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Antioxidant Vitamins in Barley Green Biomass

NATÁLIE BŘEZINOVÁ BELCREDI,*,† JAROSLAVA EHRENBERGEROVÁ,†
VLASTA FIEDLEROVÁ,§ SYLVIE BĚLÁKOVÁ,# AND KATEŘINA VACULOVÁ⊥

†Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic, §Food Research Institute Prague,
Radiová 7, 102 31 Praha 10, Czech Republic, #Research Institute of Brewing and Malting, PLC,
Malting Institute Brno, Mostecká 7, 614 00 Brno, Czech Republic, and ⊥Agrotest Fyto, Ltd,
Havlíčkova 2787/121, 767 01 Kroměříž, Czech Republic

Two malting hulled varieties (Sebastian, Malz) and one nonmalting hull-less variety (AF Lucius) were used to assess vitamins C and E in the green biomass of young plants of spring barley (*Hordeum vulgare* L.) in three stages of growth and development (BBCH 29, 31, 32−33). The samples from sampling I (BBCH 29) had statistically significantly higher vitamin C content and vitamin E activity compared to sampling I (BBCH 31). The highest average vitamin content was determined in the malting variety Sebastian (vitamin C, 520 mg 100 g^{-1} DM; vitamin E, 73.06 mg kg^{-1} DM) compared to the varieties Malz (501 mg 100 g^{-1} DM; 61.84 mg kg^{-1} DM) and AF Lucius (508 mg 100 g^{-1} DM; 67.81 mg 100 g^{-1} DM). The locality Kroměříž (Czech Republic, CR), with vitamin C and E contents of 524 mg 100 g^{-1} DM and 68.74 mg kg^{-1} DM, respectively, proved to be more suitable for growing green biomass compared to the locality Žabčice (CR) (content of vitamins C and E, 477 mg 100 g^{-1} DM and 66.39 mg kg^{-1} DM, respectively). During the research period (2005−2007), it was determined that the green mass of young plants of spring barley was a significant source of vitamins C and E in the growth stage BBCH 29; in later samplings (BBCH 32−33) the vitamin levels dropped (by as much as 48%). These vitamins are important antioxidants for human health. Therefore, “green barley” can be recommended for the preparation of natural dietary supplements and is preferred to synthetic vitamin preparations.

KEYWORDS: Green biomass; antioxidants; vitamin E; tocopherols; vitamin C; ascorbic acid; hull-less barley; spring barley

INTRODUCTION

Antioxidants have become one of the most important concerns in human nutrition because of high concentration of free radicals, both in food and after food ingestion (*1*). An imbalance between oxidants and antioxidants in favor of oxidants, potentially leading to damage, is commonly referred to as oxidative stress (*2*, *3*). Protection against oxidative damage of organisms caused by active oxygen forms is ensured by a number of antioxidant defense systems localized in various cellular structures. As oxidative stress is known to underlie many human diseases (atherosclerosis, diabetes, cancer, cardiovascular diseases, Crohn’s disease, etc.), antioxidants scavenging free radicals have been studied extensively (*2*, *4*−*7*). This free radical scavenger system can be split into enzymatic and nonenzymatic antioxidants. Superoxide dismutase, catalase, and gluthatione peroxidase are three major classes of antioxidant enzymes. Scavenging enzymes are localized mainly intracellularly, protecting thus cells. The nonenzymatic antioxidant defense system includes ascorbic acid (vitamin C), vitamin E (tocopherols and tocotrienols), glutathione, β-carotene, and vitamin A. There is a balance between both the activities and the intracellular levels of these antioxidants that is essential for the survival of organisms and their health (*2*, *7*, *8*).

Numerous studies on natural sources of phytochemicals with antioxidant capacity, such as fruits and vegetables and also field crops, have been published (*9*−*16*).

