DETERMINATION OF THE CONTENT OF RUTIN AND TOTAL POLYPHENOLS IN LEAVES OF SPINACH AND AMARANTH*

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In the years 2002 to 2004 the content of rutin and total polyphenols was determined in the leaves of spinach and amaranth using the HPLC method. The experiment included three amaranth species – love-lies-bleeding (*A. caudatus*), red-blooded amaranth (*A. cruentus*) and red-spiked amaranth (*A. hypochondriacus*) and two spinach varieties – Matador and Triptiek F1. It is evident from the results that average rutin content was lowest at the beginning of vegetation 159.11 mg.g^{-1} and the highest in the phase of elongation of stem – 294.10 mg.g^{-1} (F = 2233.89, p = 0.00). The highest average rutin content was recorded in *A. hypochondriacus* (234.54 mg.g^{-1}) and on the contrary, the lowest one in *A. cruentus* – 154.92 mg.g^{-1} (F = 608.84, p = 0.00). The difference in the content of total polyphenols in spinach leaves was statistically significantly influenced of development (F = 165.84, p = 0.00) when their lowest average content was at the beginning of spinach vegetation, i.e. 10.95 mg.g^{-1} and the highest in the 3rd sampling (formation of leaf rosette) – 15.60 mg.g^{-1}. Statistically significant differences were found between studied spinach varieties (F = 409.08, p = 0.00) compared with the variety Matador (12.38 mg.g^{-1}). Average content of polyphenols was recorded in amaranth in the phase 24 – 10.38 mg.g^{-1} and in the phase of full ripeness (15.40 mg.g^{-1}) (F = 393.60, p = 0.00). The content of total polyphenols was the lowest in *A. caudatus* (13.43 mg.g^{-1}) and the highest in *A. hypochondriacus* (15.24 mg.g^{-1}). The effect of year on studied characteristics was confirmed. Concerning nutrition, leaves of amaranth and spinach should be harvested to the onset of anthesis.

amaranth; *Amaranthus* sp.; spinach; *Spinacia* sp.; rutin; total polyphenols; vegetables

INTRODUCTION

The beginning of the 21st century is marked by increasing concern about cultivation of new species of plants that should be utilised in food, chemical, cosmetic and pharmaceutical industries or in further branches of human activities. This new and old crop such as is e.g. rediscovery of amaranth (*Amaranthus* sp.), which has universal use not only as a source of high quality flour but its leaves are utilised as a vegetable or feed. The use of amaranth as a vegetable has been still concentrated above all in the regions of the tropics and subtropics (Holubová, Hnilička, 2001). According to Shukla et al. (2006) amaranth belongs to the most important leaf vegetable in summer months in India. For example Mediterranean *Amaranthus blitum* L. and/or Asian *Amaranthus tricolor* L. are used for preparation of spinach, prepared as an asparagus or added into soups.

Amaranth leaves are an excellent source of carotenoids, iron, calcium, ascorbic acid and proteins. Their content is comparable with that of other vegetable species, in some cases even higher. According to Kalač and Moudrý (2000) the amaranth leaves contain 70–94% of water, mineral substances 7.6–22% of dry matter, proteins 17.4–38% in dry matter, fats 1.0–10.6% in dry matter and saccharides of 38–47% in dry matter. According to Petr et al. (2008) amaranth has 13.1 g dry matter, 3.5 g proteins, 6.5 g saccharides, 1.3 g fibrous material and 2.6 g ashies in 100 g. Beside these substances amaranth leaves contain bioflavonoid (vitamin P) rutin, flavonoids and polyphenols.

This study has been focused on the research of the quality indicators that should help to reveal a meaning and quality of amaranth as "a new" species of leaf vegetable on the Czech market and its comparison with traditional representative of leaf (spinach) vegetable – spinach.

MATERIAL AND METHODS

Three amaranth species were used as an experimental material: love-lies-bleeding (*Amaranthus caudatus* L.) – 'CAC 48A', *Amaranthus cruentus* L. – 'Ficha' and *Amaranthus hypochondriacus* L. – '1008'.The seeds of amaranth were obtained by GeneBank of Crop Research Institute. Spinach varieties Matador and Triptiek F1. The seeds of spinach were obtained by Sempra.

