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Nutritional quality of outer and inner leaves of green and red pigmented lettuces (*Lactuca sativa* L.) consumed as salads

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ABSTRACT

Lettuce (*Lactuca sativa* L.) is a major food crop within the European Union. The objective of this study was to test the potential nutritional quality of three types of lettuce consumed as salads: *Lactuca sativa* L. var. *longifolia* (commonly named Cogollos de Tudela) and two cultivars of *Lactuca sativa* L. var. *capitata* (Batavia Rubia Munguía and Maravilla de Verano). Distribution of sugars, proteins, minerals and antioxidant compounds (carotenoids, chlorophylls, phenolics, anthocyanins and ascorbate) between outer and inner leaves was tested. The potential benefits of each variety or cultivar were due to different compounds accumulated in leaves. Cogollos de Tudela showed higher levels of Mg and Ca in both external and internal leaves, and greater concentrations of Mn, chlorophylls and carotenoids in inner leaves than lettuces belonging to the var. *capitata*. Batavia Rubia Munguía had low quantity of Na and an important amount of K, Mg, Ca, Fe and Zn in outer leaves and it was the cultivar that accumulated the highest quantity of water in both outer and inner leaves. Maravilla de Verano showed the greatest contents of anthocyanins and ascorbate in external leaves. In the three cultivars some of the potentially beneficial compounds appeared in higher levels in the outer than in the inner leaves. Therefore, the complete elimination of the external leaves should be reconsidered when lettuces are consumed as salads or used as food crop for the 'Fourth Range' of vegetables.

1. Introduction

Lettuce (*Lactuca sativa* L.) is considered a major food crop within the European Union. Together with escarole, it is one of the most popular vegetables in salads that are consumed in increasing amounts due to their perception as "healthier" foods (Dupont et al., 2000). The healthy properties are attributed to a large supply of antioxidant compounds (e.g., vitamins C and E, carotenoids, polyphenols) and fiber content (Serafini et al., 2002; Nicolle et al., 2004a; Llorach et al., 2008). Other phytochemicals that contribute to both the sensory and health-promoting properties of lettuce are anthocyanins and chlorophylls (Li et al., 2010), being anthocyanins more abundant in the red varieties (Llorach et al., 2008). Moreover, the nutrient content of this vegetable includes useful amount of some minerals such as calcium and iron (Romani et al., 2002). However, the nutritional value of lettuce varies among different varieties. According to Mou and Ryder (2004), the lower nutritional value of some varieties is due to the high enclosure of their leaves in the head structure because most of the edible portion of head structure includes leaves that are not exposed to light (Ryder, 1997).

Lettuce is also the most used food crop for the so-called 'Fourth Range' of vegetables. The term originally meant fresh, cleaned, possibly chopped and mixed vegetables ready to be seasoned and eaten (Borghi, 2003). These vegetables are increasingly accepted by consumers because they are healthy and easy to prepare for eating. Only in the three first months of 2010, the commercialization of fruit and vegetables for the Fourth Range in Spain increased an 8.6% compared with the same period in 2009 (Eroski Consumer, 2010).

Batavia (*Lactuca sativa* L. var. *capitata*) and Cogollos de Tudela (*Lactuca sativa* L. var. *longifolia*) are highly commercialized in the North of Spain. Batavia lettuce, extensively cultivated in greenhouses, is classified as a loose-leaf lettuce or semi-heading lettuce. Batavia lettuces have an excellent shelf life, maintaining their crispness from the time they are harvested until the time they are consumed. Batavia Rubia Munguía and Maravilla de Verano are two cultivars of Batavia (*L. sativa* L. var. *capitata*) very appreciated to

Abbreviations: ASC, ascorbate; chl, chlorophyll; DHA, dehydroascorbate; FW, fresh weight; DM, dry matter; TSS, total soluble sugars; WC, water content.

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be consumed in salads. Batavia Rubia Munguía has yellow-green leaves, with very ruffled borders and a consistent, crisp texture. It develops a round, dense head. Maravilla de Verano is adapted to be grown during all year, especially in summer due to its resistance to high temperatures. It has rounded and very broad leaves with crunchy texture, green color and red pigmentation, especially in the borders of the most ruffled leaves. It develops good size, firm head. Cogollos de Tudela is a delicious variety of fan-leafed lettuce that has received its designation because it is grown in the city of Tudela and surrounding municipalities in Navarra, in the North of Spain. It is around 10 cm long and is characterized by its long, thick, strong leaves, ranging in color from the bright green of the outer leaves to the yellow of the inner ones. Its head is dense and erect.

Since outer leaves of lettuce are usually stripped off during harvest, the general objective of this study was to test the potential nutritional quality of the aforementioned varieties and cultivars of lettuce paying special attention to the distribution of the antioxidant compounds and mineral nutrients between outer and inner leaves of each cultivar.

2. Materials and methods

2.1. Experimental design

Seeds of the three aforementioned types of lettuce (Cogollos de Tudela, Batavia Rubia Munguía and Maravilla de Verano) were surface sterilized by 10% bleach for 10 min and sown on November 21st in a mixture of peat and sand (1:1, v:v). When seedlings had 2–3 fully developed leaves (December 16th), they were transferred to 1.5 L pots (one plant per pot and 10 pots per treatment) filled with a mixture of vermiculite–sand–peat (2.5:2.5:1, v:v:v). Peat was previously sterilized at 100 °C for 1 h on three consecutive days. Lettuce plants were grown in a greenhouse at 25/15 °C day/night temperatures, 50/90% day/night relative humidity (RH) and photosynthetic photon flux (PPF) of 300–400 μmol m^{-2} s^{-1} during a 14 h photoperiod. All plants were fertilized once a week with 300 mL of modified Hewitt's nutrient solution (Baslam et al., 2011) and also received 300 mL of distilled water twice a week. Plants were harvested seven weeks after transferring them to pots (on February 7th). Ten replicates per treatment were used, thus making a total of 30 pots.

