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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

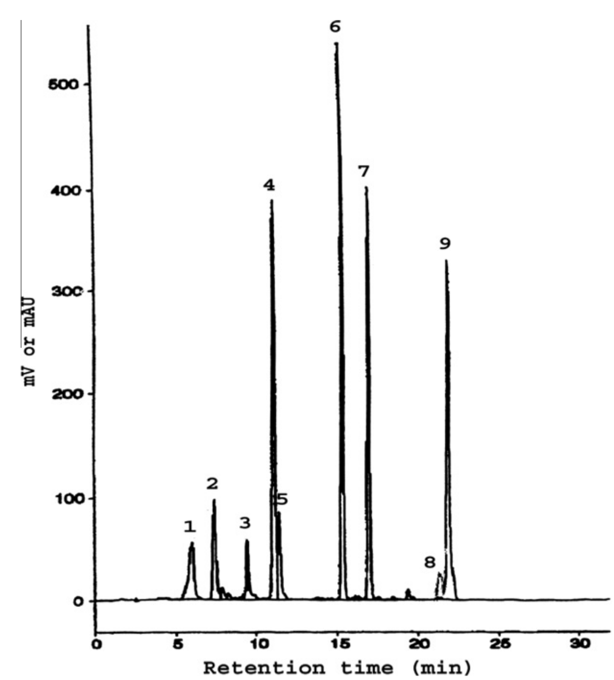

Fig. 1. Pigment profile of leafy vegetable samples by HPLC. Peak 1–9 represented the following: 1, neoxanthin; 2, violaxanthin; 3, antheraxanthin; 4, lutein, 5, zeaxanthin; 6, chlorophyll *b*; 7, chlorophyll *a*; 8, α-carotene; 9, β-carotene.

Table 1
Xanthophylls in selected leafy vegetables (means ± S.D) (mg/100 g).

	Lutein	Violaxanthin	Antheraxanthin	Zeaxanthin	VAZ[a]	Neoxanthin
Year						
2007	5.94 ± 0.96	1.63 ± 0.47	0.65 ± 0.18	0.09 ± 0.02	2.16 ± 0.62	0.83 ± 0.21
2008	5.62 ± 0.82	1.28 ± 0.32	0.52 ± 0.15	0.07 ± 0.01	1.97 ± 0.45	0.68 ± 0.15
Species						
cv. Anivip	5.91 ± 0.84	1.67 ± 0.44	0.52 ± 0.16	0.09 ± 0.02	2.28 ± 0.56	1.07 ± 0.26
cv. Monivip	3.87 ± 0.45	0.69 ± 0.27	0.38 ± 0.12	0.07 ± 0.01	1.14 ± 0.31	0.41 ± 0.12
Dandelion	5.25 ± 0.62	0.65 ± 0.18	0.47 ± 0.15	0.08 ± 0.02	1.21 ± 0.25	0.42 ± 0.14
Garden rocket	7.44 ± 0.78	1.56 ± 0.42	0.84 ± 0.21	0.06 ± 0.01	2.46 ± 0.61	0.83 ± 0.28
Wild rocket	5.82 ± 0.51	0.71 ± 0.25	0.39 ± 0.02	0.05 ± 0.01	1.15 ± 0.34	0.35 ± 0.09
Significance						
Year (Y)	NS	NS	NS	NS	NS	NS
Species (S)	**	**	*	**	**	**
Y × S	NS	NS	NS	NS	NS	NS

NS, non-significant; asterisk indicates significance at $P < 0.05$(*) or $P < 0.01$(**).
[a] Content of xanthophyll cycle pigments (violaxanthin, antheraxanthin, zeaxanthin).

9). All the leafy vegetables in our study showed nearly the same elution profiles of chloroplast pigments.

3.2. Quantitative distribution of carotenoids

The carotenoid levels in the leaves of leafy vegetables depend on several factors, including species, variety, cultivar, production practice, maturity, as well as environmental growth factors such as light, temperature, and soil properties (Van den Berg et al., 2000). Lutein and β-carotene have been widely reported as being two of the major carotenoids found in vegetables (Lakshminarayana, Raju, Krishnakantha, & Baskaran, 2005; Calvo, 2005). These two carotenoids were predominant also in the vegetables analysed in our study.

3.2.1. Xanthophylls

Five xanthophyll pigments namely: lutein, violaxanthin, antheraxanthin, zeaxanthin and neoxanthin were identified and quantified in the leaves of leafy vegetables in our study (Table 1). Xanthophylls contents differed among species/cultivars ($P < 0.01$), but not for the year and for the interaction of species/cultivars and year. We believe that the contents of xanthophylls did not differ significantly between the two experimental years because vegetables were grown in both years under very similar microclimate conditions in the greenhouse (data not shown).