Isomers of vitamin E react readily with iron ions, lipid hydroperoxides, ozone, air oxygen, and other oxidizers. Sensitivity to oxidation and biological activity declines together with a declining number of methyl groups on the chroman ring, whereas, on the contrary, the antioxidant effect increases. Reaction of vitamin E with free radical produces tocopheroxyl radical. One tocopherol molecule can react with two hydropheroxyl radicals. This reaction deactivates vitamin E, and it loses its antioxidant activity. Tocopheroxyl radical may act as prooxidant under certain conditions and damage further biomolecules. As the number of vitamin E molecules in the lipoprotein part is limited, it is crucial to secure a reverse reduction from tocopheroxyl radical, that is, regeneration of vitamin E to its original chemical structure with an active hydroxyl group on the aromatic core. For this purpose ascorbic acid is used and ascorbyl radical is formed. A chain reaction is regarded here, and a disorder conditioned by the dominance of one component can lead to the prooxidation effect. A possible undesirable prooxidation effect was also observed in higher doses of vitamins E and C (*17*−*19*).

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*Corresponding author (phone +420 545 133 134; fax +420 545 13 3302; e-mail belcr@centrum.cz).

The reaction of vitamin E with free radicals can slow lipid oxidation. One tocopherol molecule can protect about 10^3-10^8 polyunsaturated fatty acid molecules at low peroxide value (*18*).

Vitamin C (ascorbic acid) participates in significant hydroxyl reactions occurring in the human organism and in some other animals. It is also involved in the biosynthesis of mucopolysaccharides and prostaglandins, the absorption of iron ions, and their transport. Its antioxidant properties lie in reactions with active oxygen forms and free radicals. It also inhibits nitrosamine creation, acting thus as a mutagenesis and carcinogenesis modulator (*19*). In addition, ascorbic acid, an essential vitamin present in many fruits and vegetables, plays an important role in protecting plants from oxidative stress (*20*). In humans it has been associated with the prevention of chronic diseases (*10*).

The green barley biomass is considered to be a source of the above-mentioned antioxidants (*21*, *22*). Yu et al. (*23*) studied the effects of supplementation of young barley leaf extract with antioxidant vitamins C and E on the susceptibility of different low-density lipoprotein subfractions to oxidation in patients with type 2 diabetes. Tsai et al. (*24*) concluded that ingestion of 3−5% of a young barley leaf extract in the diet could decrease serum lipids and might reduce risk factors for atherosclerosis in hamsters.

Antioxidants contained in a balanced diet are biologically better accessible for the organism than those in synthetically produced preparations (*25*).

The aim of this study was to find out whether the green mass of young plants of barley varieties grown under field conditions contained bioactive substances with antioxidant properties and whether it was suitable for food supplements. In addition, we wanted to determine the effect of the genotype, plant development, year, and growing locality on the activity or content of these substances.

MATERIALS AND METHODS

Material. Two varieties of hulled two-rowed spring barley, Sebastian and Malz (registered in the European Union), and the hull-less variety AF Lucius (registered in 2009) were used as plant material. In 2005−2007, seeds were sown in plots in a field experiment with a randomized block design in three replications in two localities: Žabčice (university farm of Mendel University in Brno (MENDELU)) and Agrotest Kroměříž, Ltd., with restricted chemical input, that is, without pesticides and fertilizers.

Three samplings of green mass in the defined growth stages according to the macrophenological scale were carried out (*26*). The first sampling (I) was performed in growth stage BBCH 29, end of tillering−beginning of leaf sheath prolonging; the second sampling (II) was carried out in BBCH 31, the first node touchable above the soil surface; and the third sampling (III) in BBCH 32−33, the second node touchable. Sampling III was carried out only in 2005, and it did not prove to be useful due to a rapid decline in phytochemical concentration and higher portion of fiber in the biomass. To avoid soil contamination, harvest was carried out manually (plants with leaves and stalk height of 12−15 cm). Immediately after harvest, the collected green mass of barley was placed into special spacious and air-permeable microfilm packages suitable for delicate transport. The samples were then transported for chemical analyses in cooling boxes to laboratories.

