 13° 03′ E, altitude 4 m). The day/night air temperature and 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}, and 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 seed source are reported in Supplementary Information.

Seeds of baby leaf species were sown in March, and plants were harvested during the last week of April at the same physiological age, expressed as the standard accumulation of growing degree days after sowing. 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. 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 supplied through the irrigation system. Irrigation was delivered by a movable boom spray system (e.g. overhead irrigation). Plants were kept free from weeds, insects, and disease 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 consisted of a 8 m^2 plot area.

2.2. Collection of samples

The plant tissue samples 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 two light intensities correspond to the typical times of harvest adopted by baby leaf growers: early in the morning (low PAR at 8.00 h) and in the afternoon (high PAR at 14.00 h). 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 a light logger meter Model LI-1500 (Lincoln, NE, USA). The sensor was placed close to and at the same level as the plants being sampled. For the determination of mineral composition, nitrate content, antioxidant activity, ascorbic acid and total phenolic contents, fresh leaves were frozen in liquid nitrogen and stored at −80 °C until used. Yield was determined