The trial was established as a small plot field experiment in the years 2002 to 2004. Experimental plots were situated at the experimental field of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences in Prague-Suchdol. The area of experimental plot was 2 m^2. The trial was established by the method of the Latin square in four replications. The trial establishment and cultural practices were carried out according to methodologies of Jarošová et al. (1997).

The termopluviograms method was selected for weather valuation in the experimental years (Klabzuba et al. 1999). From weather survey results that the January 2002 was warm and droughty. The February, March, April,

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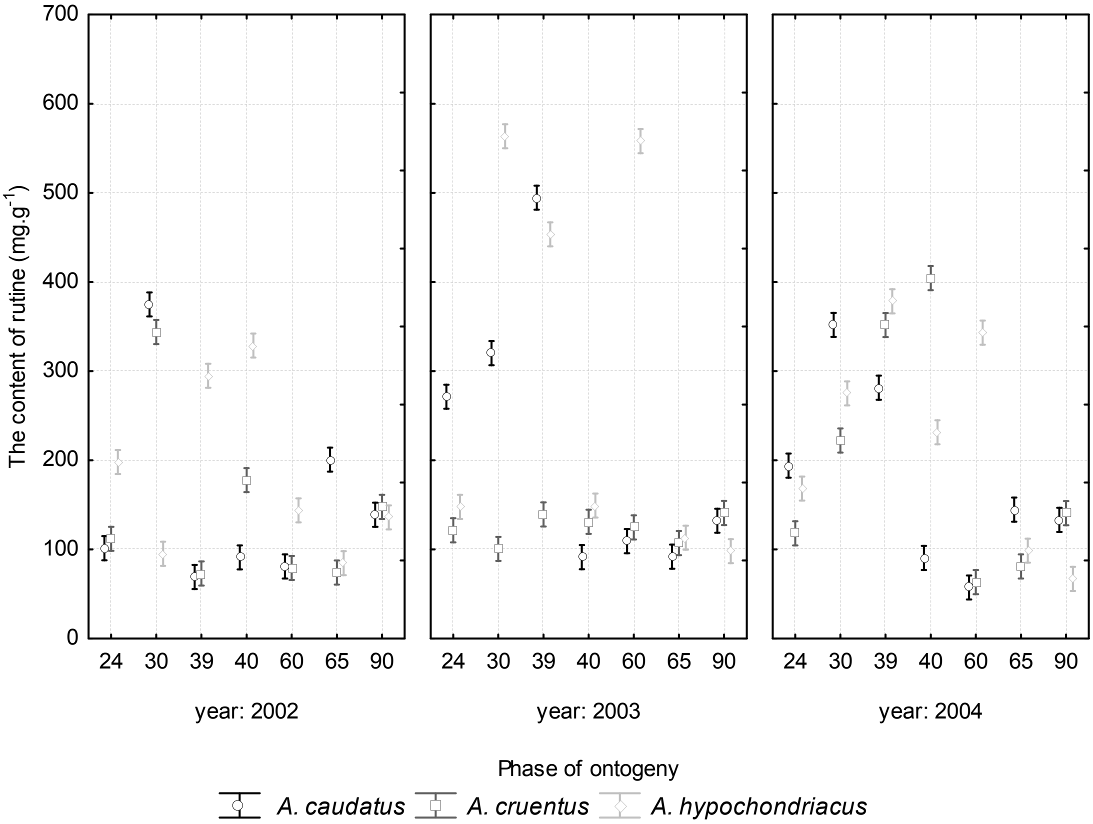

Fig. 1. The rutin content (mg.g^{-1} DM) in amaranth leaves. Vertical columns denote 0.99 intervals of reliability. Average values are determined by the method of least squares

Legend: ▫ average, □ average + standard deviation ⊥ ┬ ± confidence interval

June, July, August and December were warm and muggy. The others months from year 2002 were cold and muggy.