Description of the Experimental Localities. The MENDELU station in Žabčice is situated in the maize production area of the Czech Republic (CR), at an altitude of 184 m above sea level. Soil type at the station is classified as fluvi-eutric gleysol, a heavy (clay) to midheavy (clay-loam) soil. The climate in this area is warm, with a mean temperature of 9.3 °C. The locality is moderately dry, with a mean rainfall of 450−550 mm per year.

Agrotest Fyto, Ltd., is situated in the sugar beet production area of the CR, at an altitude of 235 m above sea level. The soil type here is classified as haplic chernozem with a medium loamy soil. The climate in this area is moderately warm, with a mean temperature of 8.7 °C, and moderately dry, with a mean rainfall of 599 mm per year.

Methods of Determination. *Vitamin C.* The reversed-phase HPLC method with UV detection was used to determine the ascorbic acid content. Samples were extracted with 3% metaphosphoric acid, homogenized in an IKA A11 grinder, and filtered. After dilution with extract agent and membrane filtration (0.45 μm), the supernatant was loaded onto the column. Chromatographical conditions were as follows: column, Lichrospher 100 RP-18 (250 × 4 mm, 5 μm) with a precolumn; mobile phase, 4% MeOH; pH 3; flow rate, 0.6 mL/min; column temperature, 35 °C; injection volume, 20 μL; detection, UV (251 nm). Evaluation was carried out with the external standard method.

Vitamin E. Determination of vitamin E activity and levels of total tocols and tocopherols (T) and tocotrienols (T3) was based on saponification and extraction of the nonsaponified portion with diethyl ether and subsequent determination using the HPLC method with fluorescent detection. Vitamin E was measured according to the European Norm EN 12822:2000 (*27*).

The sample of green mass was shock-frozen in liquid nitrogen and crushed. Two grams was immediately used for the determination of vitamin E. Long-term freezing would reduce vitamin E activity. Experimental conditions of chromatographic analysis were as follows: HPLC SpectraSystem (Thermo Separation Products, Inc., USA); pump, P2000; fluorescence detector, FL 3000; stationary phase, Nucleosil 120-5 C 18; column dimensions, 250 × 4 mm; solvent, methanol; flow, 1.0 mL/min; sample volume, 20 μL; detection, fluorometric; wavelength, $\lambda_{EX} = 290$ nm, $\lambda_{EM} = 330$ nm. A detailed description of the analysis has been given in the study of Ehrenbergerová et al. (*14*).

Vitamin E is given in milligrams of α-tocopherol equivalent, which represents the sum of the individual tocopherols and tocotrienols with respect to their biological activity (calculation after McLaughlin and Weihrauch (*28*)).

Vitamin C and E values determined in fresh mass were recalculated to dry matter (DM).

Statistical Analysis. Chemical data from vitamin C and E analyses were evaluated by a four-factor analysis of variance ANOVA using the program Statistica 7.0 (StatSoft, Inc., Tulsa, OK). The multiple-range test of mean values (LSD test, $P = 0.05$) was used as the post hoc procedure when the *F* test from ANOVA indicated significant differences among cultivars, samplings, localities, and years.

RESULTS AND DISCUSSION

Statistical analyses (**Table 1**) of main effects, barley cultivars, samplings, years, and two-way interactions (cultivars × years, samplings × years, localities × years) indicated significant differences ($P = 0.05$) in vitamin E activity. Significant effects of samplings, localities, years, and two-way interactions (cultivars × samplings, cultivars × years, localities × years) were identified for vitamin C (**Table 1**). The other effects were not statistically significant for the presence of both the vitamins in barley green biomass.

Cultivars. In the present study we determined that barley green biomass contained only the following isomers of vitamin E: α-, β+γ-, and δ-tocopherols; it did not contain tocotrienols (**Table 2**). This finding supported the study of Falk et al. (*12*), who stated that α-tocopherol was the major vitamin E form in nearly all green plant tissues. The tocopherols accumulate in that part of the grain which survives and builds up a new plant, whereas the tocotrienols are located in the endosperm and pericarp, which finally die before and during germination, respectively. It will be important to identify the exact localization of the tocotrienols in the cells of the pericarp and endosperm to better understand what makes the tocotrienols special (*12*). Cereal grain contains tocopherols and tocotrienols (*14*, *29*−*34*). Tocotrienols have been found also in vegetables, fruits, or oils (*17*, *33*−*36*).

