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Seasonal variation of ascorbic acid and nitrate levels in selected basil (*Ocimum basilicum* L.) varieties

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Abstract

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The aim of this study was to determine the levels of ascorbic acid and total nitrates in the fresh aerial parts of different varieties of basil (*Ocimum basilicum* L.) in different growing seasons. The ascorbate-nitrate index (I_{AN}) is used to determine the safety of basil for consumers. Seven basil varieties ('Ohře', 'Lettuce Leaf', 'Purple Opaal', 'Dark Green', 'Mammolo Genovese', 'Mánes' and 'Red Rubin') were cultivated as pot cultures in greenhouses during the period 2015–2016. High-performance liquid chromatography (HPLC) was used to determine the ascorbic acid content, which ranged from 34.3 to 222.0 mg/kg f. w. An ion-selective electrode was employed to determine the content of nitrates, which ranged from 237.7 to 4280.6 mg/kg f. w. The ascorbate-nitrate index reached values of between 0.016 and 0.629, which are risky-to-moderately safe ascorbate-nitrate ratios. However, the occasional use of small amounts of basil as a culinary herb is not risky for consumers. Furthermore, significant differences in the content of ascorbic acid and nitrates were found among the cultivated samples with respect to variety, year and growing season.

Keywords: culinary herbs; greenhouse production; pot culture; ascorbate-nitrate index

Basil (*Ocimum basilicum* L.) is one of the most frequently used culinary and raw materials. It contains a significant quantity of biological compounds with strong curative properties. Basil contains essential oil of varying chemical composition, chlorophyll, vitamin C, carotenoids and a wide range of phenol compounds with various antioxidant activities, depending on the basil species and variety (Politeo et al. 2007; Nurzynska-Wierdak et al. 2011). The herb is used not only for cooking but also in commercial fragrances, flavourings and for increasing the shelf life of food products (Suppakul et al. 2003). Basil is widely used in traditional medicine as a digestive tonic and for curing ailments such as warts, inflammations, colds and headaches (Chanwitheesuk et al. 2005). Basil extract has known sedative and anticonvulsant properties (Freire et al. 2006) as well as antimicrobial and antifungal activities (Holm 1999; Suppakul et al. 2003).

Ascorbic acid is among the most important and frequently monitored constituents in culinary herbs. Ascorbic acid is a very effective antioxidant (Harris 1996). According to the World Health

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Organization, 45–50 mg of vitamin C should be ingested daily (WHO and FAO 2004). To season food, normally only one spoon (about 2.5 grams) of chopped basil leaves is used. According to the USDA (2016), five leaves of fresh basil contain 0.4 mg of ascorbic acid. Favell (1998) showed that ascorbic acid can serve as a sensitive marker for determining nutritional quality. Ascorbic acid is by far the least stable nutrient during processing. It is highly sensitive to oxidation and leaching into water-soluble media during processing, storage and cooking (Franke et al. 2004). Basil is characterised by significant variability over a broad range of its constituents. There are several factors which determine the content of ascorbic acid, among them external factors (climatic and growing conditions, fertilisation, agronomic methods) as well as internal factors (genotype, development stage) (Dzida, 2011; Nurzynska-Wierdak et al. 2011; Saadatian et al. 2014; Fraszczak et al. 2015).

Basil contains not only health-promoting compounds, but also substances that may be toxic in excessive-amount. Among these dangerous substances are the nitrates. Nitrates are not usually dangerous for adults because they are relatively quickly excreted in urine. The potential danger stems from the conversion of nitrites or nitrates to nitrite. The nitrite is absorbed into blood and can cause methemoglobinemia. The European Commission (1997) established maximum permissible levels of nitrates ranging from 3,500 to 4,500 mg $N\text{-}NO_3^-$/kg f. w. for the winter season and 2,500 mg of $N\text{-}NO_3^-$/kg for the summer crops. Accumulation of nitrates is higher in the absence of light radiation (Buwalda, Warmenhoven 1999). Therefore, most greenhouse crops have higher nitrate levels compared to those grown in the field (Gruda 2005). The factors responsible for nitrate accumulation in plants are mainly nutritional, environmental and physiological. Nitrogen fertilisation (Dzida 2011; Nurzynska-Wierdak et al. 2011) and light intensity (Urbonavičiute et al. 2008) have been identified as the major factors that influence the nitrate content.