On the basis of concentration, the major xanthophyll was lutein, making up on average 48% of the total xanthophylls. The highest lutein content was measured in garden rocket (7.44 ± 0.78 mg/100 g fwt) and the lowest lutein content was measured in chicory cv. 'Monivip' (3.87 ± 0.45 mg/100 g fwt). Similar values were recorded by Kopsell, Kopsell, Curran-Celentano, and Wenzel (2009), who have shown that lutein concentrations can range from 4.8 to 13.4 mg/100 g fwt for kale and from 6.5 to 13.0 mg/100 g fwt for spinach. A similar trend was reported by Dias, Filomena, Camões, and Oliveira (2009) who have shown values from 0.52 to 6.4 mg/100 g fwt for kale and from 3.6 to 5.6 mg/100 g fwt for leaf beet and turnip greens. On the other hand, leafy vegetables in our study have higher lutein content in comparison with some vegetables in previous reports. For example, Hart and Scott (1995) found 0.103 mg/100 g fwt in Savoy cabbage and 3.046 mg/100 g fwt in greens. Granado, Olmedilla, Blanco, and Rojas-Hidalgo (1992) reported 0.185 mg/100 g fwt for Brussels sprouts and 1.503 mg/100 g fwt for leaf beet. It seems that the leafy vegetables from our study are almost the richest dietary sources of lutein among commercially available vegetables. Higher values of lutein (11.0 ± 0.70 mg/100 g fwt) were found only in 'Winterbor' kale (Lefsrud, Kopsell, Wenzel, & Sheehan, 2007).

The content of xanthophyll cycle pigments (VAZ – violaxanthin, antheraxanthin and zeaxanthin) in vegetables analysed in our study varied between 1.14 ± 0.31 (chicory cv. 'Monivip') and 2.46 ± 0.61 mg/100 g fwt (garden rocket). Since the leaves in our research were sampled early in the morning, the VAZ pool was in the epoxidised state and consequently the major cycle pigment was violaxanthin, antheraxanthin represented 22–34% and zeaxanthin represented only 2–4% of the VAZ pool.

Similar to other studies, leafy vegetables from our study represent a weak source of dietary zeaxanthin. Perry, Rasmussen, and Johnson (2009) also found a very low content of zeaxanthin in green leafy vegetables. Recent interest in zeaxanthin is due to the discovery that it represents a major carotenoid in the retinal pigment of the eye (Krinsky, Landrom, & Bone, 2003). According to Wisniewska and Subczynski (2006), the presence of zeaxanthin and/or lutein in the diet may be beneficial for reducing the incidence of the two common eye diseases of ageing, age-related macular degeneration and cataracts formation.

Neoxanthin levels ranged from 0.35 ± 0.09 to 1.07 ± 0.26 mg/100 g fwt. The best source of neoxanthin among the vegetables analysed in our study was chicory cv. 'Anivip'. The content of neoxanthin was lower than violaxanthin in all of the vegetables analysed. Similar conclusions were presented by De Sa and Rodriguez-Amaya (2003) pointing out that in green vegetables, violaxanthin usually surpasses neoxanthin.

3.2.2. Carotenes

The total carotene content significantly differed among varieties ($P < 0.01$), but not for the years 2007 and 2008 and for the interaction

Table 2
Carotenes in selected leafy vegetables (means ± S.D) (mg/100 g).

	β-carotene	α-carotene	Total carotene
Year			
2007	7.12 ± 1.56	0.26 ± 0.08	7.38 ± 1.58
2008	6.28 ± 0.82	0.18 ± 0.05	6.46 ± 0.83
Species			
cv. Anivip	7.31 ± 1.12	ND	7.31 ± 1.12
cv. Monivip	3.94 ± 0.65	ND	3.94 ± 0.65
Dandelion	6.34 ± 0.94	ND	6.34 ± 0.94
Garden rocket	7.96 ± 1.43	0.28 ± 0.04	8.24 ± 1.45
Wild rocket	7.01 ± 1.04	0.17 ± 0.06	7.18 ± 1.10
Significance			
Year (Y)	NS	NS	NS
Species (S)	**	*	**
Y × S	NS	NS	NS

NS, non-significant; asterisk indicates significance at $P < 0.05$(*) or $P < 0.01$(**); ND, below the level of detection.

Table 3
Chlorophyll in selected leafy vegetables (means ± S.D) (mg/100 g).

	Chlorophyll (mg/100 g)			Chlorophyll *a*/*b* ratio
	Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll	
Year				
2007	225.74 ± 42.16	84.28 ± 16.35	310.02 ± 51.46	2.67 ± 0.38
2008	206.38 ± 28.14	71.57 ± 12.26	277.95 ± 34.35	2.61 ± 0.26
Species				
cv. Anivip	238.31 ± 40.56	89.74 ± 24.81	328.05 ± 42.06	2.65 ± 0.31
cv. Monivip	142.26 ± 26.17	58.18 ± 12.54	200.44 ± 27.84	2.44 ± 0.16
Dandelion	180.54 ± 35.08	67.71 ± 20.12	248.25 ± 38.26	2.67 ± 0.28
Garden rocket	261.24 ± 41.26	98.38 ± 26.75	359.62 ± 48.16	2.64 ± 0.35
Wild rocket	216.01 ± 19.64	87.22 ± 23.49	303.23 ± 36.67	2.47 ± 0.24
Significance				
Year (Y)	NS	NS	NS	NS
Species (S)	**	*	**	NS
Y × S	NS	NS	NS	NS

