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Original Research Article

Chemical composition and antioxidant capacity of lettuce: Comparative study of regular-sized (Romaine) and baby-sized (Little Gem and Mini Romaine) types

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ABSTRACT

The aim of this study was to assess the differences in sugars, organic acids and main antioxidant compounds, as well as nitrate concentration, between different lettuce cultivars belonging to three main types: one regular-sized type (Romaine) and two baby-sized types (Little Gem and Mini Romaine), usually consumed as whole-head and fresh-cut lettuces. Overall, in the studied cultivars, chlorogenic acid and caffeic derivates were the major compounds among the free and bound phenolics, respectively. As regards folates, only 5-methyl tetrahydrofolate (5-MTHF) was detected in the monoglutamic form, whereas the hydrolysis of polyglutamatic forms released further 5-MTHF and tetrahydrofolate (THF). The major carotenoid found was β-carotene followed by lutein, lactucaxanthin, violaxanthin and neoxanthin. Romaine type showed the highest content of total sugars, phenolic compounds, vitamin C and folates. Mini Romaine showed the highest content of organic acids, carotenoids and chlorophylls. Finally, Little Gem presented the highest nitrate content, which can be considered a negative characteristic of this lettuce type. The differences found in colour and metabolite and nitrate concentrations could be attributed to the lettuce head structure and size that determine the penetration of the sunlight and therefore the synthesis of light-dependent metabolites and the nitrogen assimilation.

1. Introduction

Lettuce (*Lactuca sativa* L.) is one of the most popular vegetables for human consumption, especially in salad, and is considered a good source of health-promoting compounds such as phenolics, vitamin C, folates, carotenoids and chlorophylls (Nicolle et al., 2004). The protective effect of these compounds is largely due to their antioxidant effects. Phenolics have been suggested to be the main compounds responsible for the antioxidant capacity of plants, based on their ability to donate hydrogen atoms to free radicals. In addition, they possess ideal structural properties for free radical scavenging properties (Sulaiman et al., 2011); in addition to the pro-vitamin A role of β-carotene, α-carotene and β-cryptoxanthin, most of the carotenoids that we eat possess antioxidant capacity (Paiva and Russell, 1999); vitamin C is involved in several biochemical mechanisms and it also has a recognized antioxidant effect by reducing oxidative free radicals both *in vivo* and *in vitro* (Duarte and Lunec, 2005); several authors have suggested that folates (water-soluble vitamin B) may act as antioxidants (Joshi et al., 2001; Rezk et al., 2003) since they show activity values comparable to those of vitamin C and E (Gliszczyńska-Świgło, 2007); finally, dietary chlorophyll and its derivatives are known to have antioxidant capacity through their ability to reduce free radicals (Ferruzzi et al., 2002).

Lettuce is marked in two ways, as whole-heads and as fresh-cut lettuce. Indeed, in the fresh-cut industry, fresh-cut lettuce is one of the most important products. However, the physical damage or wounding caused by preparation increases the rate of biochemical reactions responsible for changes in visual quality (colour, texture and browning) and phytochemicals (vitamin C content and phenolic compounds) (Saltveit, 2003).

According to Martínez-Sánchez et al. (2012), baby leaf lettuce has several advantages as fresh-cut material compared with regular-sized lettuces. These benefits were partly attributed to the minimal oxidation and lower bruising incurred during processing, since baby leaves need little or no further preparation because the entire leaf is consumed. However, baby leaves presented certain disadvantages with regard to the shelf-life since they are immature leaves with a softer texture and higher respiration rates than those of regular-sized lettuces. Apart from these characteristics that make different lettuce types more suitable for the fresh-cut or whole-head market, depending on their shelf-life, differences in

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the phenolic, vitamin C and carotenoid contents between baby and regular lettuces in have been reported (de Azevedo-Meleiro and Rodriguez-Amaya, 2005; Martínez-Sánchez et al., 2012).

The variability in the composition and quantity of phytochemicals in lettuce indicate the importance of choosing certain types or cultivars. However, little information is available about the concentration of secondary metabolites in different lettuce cultivars and further studies are needed to improve the nutrient and antioxidant intake in the diet. In addition to these health-promoting compounds, other components with the potential to adversely affect health should be taken into account when comparing cultivars. Thus, leafy green vegetables constitute the major dietary intake source of nitrate. A low nitrate content in vegetables is very important for human health, since nitrate can be reduced to nitrite and then combined with secondary amines, increasing the risk of gastrointestinal cancer (Hord et al., 2009).

The aim of this study was to compare lettuce cultivars belonging to regular-sized (Romaine) and baby-sized (Little Gem and Mini Romaine) types, usually marketed as whole lettuce heads or fresh-cut processing. Morphologically, these types differ in the head structure and size. Romaine type presents long leaves 20–26 cm long, entire margins, and very broad midrib and open head. Mini Romaine is similar to Romaine type but smaller gauge and about 18–20 cm long. Finally, Little Gem is a long leaf lettuce that forms a compact and closed head 10–15 cm long and 8–10 cm in diameter.

These lettuce types would also be expected to present differences in the chemical composition and metabolite profiling but, in spite of being widely consumed worldwide, little information is available for these green lettuce types. Studying the chemical composition and the main antioxidant compounds they contain (polyphenols, vitamin C, folates, carotenoids and chlorophylls) could provide valuable information about their advantages and disadvantages with regard to their quality and potential health-promoting properties, as well as their suitability to be used as whole heads or fresh-cut lettuce.