In experimental year 2003 were most of the months warm and droughty except February and October, that were drought and cold. At the other side the months January, May and July were warm and muggy. January and April of the year 2004 were cold and muggy. The part of the year 2004 (February, July, August, September and October) was warm and drought. May was cold and drought and March, June, December and November were warm and muggy.

Samples of amaranth plants were sampling in selected growth stages according to H r a d e c k á , B u r e š o v á (1994); the first four leaves (phase 24), intensive prolongation of the stem (phase 39), formation of the apex of main panicle (phase 40), full anthesis (phase 60), start of seeds formation (phases 61–68) and full ripeness of seeds (phase 90).

Spinach plants were sampled a weekly intervals from formation of 5 leaves up to anthesis. In 7 partial samplings were always done experimental years, four samples for each variety and species of amaranth.

The rutin identification from water-ethanol extracts, highly efficient liquid chromatography (HPLC) was chosen by isocratic elution manufactured by the company Waters^{TM} (prefabricated system – pump Waters^{TM} 600S, auto sampler Waters^{TM} 717 plus, detector PDA 996 – UV-VIS) for identification in UV and visible region. The method used for determination of rutin was according to F a u s t u s o v á (2000).

The content of total polyphenols such as gallic acid were determined in homogenised leaves calculated on dry matter (L a c h m a n et al., 1997).

The results obtained were statistically evaluated by analysis of variance of multiple classification on the significance level $\alpha = 0.01$. The presented statistical evaluation was processed using the computer program "Statistica CZ version 7".

RESULTS AND DISCUSSION

The content of total polyphenols and rutin was determined in leaves of amaranth and spinach during their ontogeny. Rutin was not confirmed in spinach leaves. Changes in the content of rutin in amaranth leaves during their ontogeny demonstrate the fact that the least rutin content was found in leaves at the end of vegetation where gradual limitation of metabolic activity of leaves and transport of assimilates into seeds occur. On the other side, the greatest amount of rutin is in the period of prolongation of stem and formation of the apex of main panicle. According to H a g e l s et al. (1995) and L a c h m a n et al. (2000c) the highest amount of rutin is in buckwheat in the time of anthesis. This conclusion was not confirmed in case of amaranths. The deduction of K a l i n o v á , D a d á k o v á

