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Carotenoid and chlorophyll composition of commonly consumed leafy vegetables in Mediterranean countries

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ABSTRACT

Major chloroplast pigments in five leafy vegetables (chicory-*Cichorium intybus*, cv. 'Anivip' and cv. 'Monivip', dandelion-*Taraxacum officinale*, garden rocket-*Eruca sativa* and wild rocket-*Diplotaxis tenuifolia*), commonly consumed in Mediterranean countries, have been separated by high-performance liquid chromatography (HPLC) on a reversed-phase column. Three classes of pigments were identified and quantified: xanthophylls (oxygenated carotenoids), carotenes (hydrocarbon carotenoids) and chlorophylls. The contents of the pigments in the analysed leafy vegetables varied significantly. The results indicated that selected leafy vegetables were moderately rich in xanthophylls, primarily lutein (3.87–7.44 mg/100 g fwt). Other xanthophylls were detected in relatively small quantities. The provitamin A carotenoids (α- and β-carotene) were also detected, but α-carotene were not present in chicory cultivars and in dandelion. The ratio of chlorophyll *a*/*b* varied from 2.44 to 2.67 depending on the species. The highest content of all the analysed constituents was found in the garden rocket.

1. Introduction

In recent years, leafy vegetables, such as chicory (*Cichorium intybus* L.), dandelion (*Taraxacum officinale* Waggner), garden rocket (*Eruca sativa* Mill.) and wild rocket (*Diplotaxis tenuifolia* DC.) have become widely used in Mediterranean countries for various kinds of salads, such as fresh, mixed or garnish salad. Over the last decade an abundance of research has shown that fresh leafy vegetables constitute important functional food components by contributing vitamins, minerals and biologically active compounds which are associated with dietary activities (Kimura & Rodriguez-Amaya, 2003; Kmiecik, Lisiewska, & Jaworska, 2001; Su, Rowley, Itsiopoulos, & O'Dea, 2002). Leafy vegetables also contain several types of photosynthetic pigments, that are chlorophylls and carotenoids (Kimura & Rodriguez-Amaya, 2002). The composition of these pigments produces specific colouration of the food, which is one of the assessed visual quality attributes (Xue & Yang, 2009). In addition, chlorophyll and carotenoid concentration correlate to the photosynthetic potential of plants giving some indication of the physiological status of the plant (Gamon & Surfus, 1999). However, the content of pigments in plants is important, not only due to the colouration and physiological function, but also due to their acknowledged roles in health (Liu, Perera, & Suresh, 2007; Niizu & Rodriguez-Amaya, 2005). For example, carotenes are the sources of vitamin A (Olson, 1994). Lutein and zeaxanthin are important factors for human vision (Wisniewska & Subczynski, 2006). Carotenoids and chlorophylls have an important role in the prevention of various diseases associated with oxidative stress, such as cancer, cardiovascular diseases and other chronic diseases (Sangeetha & Baskaran, 2010). Humans cannot synthesise both pigments but are able to deposit dietary pigments as absorbed or with slight modification of their structure (Larsen & Christensen, 2005).

Among the carotenoids in leafy vegetables, zeaxanthin, lutein and β-carotene have been intensively studied with regard to their effects on human health (Landrum & Bone, 2001). The interest in new data on carotenoids in edible plants is increasing due to a more extensive use of natural compounds in the food, following the directives of European Community in favour of natural rather than synthetic compounds (Xu et al., 2006).

The present study was undertaken with an aim to evaluate the pigments content of leafy vegetables commonly consumed in the Mediterranean part of Europe. Although there have been a number of studies of the carotenoid and chlorophyll content of vegetables, there are just a few studies on pigment profiles in the vegetables included in our study. The results of our study can be used as fundamental data for dietary recommendation to help the consumers to select appropriate types of vegetables to meet their nutrient and health needs.

2. Materials and methods

2.1. Plant culture

The greenhouse experiment was conducted in the experimental field (46° 04′ N, 14° 31′ W, 320 m above sea level) of the

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Biotechnical Faculty in Ljubljana, Slovenia. Trials were carried out during two seasons (2007 and 2008). Leafy vegetables used in this study include chicory (*C. intybus* L. cv. 'Anivip' and cv. 'Monivip'), dandelion (*T. officinale* Waggner), garden rocket (*E. sativa* Mill.) and wild rocket (*D. tenuifolia* DC.). These vegetables are easily grown in a greenhouse and are thus suitable as experimental plants. Seeds were purchased from a commercial seedling company.

Sowing took place on 24 March 2007 and 26 March 2008 in plastic pots (8 cm in diameter and 6 cm in height, one seed per pot) filled with a commercial peat-based potting medium (Klasmann Tray substrate; pH 6–6.5; N 180 mg/l; P_2O_5 210 mg/l; K_2O 250 mg/l; MgO 85 mg/l + microelements). The pots were placed on the rolling benches in a glasshouse compartment. The experiment was made in a complete randomised block design on four benches, each bench representing a block/replication.

After seedling emergence, the plants were uniformly irrigated as needed with tap water (generally two times per day), by overhead misters. When plants reached two fully expanded leaves, they were fertigated weekly, with 25 mg/l of a water-soluble fertilizer Peters Professional, Scotts Company (0.75 g N, 0.55 g P_2O_5 and 1.45 g K_2O/l).

Average maximum and minimum temperatures of 34.5 and 12.8°C, respectively, and average maximum and minimum relative humidity of 86.2% and 24.7%, respectively were recorded during the experiment. The average light intensity (PAR) in day time was approximately 420 μmol/m^2/sec. The data were obtained from a weather shelter located half metre above the flats at the center of the experiment benches.