The variety Sebastian (**Table 2**) had the highest average content of α-T (71.15 mg kg^{-1} DM). α-T contributes to vitamin E activity and total tocol content significantly (83%); therefore, the variety

Table 1. Mean Squares from Analysis of Variance for Vitamins E and C[a]

	vitamin E					vitamin C	
	df	$MS_{\alpha\text{-T}}$	$MS_{\beta+\gamma\text{-T}}$	$MS_{\delta\text{-T}}$	$MS_{\text{vitamin E}}$	df	MS
cultivars	2	1577**	105***	7.71**	1512**	2	3120
samplings I, II	1	5954***	89.6***	25.5***	6972***	1	200623***
sites	1	78.5	2.1	3.8	199	1	100078***
years	2	8912***	62.1***	5.1*	8076***	1	226282***
interactions							
cultivars × samplings	2	76.1	3.32	2.5	85.4	2	22424***
cultivars × sites	2	420	2.32	8.2**	426	2	3923
samplings × sites	1	188	103***	4.8	410	1	536
cultivars × years	4	611*	6.61	4.1*	622*	2	14103**
samplings × years	2	3372***	229***	20.2***	3141***	1	3165
sites × years	2	1284**	192***	3.8	1281**	1	46157***
error	124	231	5.75	1.6	240	81	2741

[a] *, $P = 0.05$; **, $P = 0.01$; ***, $P = 0.001$.

Table 2. Mean Contents of Vitamins C and E in Varieties/Line, Samplings, and Locations in 2005−2007[a]

	mg kg^{-1} DM					mg 100 g^{-1} DM
	α-T	β+γ-T	δ-T	total tocopherols	vitamin E	vitamin C
Malz	59.69a	11.60b	1.74a	73.02a	61.84a	501a
AF Lucius	65.63ab	12.67c	2.42b	80.72b	67.81ab	508a
Sebastian	71.15b	9.74a	1.72a	82.61b	73.06b	520a
sampling I (BBCH 29)	71.92b	12.12b	1.54a	85.58b	74.53b	555b
sampling II (BBCH 31)	59.06a	10.55a	2.38b	71.99a	60.61a	464a
2005	73.07b	12.64b	1.91ab	87.63b	74.92b	not valid
2006	73.63b	10.57a	2.31b	86.51b	75.19b	558b
2007	49.76a	10.79a	1.66a	62.21a	52.59a	461a
Žabčice	64.75a	11.21a	2.12a	78.09a	66.39a	477a
Kroměříž	66.23a	11.46a	1.80a	79.48a	68.74a	542b

[a] Values within each cultivar, growth stage, location, and year followed by the same letter are not significantly different ($P = 0.05$).

Sebastian also had the highest activity of vitamin E and total T content. This variety was not statistically different from the variety AF Lucius in this measurement. The variety Sebastian contained statistically significantly lower content of minority β+γ-T (9.74 mg kg^{-1} DM) compared to both of the other the cultivars (**Table 2**). The lowest content of α-T (59.69 mg kg^{-1}DM), and thus total T and vitamin E activity (73.02 and 61.84 mg kg^{-1} DM), was determined in the variety Malz (**Table 2**). Lower α-T content was found also in potatoes (0.5−2.8 mg kg^{-1}) (*13*).

The level of α-T in tomatoes varied from December (5.4 mg kg^{-1}) to June (130 mg kg^{-1}) in a study by Raffo et al. (*16*). A much higher content of total tocols was detected in hazelnuts (269 mg kg^{-1}) (*15*) compared to barley biomass.