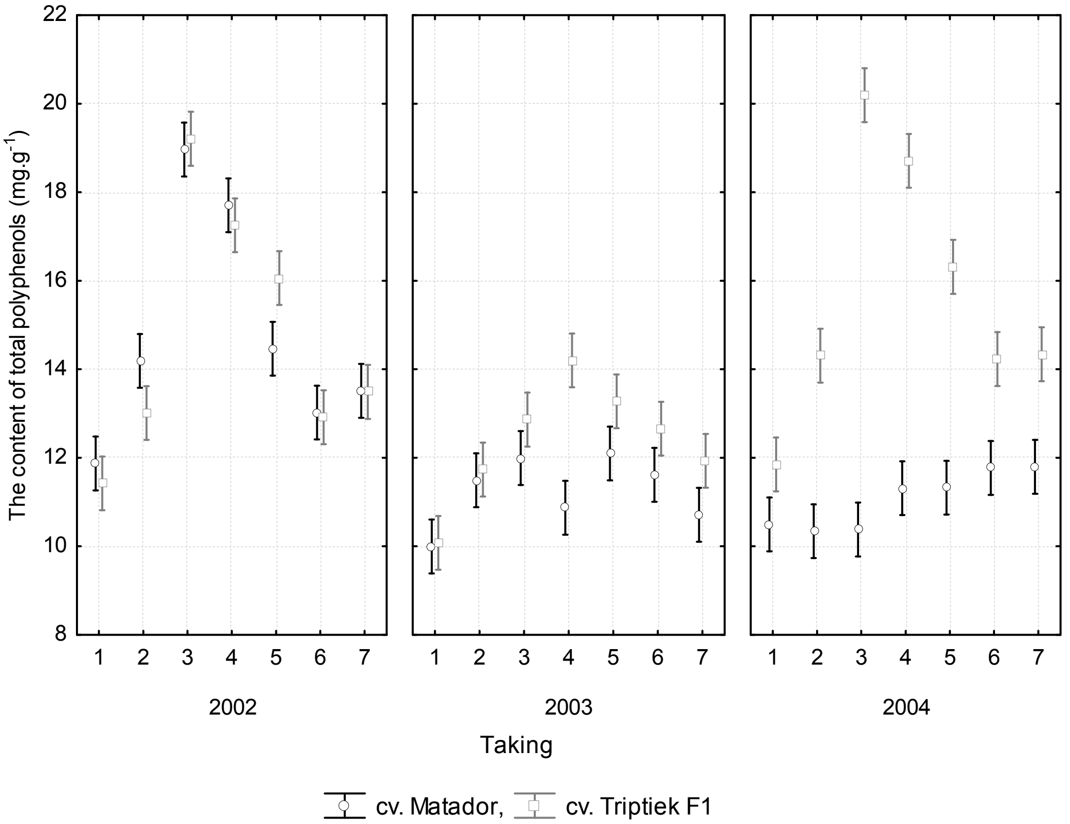

Fig. 2. The content of total polyphenols (mg.g^{-1} DM) in spinach leaves. Vertical columns denote 0.99 intervals of reliability. Average values are determined by the method of least squares

Legend: See Fig. 1

(2006) accounted, that the rutin content is highes at flover beginnig in the buckwheat. This idea was not validationed.

The differences in the rutin content between phases of ontogeny were statistically significant (Fig. 1). The average content of rutin was 159.11 mg.g^{-1} at the beginning of vegetation and 126.02 mg.g^{-1} at the end of vegetation. The highest average rutin content was measured in the phase of elongation of stem (294.10 mg.g^{-1}). Average content of rutin in amaranth leaves was in the time of anthesis 173.29 mg.g^{-1}. The rutin content in amaranth leaves was increasing up to the phases 30 and 39, then the fall was recorded. Red-spiked amaranth (*A. hypochondriacus*), in which further maximum of rutin content was obtained in the phase 60. L a c h m a n et al. (1998) e.g. present changes of rutin content in buckwheat in their study. The differences were found among phases of ontogeny K a l i n o v á et al. (2006), too. The congruent results were founded in amaranths.

Statistically significant differences in the values of rutin content in leaves were recorded among different species (Fig. 1). The highest average rutin content was demonstrated by *A. hypochondriacus* out of studied species (234.52 mg.g^{-1}) where the rutin content ranged in the interval of values from 98.75 mg.g^{-1} (phase 65) up to 375.52 mg.g^{-1} (phase 39). On the contrary, the lowest content was found in *A. cruentus* (154.92 mg.g^{-1}). In this species the rutin content was from 82.84 mg.g^{-1} (phase 60) up to 348.92 mg.g^{-1} (phase 30). K a l i n o v á, D a d á k o v á (2006) found the differences in the buckwheat's leaves among its cultivars. This conclusion were valited our results with amaranths. These results that the values are higher than for leaves of pagoda tree (61.76 mg.g^{-1}), tops of buckwheat (55.99 mg.g^{-1}) and fringed rue (9.68 mg.g^{-1}) (L a c h m a n et al., 2000a). After B i l b a o et al. (2007) citrus fruits contain 3.26 mg.g^{-1} of rutin. This amount of rutin is much lower than it was determined for amaranth leaves.

Š e v č í k et al. (2001) states that rutin content embody wide variability from 200 to 400 mg.g^{-1} in buckwheat achene. Presented values are higher than rutin content in the amaranth leaves. According to K r a f t et al. (2002) that rutin accumulation is cultivationed by drought weather, but experimental years were in the vegetation master warm, that why does not this conclusion validated neither controvert.

In addition, the content of total polyphenols was studied in the leaves of experimental plants. The content of total polyphenols in amaranth and spinach leaves was lowest in spinach in the period of 4 right leaves (sampling 1) – 10.38 mg.g^{-1}.