NS, non-significant; asterisk indicates significance at $P < 0.05$(*) or $P < 0.01$(**).

of species/cultivars and year (Table 2). The highest content of total carotene (8.24 ± 1.45 mg/100 g fwt) in garden rocket was twofold higher than the lowest content in chicory cv. 'Monivip' (3.94 ± 0.65 mg/100 g fwt). These results showed that the carotene concentration in the leafy vegetables is determined by the species.

A major contributor to the total carotene content of vegetables in our study was β-carotene which is the main carotenoid with pro-vitamin A activity (Olson, 1994). Regarding the concentration of this pigment Podsedek (2007) reported that leafy vegetables are indeed a richer source of β-carotene than other crops. Bhaskarachary, Ananthan, and Longyah (2008) also demonstrated similar domination of β-carotene in 17 species of leafy vegetables.

Among the vegetables analysed in our study, garden rocket had the highest content of β-carotene (7.96 ± 1.43 mg/100 g fwt), followed by chicory cv. 'Anivip' (7.31 ± 1.12 mg/100 g fwt), wild rocket (7.01 ± 1.04 mg/100 g fwt), dandelion (6.34 ± 0.94 mg/100 g fwt) and chicory cv. 'Monivip' (3.94 ± 0.65 mg/100 g fwt). However, the difference was not statistically significant except in the case of garden rocket. The values in our study are in agreement with the data of Sangeetha and Baskaran (2010) who analysed the β-carotene content in a number of vegetables. On the other hand, some of our findings did not agree with previous reports. For example, the β-carotene content in leafy vegetables analysed in our study was higher than that of the lettuce, cress and chicory (Kimura & Rodriguez-Amaya, 2002), of the cabbage and Chinese cabbage (Singh, Kawatra, & Sehgal, 2001), of the rocket and spinach (Burns et al., 2003).

In leaves of chicory cultivars and in dandelion, α-carotene was below the detection limit. Low contents of α-carotene were detected in the leaves of garden (0.17 ± 0.06 mg/100 g fwt) and wild rocket (0.28 ± 0.04 mg/100 g fwt). This observation is consistent with the results of Yang, Huang, Peng, and Li (1996), who found only minor quantities of α-carotene in several green vegetables.

3.3. Quantitative distribution of chlorophyll

On the basis of concentrations, chlorophyll was the most abundant pigment observed among species/cultivars (Table 3).

The contents of chlorophylls did not significantly change for the species/cultivars from year 2007 to 2008. On the other hand, significant differences ($P < 0.01$) in the total leaf chlorophyll content among the five vegetables analysed in our study, were found. The contents of total chlorophyll are as following: garden rocket > chicory cv. 'Anivip' > wild rocket > dandelion > chicory cv. 'Monivip'.

On average of the two years, the chlorophyll *a* concentration varied from 206.38 ± 28.14 to 225.74 ± 42.16 mg/100 g fwt, the chlorophyll *b* concentration from 71.57 ± 12.26 to 84.28 ± 16.35 mg/100 g fwt and the total chlorophyll from 277.95 ± 34.35 to 310.02 ± 51.46 mg/100 g fwt.

Although the total chlorophyll, chlorophyll *a* and *b* contents varied between species/cultivars, the chlorophyll *a*/*b* ratio was found to be similar. As expected, all species/cultivars had significantly higher chlorophyll *a* contents compared to chlorophyll *b*. The mean ratio of chlorophyll *a* to *b* was similar to the values reported for other dark green leafy tissues (Schwartz & Von Elbe, 1983).

We can conclude that the leafy vegetables analysed in our study are the crops with a relatively high content of chlorophylls, similar to that in kale (Kopsell, Kopsell, & Lefsrud, 2004) and exceeding that in spinach (Jaworska & Kmiecik, 1999).

Species/cultivars with high levels of chlorophylls also had relatively high amount of lutein and total carotenes. The positive correlation between the contents of chlorophyll and carotenoids have been also reported for other leafy crop species, like as kale (Kopsell et al., 2004), Swiss chard (Ihl, Shene, Scheuermann, & Bifani, 2006) and lettuce (Caldwell & Britz, 2006).

4. Conclusions

In conclusion, this study focused on the quantification of pigments of leafy vegetables commonly consumed in the Mediterranean countries. There is still very limited information on selected leafy vegetables analysed in this study. In general, the obtained results agree with those reported in the literature and the values fall within the wide ranges of data found in the literature. The data generated on the composition of carotenoids and chlorophylls in leafy vegetables could be the basis for suggesting the inclusion of these leafy vegetables in a daily diet to overcome health problems, such a vitamin A deficiency and age-related macular degeneration.

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