Classification based on vitamin C content listed the varieties in a similar order (**Table 2**). The highest average content was determined in the variety Sebastian (520 mg 100 g^{-1} DM) and the lowest in the varieties AF Lucius (508 mg 100 g^{-1} DM) and Malz (501 mg 100 g^{-1} DM). However, the varieties did not differ statistically significantly in values of vitamin C. Average vitamin C content of the whole set was 500 mg 100 g^{-1} DM. Comparable contents of vitamin C were detected in cabbage (*33*), tomatoes (*16*), and fresh spinach (*37*). Leaves and roots of rocket salad (*38*), peel (116−228 mg kg^{-1}) and flesh (28 − 53 mg kg^{-1}) of six pear cultivars (*20*), and germinated cowpea seeds (15−17 mg 100 g^{-1} DM) (*39*) contained less vitamin C (87−181 mg 100 g^{-1}). Our results of barley green biomass were several times higher (**Table 2**) than the content of vitamin C in garden fresh peas (25.6−30.9 mg 0.100 g^{-1}), broccoli (77.1−93.1 mg 0.100 g^{-1}), green beans (11.8−15.1 mg 0.100 g^{-1}), carrots (3.7−4.4 mg 0.100 g^{-1}) (*9*), or hazelnuts (24.5 mg kg^{-1}) (*15*). During the 3 months of storage (freezing) of the grown green barley mass, a 42% loss of vitamin C compared to the original vitamin C content in the fresh sample occurred. Favell et al. (*9*) gave a similar average (30%) of vitamin C losses by freezing depending on the vegetable.

Table 3. Declining Vitamin E Activity and Vitamin C Content with Plant Development in 2005

	vitamin E (mg kg^{-1} DM)			vitamin C (mg 100 g^{-1} DM)		
variety	sampling I	sampling II	sampling III	sampling I	sampling II	sampling III
Malz	93.10	41.61	55.52	567	309	272
AF Lucius	102.39	55.72	61.72	495	393	165
Sebastian	117.90	58.91	53.67	538	364	241

Samplings. In terms of sampling time, all of the studied vitamins on the average of all factors were significantly higher in sampling I, that is, in plants less developed and thus probably less resistant to environment-induced stresses, compared to sampling II (**Table 2**). The only exception was isomer δ-T of vitamin E. Its content was statistically significantly higher only in sampling II (2.83 mg kg^{-1}DM) compared to sampling I (1.54 mg kg^{-1} DM). Falk et al. (*12*) proved increased γ-tocopherol content compared to α-tocopherol during tobacco leaf aging. They explained that the enzyme γ-tocopherol-methyltransferase (γ-TMT) declined with senescence. γ-TMT catalyzed the final conversion of γ-tocopherol to α-tocopherol. In this experiment, α-tocopherol declined significantly during the higher growth stage (**Table 2**).

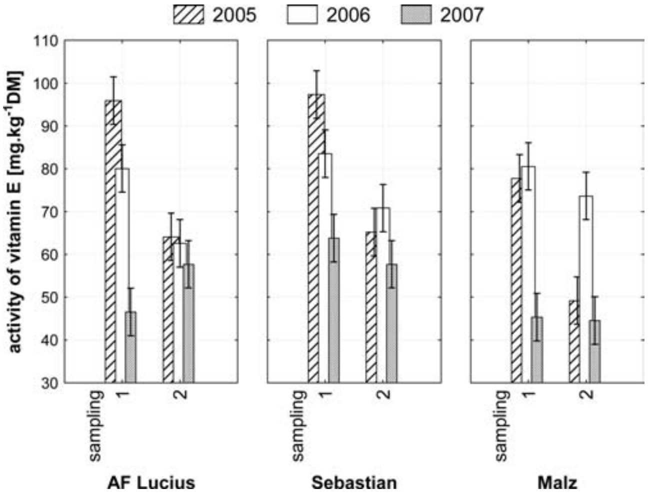

Figure 1. Average vitamin E activity in varieties in the individual green biomass samplings and in the studied years.