The harvesting was carried out after 40 days, when the plants had reached commercial maturity. Plant samples were picked randomly by hand with four repetitions. The first measure was to separate the leaves from the remaining parts of the plants by cutting the leaves at the root–shoot junction.

2.2. Sample preparation

The uniform, non-senescent, and undamaged leaves were washed with deionised water and the roots were discarded. Leaves were collected in the morning (6:00–8:00 solar time) and were immediately wrapped in aluminium foil to avoid degradation of pigments by light. They were transported in a portable refrigerator to the laboratory (the raw material did not exceed 2 h), where leaves were frozen in liquid nitrogen, lyophilised, ground to a fine powder using a planetary micro mill (FRITSCH, Pulversitte 7) and stored at −20°C in humidity-proof plastic containers until analysis. Before and after lyophilisation samples were weighed in order to recalculate data obtained from biochemical analyses from mg per dry weight (dwt) to mg per 100 g fresh weight (fwt). The foliar moisture content was 88.1 ± 2.1 (chicory cv. 'Anivip'), 87.4 ± 1.6 (dandelion), 89.2 ± 3.4 (chicory cv. 'Monivip'), 85.7 ± 2.6 (wild rocket) and 86.8 ± 2.9 (garden rocket) g/100 g fwt.

2.3. Pigments determination

Chloroplast pigments were determined using the method described in Šircelj and Batič (2007). Pigments were extracted from 100 mg of the dry leaf powder with 5 ml of ice-cold acetone on an ice bath, using T-25 Ultra-Turrax (Ika-Labortechnik, Staufen, Germany) homogenizer for 25 s. All extraction procedures were performed in dim light. Acetone extracts were filtered through 0.2 μm Minisart SRP 15 filter (Sartorius Stedim Biotech GmbH, Goettingen, Germany) and then subjected to HPLC gradient analysis (a Spherisorb S5 ODS-2 250 × 4.6 mm column with an S5 ODS-2 50 × 4.6 mm precolumn (Alltech Associaties, Inc., Deerfield, USA)), using the following solvents: solvent A; acetonitrile/methanol/water (100/10/5, v/v/v); solvent B; acetone/ethylacetate (2/1, v/v), at a flow rate of 1 ml/min, employing linear gradient from 10% solvent B to 70% solvent B in 18 min, with a run time 30 min, and photometric detection at 440 nm. The HPLC analysis was performed on a Spectra-Physics HPLC system with Spectra Focus UV–VIS detector (Fremont, USA). Identification of compounds was achieved by comparing the retention times and the spectra as well as by the addition of standards. The concentrations of pigments were calculated with the help of corresponding external standards. The following standards were used for the determination of photosynthetic pigments: α-, β-carotene, neoxanthin, violaxanthin, antheraxanthin, zeaxanthin, lutein and chlorophyll, all from DHI LAB products (Hoersholm, Denmark). All standards were highly purified. The solvents acetone, ethylacetate, methanol and acetonitrile were from Merck, all HPLC grade.

2.4. Statistical analysis

All measurements were performed in triplicates ($n = 3$) and the values were averaged and reported along with the standard deviation (±S.D). Statistical analysis was performed using the Statgraphics programme, version 4.0. The differences between the means were analysed by ANOVA test followed by the posthoc test Tukey's LSD. A significant difference was considered at the level of $P < 0.05$.

3. Results and discussion

3.1. Chromatographic profiles of pigments

The chromatogram of the most common pigment pattern presented in investigated leafy vegetables is shown in Fig. 1. Three classes of pigments namely: xanthophylls, carotenes and chlorophylls were identified and quantified under the HPLC conditions used in our research. Pigments were eluted in the following order: neoxanthin (peak 1), violaxanthin (peak 2), antheraxanthin (peak 3), lutein (peak 4), zeaxanthin (peak 5), chlorophyll *b* (peak 6), chlorophyll *a* (peak 7), α-carotene (peak 8) and β-carotene (peak

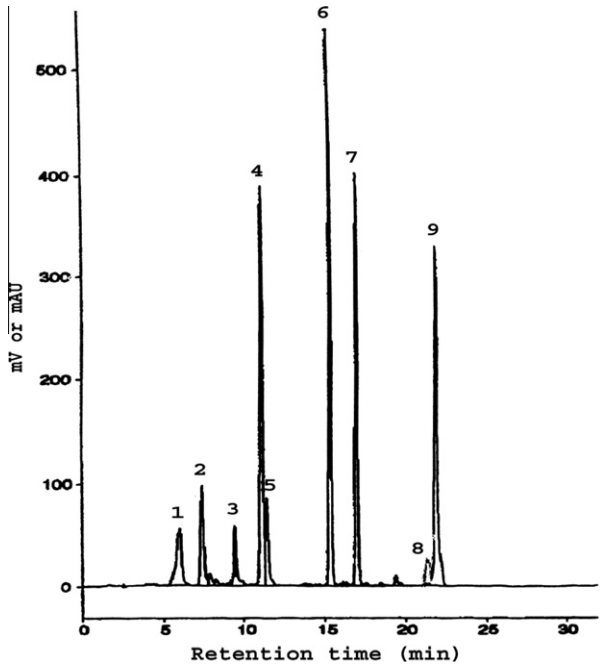

Fig. 1. Pigment profile of leafy vegetable samples by HPLC. Peak 1–9 represented the following: 1, neoxanthin; 2, violaxanthin; 3, antheraxanthin; 4, lutein, 5, zeaxanthin; 6, chlorophyll *b*; 7, chlorophyll *a*; 8, α-carotene; 9, β-carotene.