The differences in the content of total polyphenols in spinach leaves were statistically significantly influenced by ontogeny development of the studied plants (Fig. 2). Average content of total polyphenols at the beginning of vegetation of spinach was 10.95 mg.g^{-1} and the end of vegetation 12.63 mg.g^{-1}. The highest average content of

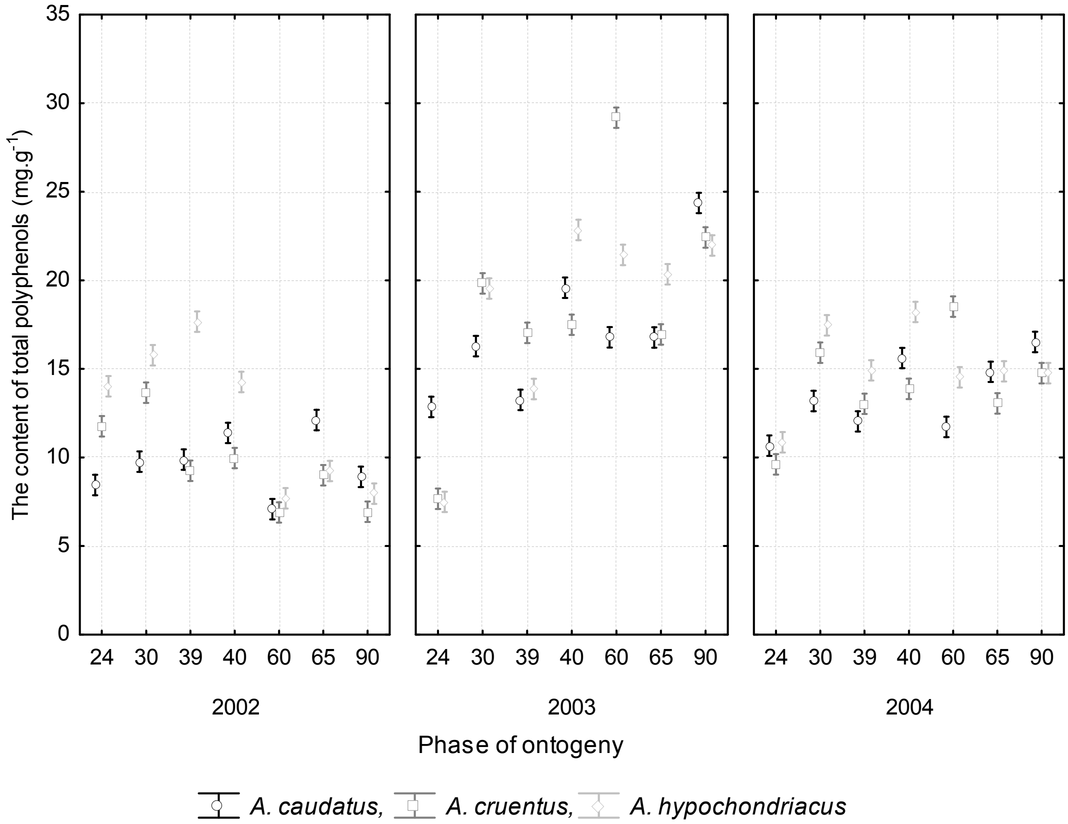

Fig. 3. The content of total polyphenols (mg.g^{-1} DM) in amaranth leaves. Vertical columns denote 0.99 intervals of reliability. Average values are determined by the method of least squares

Legend: See Fig. 1

Table 1. The effect of phase of development on the content of total polyphenols of amaranth leaves (mg.g^{-1} DM) calculated by the method of the least squares.