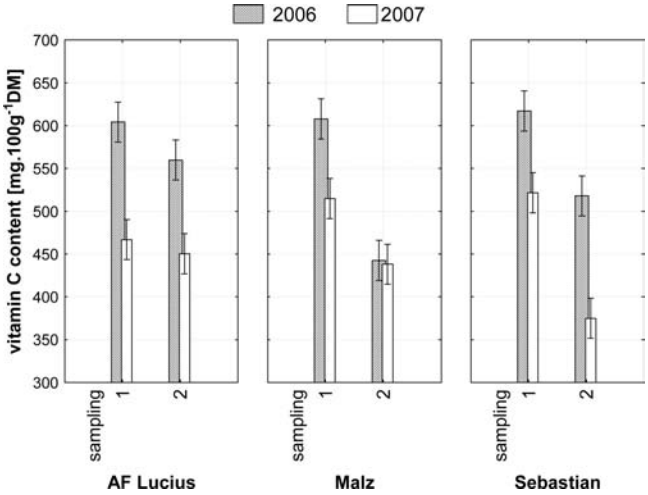

Figure 2. Average vitamin C content in the individual samplings of green biomass in 2006−2007.

The decline in the sum of β+γ-T was not so considerable. A marked decline in vitamin E activity was recorded in sampling III (**Table 3**), on average by 45% versus sampling I. The highest decline in the activity of vitamin E was recorded in the variety Sebastian (by 55%); the smallest decline was determined in the variety Malz (by 41%) and in the variety AF Lucius by 40%. Green biomass in the third sampling already contained α-tocotrienol (26.19 mg kg^{-1} DM).

Average vitamin C content (**Table 2**) in the varieties was statistically significantly higher in sampling I (555 mg 100 g^{-1} DM) compared to sampling II (464 mg 100 g^{-1} DM). Results of vitamin C from both localities (**Figure 1**) showed the highest values in the varieties Malz and Sebastian in sampling I. Lisiewska et al. (*40*) also reported the content of vitamin C decreased with plant growth in all parts of dill plants.

With further growth and plant development according to the decadic scale (BBCH), the content of vitamin C declined in all of the varieties (**Table 3**). The highest decline was recorded in sampling III (BBCH 32−33), when the values in both varieties were by > 50% lower versus those of sampling I (**Table 3**). The decline in vitamin C content was highest in the variety AF Lucius (77%). For this reason mass was not taken in sampling III in 2006 and 2007. In sampling III high declines not only in the content of vitamins C and E but also in other substances were recorded (*22*).

Years. In 2007 (**Table 2**), on the average of varieties, statistically significantly lower contents of α-T (49.76 mg kg^{-1} DM) and total T (62.21 mg kg^{-1} DM) and activities of vitamin E (52.59 mg kg^{-1} DM) and vitamin C (461 mg 100 g^{-1} DM) versus 2005 and 2006 were determined. The presence of β+γ-T was significantly higher in 2005 compared to 2006 and 2007. In 2007 the content of δ-T did not differ statistically significantly from that in 2005; however, in 2006 it was significantly higher compared to 2007.

The vitamin C results from sampling I (2005) were not included in this study. This vitamin was very unstable, and even drooping and creasing of the biomass led to its fast enzymatic oxidation. Our results correspond to those of Kalt (*41*), who ranked vitamin C, compared to other antioxidants, for example, carotenoids and phenolics, as the most labile substance and assumed its rapid decline during processing and storage.

Statistically significantly higher average vitamin E activity (**Figure 1**) and total T content were achieved by the varieties Sebastian and AF Lucius (97.33 and 111.88 mg kg^{-1} DM and 95.89 and 113.23 mg kg^{-1} DM, respectively) in sampling I in 2005 versus the other results determined. A significantly higher content of vitamin C (**Figure 2**) was found in sampling I in 2006 in all of the studied varieties (604−617 mg 100 g^{-1} DM) compared to average contents of both samplings in 2007 and sampling II in 2006. These results correspond to the statistically significant interactions determined for samplings × years (**Table 1**).

