Scientia Horticulturae 204 (2016) 106–115

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Different agronomic and fertilization systems affect polyphenolic profile, antioxidant capacity and mineral composition of lettuce

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ARTICLE INFO

Article history:
Received 18 November 2015
Received in revised form 3 April 2016
Accepted 4 April 2016
Available online 14 April 2016

Keywords:
Flavonols
Lettuce
Minerals
Organic farming
Phenolic acids

ABSTRACT

The present paper aims to investigate phenolic profiles, antioxidant capacity and mineral composition of lettuce (*Lactuca sativa* L., var. 'Maravilla de Verano') grown under conventional (CON) and an organic (ORG) systems with four different fertilization treatments. The polyphenolic profiles of leaf extracts were determined by ultra-high-performance liquid chromatography (UHPLC), the levels of mineral elements by means of inductively coupled plasma mass spectrometry, whereas total phenolic content and antioxidant capacity were determined spectrophotometrically. Yield, soil and meteorological parameters were measured. In all the fertilization treatments, total phenolic acids and flavonols in CON were significantly higher compared to ORG. A trend parallel to that of single phenols was observed for total phenolic content and total antioxidant capacity. Plant mineral distribution revealed significant changes between CON and ORG systems in some plant macronutrients (N, Mg, S, Na, Fe) and micronutrients (Se, Mn, Mo). The differences among fertilization treatments for all the parameters considered were also discussed. From the overall analysis of the results, the higher content of phenolics observed in CON system could be associated to the presence of more stressful conditions, in terms of plant and/or soil mineral deficits. On the other hand, the adoption of an organic management determined higher yields and a better plant mineral balance.

1. Introduction

Green lettuce (*Lactuca sativa* L.) is primarily consumed as whole heads or fresh-cut product (Romani et al., 2002). As lettuce worldwide consumption has steadily increased in the last decades (Heimler et al., 2012), a series of studies have been recently conducted for studying its nutraceutical and health-promoting compounds (Ribas-Agustí et al., 2011; Heimler et al., 2012; Abu-Reidah et al., 2013; Durazzo et al., 2014; Pepe et al., 2015).

It was demonstrated that lettuce is particularly rich of some classes of polyphenols, plant secondary metabolites with free radical-scavenging properties that have evolved for facing abiotic and biotic stresses experienced by the plants in the surrounding environment (Romani et al., 2002; Oh et al., 2009a). Polyphenols are also important in human diet, preventing cancer and cardiovascular diseases (Manach et al., 2004; Hooper and Cassidy, 2006). The health-promoting properties of lettuce polyphenols were recently tested by Pepe et al. (2015) and Adesso et al. (2016), who demonstrated that lettuce leaf extracts are able to reduce both the inflammatory and oxidative stress in murine monocyte macrophage cells, by decreasing reactive oxygen species, nitric oxide release, inducible nitric oxide synthase and cycloxygenase-2 expression, and promoting the nuclear translocation of the nuclear

Abbreviations: CON, conventional soil management; DPPH•, 2,2-diphenyl-1-picrylhydrazyl radical; ORG, organic soil management; DW, dry weight; FRAP, ferric reducing ability of plasma; FW, fresh weight; GAE, gallic acid equivalents; PAR, photosynthetically active radiation; TE, Trolox equivalents; UHPLC, ultra-high-performance liquid chromatography.

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http://dx.doi.org/10.1016/j.scienta.2016.04.003
0304-4238/

Table 1
Compost and rock dust applied every year (2011–2013) for the different fertilization treatments.

	Fertilization treatment	Compost (t ha^{-1} yr^{-1})	Rock dust (t ha^{-1} yr^{-1})	Calcium nitrate (kg N ha^{-1} yr^{-1})
A	No fertilization	0	0	0
B	Manure	12	0	0
C	Rock dust	0	28.8	0
D	Manure + rock dust	12	28.8	0
K	Mineral fertilization (control)	0	0	150

factors Nrf2 and NF-κB. Considering that lettuce is a cheap and popular food widely consumed fresh worldwide and that the lack of cooking preserve the degradation of the more thermolabile chemical species and avoid the loss of water-soluble compounds, this vegetable is of key importance for the dietary supply of natural antioxidants (Pepe et al., 2015).

In lettuce, two classes of polyphenols are mainly present: phenolic acids and flavonols (Manach et al., 2004; Baslam et al., 2013; López et al., 2014). Phenolic acids are rarely found in lettuce in the free form, being mostly present as bound forms, mainly glycosylated derivatives or esters (e.g. chlorogenic acid, from the combination of caffeic and quinic acids) (DuPont et al., 2000; Mulabagal et al., 2010; Ribas-Agustí et al., 2011; Mai and Glomb, 2013). Flavonols are very frequent in lettuce, and their main representatives are the glycosylated derivatives of quercetin and kaempferol (Romani et al., 2002; Hooper and Cassidy, 2006).