Year	Total polyphenols			Rutine		
	average content (mg.g^{-1})	average content – 99% deviation	average content + 99% deviation	average content (mg.g^{-1})	average content – 99% deviation	average content + 99% deviation
2002	10.51^{A}	10.41	10.61	157.79^{A}	155.33	160.26
2003	18.00^{B}	17.90	18.10	212.28^{A}	209.33	215.23
2004	14.24^{C}	14.14	14.34	199.77^{A}	197.31	202.24

^{A,B,C} – denoted differences are statistically significant on the level of significance α = 0.01

Table 2. The effect of phase of development on the content of total polyphenols of spinach leaves (mg.g^{-1} DM) calculated by the method of the least squares

Year	Total polyphenols		
	average content (mg.g^{-1})	average content – 99% deviation	average content + 99% deviation
2002	14.79^{A}	14.68	14.91
2003	11.87^{B}	11.75	11.99
2004	13.61^{C}	13.52	13.75

$^{A, B, C}$ – denoted differences are statistically significant on the level of significance α = 0.01

total polyphenols was found at 3rd sampling (formation of leaf rosette) – 15.60 mg.g^{-1}. The content of total polyphenols decreased to the value 12.63 mg.g^{-1} in the period of anthesis.

Statistically significant differences were recorded among studied spinach varieties (Fig. 2). The highest average content of total polyphenols had more modern variety Triptiek F1 (14.29 mg.g^{-1}) compared to the Matador variety (12.38 mg.g^{-1}). In the variety Triptiek F1 the content of polyphenols ranged between 11.12 mg.g^{-1} (sampling 1) and 17.42 mg.g^{-1} (sampling 3).

In the case of evaluation the content of total polyphenols in amaranth leaves can be statistically significantly higher than in spinach leaves. Average content of total polyphenols in spinach leaves was 13.33 mg.g^{-1} and in amaranth leaves it was 14.27 mg.g^{-1}.

Content of total polyphenols in amaranths leaves was save as in case of spinach variant in dependence on plant ontogeny (Fig. 3). Average content of studied substances was in amaranth in the phase 24 – 10.38 mg.g^{-1} and in the time of full ripeness it was 15.40 mg.g^{-1}. Compared to spinach leaves in the case of amaranth species, it is not possible to observe unambiguous tendency in the growth or drop in the content of polyphenols. The content of total polyphenols in all studied amaranth species showed identical tendency. The increase occurred always between two phases of development and then decrease polyphenols content. Compared to the remaining amaranth species, the content of polyphenols was increasing only in love-lies-bleeding (*A. caudatus*) from the onset of anthesis (11.87 $mg.g^{-1}$) to full ripeness (16.6.0 $mg.g^{-1}$). Despite of this, it is possible to mention that the highest average content of studied substances was found in the period of intensive elongation of stem (15.71 $mg.g^{-1}$) to formation of apex of main panicle (15.92 $mg.g^{-1}$). No significant differences were found among these growth stages.

Significant differences among studied amaranth species are probably given by their earliness when the earliest species is love-lies-bleeding (*A. caudatus*), and on the contrary, the late one is red-spiked amaranth (Fig. 3). The content of total polyphenols was the lowest in love-lies-bleeding (*A. caudatus*) (13.43 $mg.g^{-1}$) and the highest one in red-spiked amaranth (15.24 $mg.g^{-1}$).

Regarding the obtained results, it is evident that amaranth and spinach are the richest source of polyphenols than carrot, celery leaves, red rape (Lachman et al., 2000a) and pepper (Lachman et al., 2000a; Haque et al., 2006). According to Sellapan et al. (2006) cranberries and blackberries contain 0.0019 to 2.559 mg of polyphenolic substances per g^{-1} of fresh mass, then less than they comprise in leaves of spinach of amaranth.

On the other side, the content of total polyphenols in spinach and amaranth leaves is lower than that in onion and tomatoes, as presented Lachman et al. (2000b). Leaves of spinach and amaranth contain also less polyphenols than polyphenols content of citrus peels presented by Alicia et al. (2005) . The content of extracted polyphenols for *Citrus reticulata* was 76.00 $mg.g^{-1}$, *Citrus sinensis* 43.30 $mg.g^{-1}$ and 51.00 $mg.g^{-1}$ for *Citrus paradisi*.