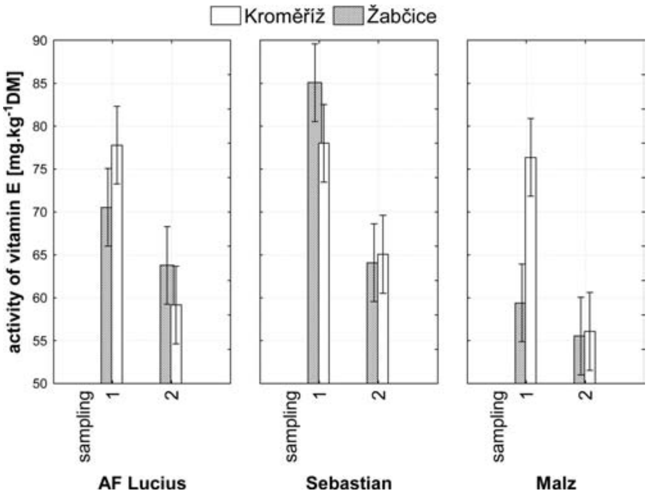

Figure 3. Average values of vitamin E activity in varieties according to samplings in the localities (as an average of years).

Localities. Samples from two localities did not differ statistically significantly in average content of isomers, total tocopherols, and vitamin E activity (**Table 2**; **Figure 3**). Higher average values were determined in Kroměříž with the exception of isomer δ-T. This small difference between the localities can be explained by the fact that the content of vitamin E was more associated with a genotype and year (see interactions of cultivars × years in **Table 1**). Higher values were obtained in a colder and wetter year. Vitamin E activity and content of its isomers were not affected significantly by the localities but by the other factors given in **Table 1**. Our data correspond to those of Nishiba et al. (*35*) and Britz et al. (*42*). They found that the effects of a locality or a sampling date were small compared to those of a genetic line. According to Ehrenbergerová et al. (*14*), tocol content did not differ statistically significantly in various chemical treatments either. The highest value of vitamin E activity on the average of years and samplings was determined in the variety Sebastian in the Žabčice locality (74.57 mg kg^{-1} DM) in biomass sampling I.

However, according to the analysis of variance (**Table 1**), vitamin C content was statistically highly significantly influenced by the locality (unlike vitamin E activity). Its content (**Table 2**; **Figure 4**) was statistically significantly higher in all varieties, on the average of samplings and years, in the locality of Kroměříž versus Žabčice. These results correspond to statistically significant interactions of cultivars × samplings, cultivars × years, and localities × years (**Table 1**). Lachman et al. (*43*) stated that high-altitude potato-growing areas showed an apparent tendency to higher total polyphenol contents in the analyzed varieties. Higher areas in the CR represent the areas with lower average temperature and higher precipitation volume. These results correspond to a higher finding of vitamins C and E under the wetter and colder conditions of the locality Kroměříž in 2006 (**Table 4**). Statistically significantly higher vitamin C content in the locality Kroměříž in 2006 could be caused by a higher amount of precipitation in the period of biomass formation (266 mm, vs 171 mm in Žabčice). This is also documented by the calculated hydrothermic coefficient (HTC) according to Seljaninov (*44*), which was 2.73 for the locality Kroměříž (**Table 4**); this indicates excess moisture, compared to the value of only 1.62 in the locality Žabčice, that is, sufficiency of moisture. Comparison of temperatures in the period of biomass formation did not show any substantial differences in the localities, but the sum of effective temperatures (SET) in Kroměříž was lower than in Žabčice (**Table 4**). Similarly, it is also possible to explain significantly higher vitamin E content in years with higher precipitations, that is, 2005 and 2006, in both the localities in the period of biomass formation compared to 2007 when hydrothermic coefficients in both localities characterized this period as dry (**Table 4**).

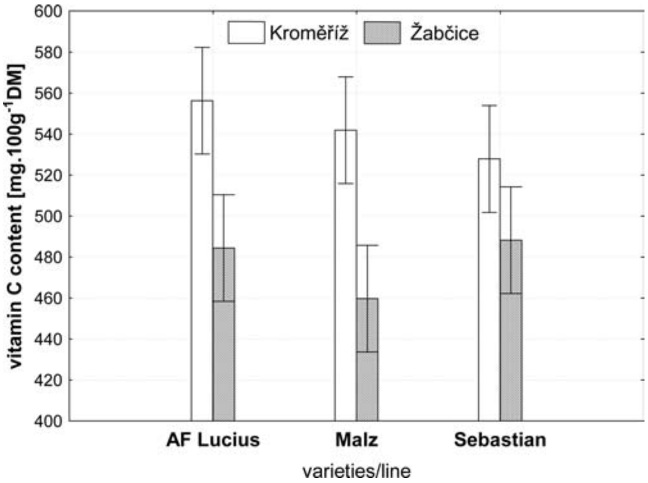

Figure 4. Average values of vitamin C content according to locality (as an average of samplings and years).