Samples for analytical determinations were collected from both inner (internal zone) and outer (external zone) leaves of lettuces. Both zones were visually delimited. The internal zone was quite close to the meristematic tip of the shoot and included light green leaves. The harvested inner leaves were placed midway between the center of the head and the outer portion. Outer leaves exhibited darker color and larger size than inner leaves and were not compact in the lettuce head.

2.2. Growth parameters and leaf water content (WC)

Dry matter (DM) of the aerial part was determined after drying the plant material at 80 °C for 2 days. Leaf WC was calculated as (FW–DM)/DM, where FW and DM denote fresh weight and dry matter, respectively.

2.3. Elemental analyses

For elemental analyses, samples (0.5 g DM) were dry-ashed and dissolved in HCl according to Duque (1971). Phosphorus, potassium, magnesium, calcium, manganese, iron, zinc, copper and sodium were determined using a Perkin-Elmer Optima 4300 inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin-Elmer, USA). The operating parameters of the ICP-OES were: radio frequency power, 1300 W; nebulizer flow, 0.85 L min^{-1}; nebulizer pressure, 30 psi; auxiliary gas flow, 0.2 L min^{-1}; sample introduction, 1 mL min^{-1} and 3 replicates. Total nitrogen and sulfur were quantified after combustion (950 °C) of leaf DM with pure oxygen by an elemental analyzer (Carlo Erba CHNS-O EA1108, Carlo Erba Instruments, Italy).

2.4. Total soluble sugars (TSS) and total soluble proteins in leaves

Total soluble sugars (TSS) and total soluble proteins were quantified in potassium phosphate buffer (KPB) (50 mM, pH = 7.5) extracts of fresh leaves (1 g). These extracts were filtered through four cheese cloth layers and centrifuged at 38,720 × g for 10 min at 4 °C. The supernatant was collected and stored at 4 °C for protein and TSS determinations. Total soluble sugars (TSS) were analyzed with the anthrone reagent in a Spectronic 2000 (Bausch and Lomb, Rochester, NY) (Yemm and Willis, 1954). Leaf soluble proteins were measured by the protein dye-binding method of Bradford (1976) using bovine serum albumin (BSA) as standard. Results were expressed as mg of total soluble proteins or TSS per g of DM.

2.5. Chlorophylls and carotenoids

Contents of chlorophylls (chl *a* + chl *b*) and total carotenoids were determined according to Séstak et al. (1971). Samples of fresh outer or inner leaves (1 cm^2, equivalent, respectively, to 5.6 and 3.5 mg of DM in outer and inner leaves of Cogollos de Tudela; 3.8 and 3.2 mg of DM in outer and inner leaves of Batavia Rubia Munguía; and 4.1 and 3.2 mg of DM in outer and inner leaves of Maravilla de Verano) were immersed in 5 mL of 96% ethanol at 80 °C during 10 min to extract the pigments. The absorbance of extracts was measured at 470, 649, 665 and 750 nm using a Spectronic 2000 (Bausch and Lomb, Rochester, NY). Estimation of chl *a* and chl *b* and total carotenoids in the same extract solution was performed by using the extinction coefficients and equations established by Lichtenthaler (1987). Results were expressed as mg of total chlorophylls (*a* + *b*) or carotenoids per g of DM.

Individual carotenoids were determined using 1 cm^2 discs of outer or inner leaves. The leaf discs were ground in a mortar with pure acetone and a pinch of sodium ascorbate, as described by Abadía et al. (1999). Pigment extracts were then analyzed using HPLC (De las Rivas et al., 1989) with some modifications (Larbi et al., 2004). Results were expressed as mg of each type of carotenoid per g of leaf DM.

2.6. Total phenolics and anthocyanins

Total phenolic compounds were extracted according to Chapuis-Lardy et al. (2002) with slight modifications. Samples (0.5 g FW) were pulverized in liquid nitrogen, mixed with 20 mL of 80% methanol, and homogenized at room temperature for 1 min. After filtration, 0.5 mL of each sample were mixed with 10 mL of distilled water. Total phenolic content was determined from aqueous solutions by spectrophotometric analysis at 760 nm with Folin–Ciocalteu reagent (Waterman and Mole, 1994). Although it is not completely specific for phenolic compounds (e.g., it is affected by other constituents) and not all phenolic compounds exhibit the same level of activity in the assay (Kang and Saltveit, 2002), the Folin–Ciocalteu method is commonly used to measure phenolic content. Results were expressed as mg of gallic acid per g of DM of either outer or inner leaves.

Anthocyanins were analyzed according to Cevahir et al. (2004) with some modifications (Pietrini and Massacci, 1998). Samples of 1 cm^2 of leaves were collected and homogenized in 1 mL of acidified methanol (2.27 mL HCl 37% + 97.73 mL methanol) and maintained at 4 °C overnight in the dark to avoid degradation of chlorophylls whose products may interfere with the absorption of anthocyanins