The effect of the year is evident from Tables 1 and 2 as affected studied indicators in both plant species. It followed from statistical analysis that the content of total polyphenols and rutin in amaranth leaves had been significantly lowest in 2002 (10.51 $mg.g^{-1}$ and 157.79 $mg.g^{-1}$) and on the contrary, it was the highest in 2003 (18.00 $mg.g^{-1}$ and 259.90 $mg.g^{-1}$) – Table 1. On the contrary, the content of total polyphenols was the highest in spinach leaves in 2002 (14.79 $mg.g^{-1}$) and the lowest in 2003 (11.87 $mg.g^{-1}$) as presented in Table 2. It follows from the results obtained that warmer and drier years are much suitable for amaranth plants (2003), whereas spinach prefers warmer but moister years (2002). According to Pospisil et al. (2006) is yield formation of amaranth in dependence on the years. This conclusion was confirming in our experiment.

On the basis of our trials, it would be suitable to modify our eating habits and supplement of our food by "new" plant species that fulfil criteria of healthy nutrition. Except buckwheat that is highly valued for its rutin content, we can use amaranth leaves as a source of these substances. The content of rutin is higher in amaranth leaves than e.g. in buckwheat and rue. Compared to rue, amaranth leaves do not contain photo-sensible furanocoumarins. The content of total polyphenols in leaves of studied plants is comparable with other vegetable species. Spinach and amaranth leaves can be harvested from the beginning of anthesis. In the period of bud formation, anthesis and ripening of seeds, the share of dry matter is increasing, worse digestibility of leaves occur, and in case of amaranth leaves grow their bitter.

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Stanovení obsahu rutinu a celkových polyfenolů v listech špenátu a laskavce.

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V letech 2002 až 2004 byl v listech laskavce a špenátu stanovován metodou HPLC obsah rutinu a celkových polyfenolů. Do pokusu byly zařazeny tři druhy laskavce – laskavec ocasatý, laskavec krvavý, laskavec červenoklasý, a dvě odrůdy špenátu – Matador a Triptiek F1. Z výsledků je patrné, že průměrný obsah rutinu byl nejnižší na počátku vegetace – 159,11 $mg.g^{-1}$ a nejvyšší ve fázi prodlužování stonku – 294,10 $mg.g^{-1}$ ($F = 2233{,}89$, $p = 0{,}00$). Nejvyšší průměrný obsah rutinu měl laskavec červenoklasý (234,52 $mg.g^{-1}$) a naopak nejnižší laskavec krvavý – 154,92 $mg.g^{-1}$ ($F = 608{,}84$, $p = 0{,}00$). Rozdíl v obsahu celkových polyfenolů v listech špenátu byl statisticky průkazně ovlivněn fází vývoje ($F = 165{,}84$, $p = 0{,}00$), kdy nejnižší průměrný obsah byl na počátku vegetace špenátu – 10,95 $mg.g^{-1}$ a nejvyšší ve 3. odběru (vytvoření listové růžice) – 15,60 $mg.g^{-1}$. Statisticky průkazné diference byly nalezeny mezi sledovanými odrůdami špenátu ($F = 409{,}08$, $p = 0{,}00$), neboť nejvyšší průměrný obsah celkových polyfenolů měla odrůda Triptiek F1 (14,29 $mg.g^{-1}$) ve srovnání s odrůdou Matador (12,38 $mg.g^{-1}$). Průměrný obsah polyfenolů byl u laskavce ve fázi 24 – 10,38 $mg.g^{-1}$ a v období plné zralosti 15,40 $mg.g^{-1}$ ($F = 393{,}60$, $p = 0{,}00$). Obsah celkových polyfenolů byl nejnižší u laskavce ocasatého (13,43 $mg.g^{-1}$) a nejvyšší u laskavce červenoklaséo (15,24 $mg.g^{-1}$). Byl prokázán vliv ročníku na sledované charakteristiky. Z hlediska nutričního je vhodné sklízet listy laskavce a špenátu v období do počátku kvetení.

laskavec; *Amaranthus* sp.; špenát; *Spinacia* sp.; rutin; celkové polyfenoly; zelenina

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