The highest content of vitamin C (**Figure 4**) in the locality Kroměříž was achieved by the variety AF Lucius (556 mg 100 g^{-1} DM).

Conclusion. Our results indicate that green biomass of spring barley is a good source of antioxidant vitamins C and E. In the first two growth stages of the macrophenological scale BBCH 29 (i.e., end of tillering, sampling I) and BBCH 31 (the first node touchable above the soil surface, sampling II), the green biomass contained only tocopherols. Tocotrienols were detected only in growth stage BBCH 32–33 (the second node touchable, sampling III). Sampling III, however, exhibited statistically significantly lower activity of vitamin E, total tocopherols, and vitamin C contents, and it was more demanding for further processing (higher content of fiber); therefore, it was abandoned in subsequent years. The highest contents of both vitamins were at the end of tillering in sampling I. Factors (years and samplings) and interactions of localities and years were significant sources of vitamins C and E variability.

The highest contents of ascorbic acid and total tocols were determined in the malting variety Sebastian. The localities did not statistically significantly affect vitamin E activity and content of individual isomers, unlike vitamin C. Significantly higher vitamin C content was determined in the locality Kroměříž compared to Žabčice. In addition, statistically significant effects of years on content of vitamin C, activity of vitamin E, and content of its isomers were determined.

We can summarize that the growing and harvesting methods were appropriate for preserving both antioxidant vitamins in young green biomass. This study indicates that the varieties Sebastian and AF Lucius harvested in BBCH 29 (sampling I) showed the greatest abundance of both vitamins. The localities and growing years also affected contents of vitamins C and E.

Table 4. Weather Conditions in the Growing Localities[a]

		av temp (°C)		sum of precipitation (mm)		hydrothermic coeff (HTC)	
		Kroměříž	Žabčice	Kroměříž	Žabčice	Kroměříž	Žabčice
2005	March	2.1	2.6	13.5	5.8	1.58	1.49
	April	10.5	11.0	66.3	49.5		
	May	14.7	15.0	53.6	66.8		
		$\bar{x}$ = 9.1	$\bar{x}$ = 9.5	Σ133.4	Σ122.1	sufficiency of water	sufficiency of water
		SET = 776	SET = 790				
2006	March	1.5	1.9	61.6	46.2	2.73	1.62
	April	10.6	11.1	89.7	50.5		
	May	14.4	14.7	115.3	75.3		
		$\bar{x}$ = 8.8	$\bar{x}$ = 9.2	Σ266.6	Σ172	excess of water	sufficiency of water
		SET = 750	SET = 779				
2007	March	6.7	7.1	73.0	80.8	0.70	0.33
	April	11.6	12.2	3.7	4.4		
	May	16.0	16.7	54.8	24.8		
		$\bar{x}$ = 11.4	$\bar{x}$ = 12.0	Σ131.5	Σ110.0	lack of water	drought
		SET = 840	SET = 882				

[a] $\bar{x}$, average temperature for the studied period March–May in the given year. Σ, sum of precipitation for the studied period March–May in the given year. SET, sum of effective temperatures (>5 °C) for the studied period April–May in the given year (in the vegetative period). HTC, Seljaninov's hydrothermic coefficient.

ABBREVIATIONS USED

BBCH, Biologische Bundesanstalt Bundssortenamt and Chemical Industry; MENDELU, Mendel University in Brno, Czech Republic; HTC, hydrothermic coefficient; SET, sum of effective temperatures; γ-TMT, γ-tocopherol-methyltransferase.

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