2. Materials and method

2.1. Experimental design

Lettuce plants (*Lactuca sativa* L.) belonging to three different types of regular (Romaine), intermediate (Mini-romaine) and low (Little gem) size were cultivated in greenhouse conditions. A total of sixteen cultivars were studied in Romaine (Carrascoy, Espuña, Aitana, Collado, Alhama, Isasa, Ar-29213), Little gem (Ricote, Petra, Etna, Urbion, Sandra, Maite, Ferro) and Mini Romaine (Marta and Ar-29232) lettuces. There were four replicates per cultivar in a randomized design. Plants were irrigated with a nutritive solution containing (mM): 6 N, 7 K, 4 Ca, 1 Mg, 1 P, 1 S; and (μM) 2.24 Fe, 0.11 Mn, 0.27 B, 0.13 Zn, 0.032 Cu and 0.05 Mo. Sixty days after transplanting, plants were harvested and washed with deionised water. The colour of each sample was measured by reflectance in the inner and outer leaves, using a Minolta CR-200 (Minolta, Ramsey, NJ, USA) colorimeter through direct reading in three different areas of the surface of randomly selected leaves, taking the mean of the three measurements as the definitive. Colour data are provided as hue angle ($Hue = \tan^{-1} b^*/a^*$) and chroma [$Chr = (a^{*2} + b^{*2})^{1/2}$]. Finally, the samples (500 g per replicate) were powdered with liquid N_2 and frozen at −80 °C until subsequent analysis.

2.2. Analysis of metabolites and nitrate

An overall extraction of water- and ethyl acetate-soluble compounds was performed according to Cano et al. (2002). Aliquots of 5 g of powered sample were homogenized with 5 mL of phosphate buffer (pH = 7.5) in a Polytron (PT-MR 3100, Littau-Luzern, Switzerland). Ethyl acetate (10 mL) was added and the mixture was homogenized again. The aqueous fraction was used for analysing soluble sugars, organic acids and antioxidant capacity of the water-soluble fraction (W-ABTS and W-FRAP), while the ethyl acetate fraction was used for analysis of the antioxidant capacity of the organic fraction (EA-ABTS and EA-FRAP). Soluble sugars were analysed in the aqueous phase using a Hewlett-Packard model 1100 HPLC system liquid chromatograph (Waldbronn, Germany) equipped with a refraction index detector. The separation was performed in a 300 × 7.8 mm i.d., CARBOSep CHO-682 LEAD column (Transgenomic, Omaha, NE, USA) with ultrapure water as mobile phase at a flow rate of 0.4 mL/min. Standard solutions of sucrose, glucose and fructose (purity >99.5%) from Sigma (Steinheim, Germany) were injected at concentrations of 1–10 g L^{-1} to obtain the linearity of the detector response and the detection limits of the studied sugars. Organic acids were analysed according to Flores et al. (2012) using a liquid chromatograph (Agilent Series 1200, Santa Clara, CA, USA) equipped with a Prontosil C_{18} analytical column of 250 mm × 3 mm and 3 μm particle size (Bischoff, Leonberg, Germany). The injected sample volume was 20 μL, while the mobile phase was 0.1% (v/v) formic acid at a flow rate of 0.4 mL/min. The mass spectral analysis was performed on a G6410A triple quadrupole mass spectrometer from Agilent equipped with an ESI interface operating in negative ion mode. The effect of co-eluting matrix components, which can cause ion suppression or enhancement of the analyte, was eliminated following the standard additions approach. For that, standard solutions of glutamic, tartaric, α-ketoglutaric, malic, shikimic, succinic, fumaric, citric, malonic and quinic acids (purity 99%) from Sigma–Aldrich (Steinheim, Germany) were added to the matrix at concentrations 0.05–2000 μg mL^{-1} for each organic acid.

Free phenolics were extracted (3 g of fresh powdered sample) with methanol:formic acid (97:3) according to Cantos et al. (2002). For the determination of bound compound, extraction and simultaneous hydrolysis were performed according to Luthria et al. (2006) with 2 N NaOH containing 10 mM EDTA and 1% ascorbic acid as antioxidants, and subsequent acidification with HCl. Afterwards, free phenolic acids were extracted twice with ethyl acetate, the organic layer was evaporated to dryness under vacuum and the residue was re-dissolved in methanol. Both extracts (methanolic and hydrolysed) were injected in a liquid chromatograph (Agilent Series 1200, Santa Clara, CA, USA) equipped with a triple quadrupole mass spectrometer detector and ESI interface operating in negative ion mode, using N_2 as nebulizer and drying gas and the following operation parameters: 2000 V capillary voltage, 60 psi nebulizer pressure, 13 L/min drying gas flow and 350 °C drying gas temperature. Separation was achieved in a Lichrosphere C_{18} analytical column of 250 mm × 4 mm and 5 μm particle size (Agilent Technologies, Waldbronn, Germany) with 5% formic acid (solvent A) and acetonitrile (solvent B) as the mobile phase at a flow rate of 1 mL/min. The gradient began with 5% B, reaching 10% B in 9 min, 30% B in 50 min, increased to 100% in 2 min and held at 100% B for an additional 3 min, returning to initial conditions in 1 min and remaining isocratic for 6 min. Standards of protocatechuic acid, chlorogenic acid, caffeic acid, p-coumaric acid, rutin (quercetin 3-*O*-rutinoside), phloridzin (phloretin 6′-*O*-glucoside), luteolin, apigenin and kaempferol were purchased from Sigma–Aldrich (Steinheim, Germany) (purity >99%). Phenolic compounds in the samples were identified by comparison of retention times and quantifier transition with those of the corresponding standard. Quantification was performed in single reaction monitoring (SRM) mode at *m/z* 153→109 (protocatechuic acid), *m/z* 353→191 (chlorogenic acid), *m/z* 179→135 (caffeic acid), *m/z* 163→119