Greenhouse and soilless lettuce culture systems are today increasingly widespread than open-air-grown lettuce (Romani et al., 2002; Heimler et al., 2012; Durazzo et al., 2014). While this warrants a higher yield and better climatic, phytopathogical, water and nutritional control, it not always ensures a good phytochemical profile in the final product (Tomas-Barberan et al., 1997; Llorach et al., 2008). Indeed, the adverse agronomic and environmental factors experienced by plants grown in open field, such as high or low temperature, ultraviolet light, insect attack, pathogen infection and eventual nutrient deficiency can increase the amounts of phenolics and/or change their profiles, even with a decrease of crop productivity (Tomas-Barberan et al., 1997; Llorach et al., 2004, 2008; Oh et al., 2009b). For instance, flavonols are yellow pigments generally considered to act as UV protectants and free radical scavengers, so their biosynthesis is stimulated in the outer and aerial tissues (stem and leaf epidermis) under excess light (Sofo et al., 2012). In addition, the levels of phenolic acids in lettuce are sensitive to environmental conditions (Liu et al., 2007; Mai and Glomb, 2013). For all these reasons, it is important to find a compromise between convenient agronomic practices and food nutritional properties. On the other hand, to obtain top yields of high quality and preserve environmental sustainability, it is indispensable to adopt sustainable agricultural techniques. Among open field agronomic techniques, organic farming offers more benefits than a conventional approach in terms of sustainability, enhanced physico-chemical and microbiological soil fertility, and absence of synthetic fertilizers and chemical pesticides (Heimler et al., 2012; Durazzo et al., 2014)

Other than phenolics, lettuce chemistry at the elemental level, that includes the content of all mineral nutrients and trace elements, is of key importance (Kelly and Bateman, 2010). Indeed, it is known that some inorganic cations, naturally present at non-toxic concentrations in lettuce, such as Fe, Cu and Zn, participate in the radical-scavenging reactions for their role of cofactors in some antioxidant enzymes, Fe/S proteins and cytochromes. In the case of K, Na, Ca, Mg, S and P, they are essential to the human organism together with some micronutrients (e.g., Mn, Se and Co) that act as cofactors in human vitamins and enzymes.

In the last years, great importance has been devoted to the consumption of lettuce since it is a source of natural antioxidants (Llorach et al., 2008; Kelly and Bateman, 2010; Heimler et al., 2012; Abu-Reidah et al., 2013; Lee and Scagel, 2013). Based on this accepted knowledge, the present paper aims at investigating phenolic profiles, total phenol content, total antioxidant capacity and mineral composition of lettuce grown under two different farming systems (conventional and organic) and under different fertilization treatments in order to establish if these management practices can modify its nutritional quality.

2. Materials and methods

2.1. Experimental site and plant material

The experiment was conducted in 2011, 2012 and 2013 in two open-field farms at Järna, in central Sweden, not distant each other and have similar climatic conditions. The first farm ('Gerstaberg'; CON) was managed conventionally for over 50 years, cultivated with a rotation potato/rye in the last 12 years (fertilization using 35 kg N-NO_3 ha^{-1} yr^{-1} and 20 kg N-NH_4 ha^{-1} yr^{-1}, crop residue shredding, and ploughing twice a year at a soil depth of 30 cm). The second one was an organic farm ('Nibble garden'; ORG) amended with different types of animal manure and without any synthetic fertilizers or chemical pesticides since its inception in 1966, and cultivated with a rotation of with potato/clover/barley in the last 12 years (minimum tillage, crop residues shredded and incorporated into the soil after a light harrowing at 10-cm soil depth).

The lettuce (*L. sativa* L.) variety used in this study was 'Maravilla de Verano'. Starting from 2011, both farms (CON and ORG) were divided in four blocks: one unfertilized control (treatment A) and three fertilization treatments (treatments B, C and D) with different types of soil amendments (Table 1). An additional mineral fertilized treatment (K) was included in CON field whereas it was not possible to include it in the ORG system due to the strict restrictions in the use of synthetic fertilizers.

Cattle manure composted for six months was used for treatment B. Rock dust (treatment C) was obtained by finely grinding (approximately 0.5 mm of diameter) local rocks using a jaw crusher (Model Nordberg C100; Metso Minerals Ltd., Tampere, Finland). Compost mean composition was the following: water 248 g kg FW^{-1}, pH 7.98, total N 18 g kg DW^{-1}, organic C 338 g kg DW^{-1}, organic matter 583 g kg DW^{-1}, humus 104 g kg DW^{-1}, P_2O_5 6.8 g kg DW^{-1}, K_2O 14 g kg DW^{-1}, Zn 0.112 g kg DW^{-1}, Fe 5.53 g kg DW^{-1}, 0.065Cu g kg DW^{-1}, Mn 0.114 g kg DW^{-1}. Rock dust mean composition (% w/w) was the following: SiO_2 65.21, K_2O 11.41, CaO 9.06, Al_2O_3 5.42, MgO 1.96, Na_2O 1.65, P_2O_5 1.28, FeO 1.21, Fe_2O_3 1.02, MnO 0.07, other minerals 1.71. Compost stone meal (treatment D) was prepared by mixing the stone powder with cattle manure (<0.2 mm) from diabase, after which the mixture was composted for 6 months. Fertilizers amounts of the treatment K (Table 1) were calculated on the basis of the values of soil texture and N content (Table 2). At the establishment of the treatments, ten composite soil sample were taken by randomly collecting three soil cores per each farming system and fertilization treatment. On these samples, soil texture was determined, soil N was measured by the analytic kit Reflectoquant 10© (Merck, NJ, USA) combined to the Kjeldahl