The ratio of ascorbic acid to nitrate content, the so-called ascorbate nitrate index (I_{AN}), is a parameter that can be used to evaluate the nutritional quality and safety of horticultural products. Horticultural products with a higher content of ascorbic acid compared to nitrate are safer and more suitable for human nutrition (Pokluda 2005). Lachman et al. (1997) categorised vegetables according to this index into three groups: risk species with values below 0.5, neutral species with values ranging from 0.5 to 1.0 and positively beneficial species with values over 1.0.

The aim of this research was to determine the levels of ascorbic acid and total nitrates in different varieties of basil in different growing seasons. The I_{AN} was calculated to evaluate the safety of the basil for human diets.

MATERIAL AND METHODS

Plant material and cultivation conditions. The control variety 'Ohře' produced by Seva Moravia (Czech Republic) and six varieties ('Lettuce Leaf', 'Purple Opaal', 'Dark Green', 'Mammolo Genovese', 'Mánes' and 'Red Rubin') produced by Semo Smržice Ltd. (Czech Republic) were used in this experiment (Fig. 1).

Fig. 1. Varieties of basil just before harvest from left to right: 'Ohře', 'Lettuce Leaf', 'Purple Opaal', 'Dark Green', 'Mammolo Genovese', 'Mánes', 'Red Rubin'

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Table 1. Cultivation details for basil plants

Time of sowing and harvesting	2015	2016
Pot culture 1	23.3.–9.4.	8.2.–25.4.
Pot culture 2	27.4.–14.7.	28.4.–20.6.
Pot culture 3	1.9.–26.10.	15.8.–17.10.

The plants were grown in a greenhouse of Mendel University in Brno, Faculty of Horticulture in Lednice, as pot plants during the period 2014 to 2016 (Table 1). Greenhouse experiments were performed in four replicates for each variety with 30 plants for each variant.

Basil was grown in 0.5 dm^3 plastic pots, at 20 plants per pot. Plants were cultured in medium (Horticultural substrate B with active humus, Rašelina Soběslav Ltd., Czech Republic), irrigated as needed and were protected from pests and diseases. Fertilisation (Kristalon Gold, Agro CS, Czech Republic; dosage: 10 g of fertiliser per 10 l of water) was carried out once. Plants were harvested for determination of ascorbic acid at around 7.00 a.m., for determination of nitrates at around 7.00 a.m. (in 2014) and at around 11.00 a.m. (years 2015–2016) before their flowering (they were planted as culinary herbs). Samples were immediately transported to a laboratory for chemical analysis.

Extraction and determination of ascorbic acid. The concentration of ascorbic acid was determined using HPLC according to Arya et al. (2000). Aerial parts of basil (10 g) were homogenised in a blender with 30 ml of 0.1 M oxalic acid. The homogenate was topped up with oxalic acid to a volume of 100 ml, filtered, centrifuged (3,800 rt/min for 10 min at room temperature) and the supernatant was used for measurement. The analyses were performed on a RP-HPLC (ECOM, Praha, Czech Republic) at 254 nm using a UV-VIS detector. All samples were measured in triplicate. The amount of ascorbic acid was expressed in mg/kg f. w.

Extraction and determination of nitrates. The content of nitrates was determined using an ion-selective electrode according to Javorský et al. (1987). Twenty grams of the sample were mixed with 20 ml of aluminium sulphate. Homogenised sample (20 g) was accurately weighed and boiled for 5 min with 20 ml of aluminium sulfate and 1 ml of 30% hydrogen peroxide. After cooling, the solution was made up to 100 ml aluminium sulphate in an Erlenmeyer flask. Measurements were carried out on the Ionalyzer MPH 171 device (Monokrystaly, Turnov, Czech Republic). The potential was recorded and compared with a calibration graph. Nitrate content was expressed as mg NO_3/kg f. w.

Statistical analysis. The Statistica Cz v. 12 (StatSoft) programme was used for statistical evaluation of the results. Data are expressed as means. Differences were analysed using the Kruskal-Wallis test.

RESULTS AND DISCUSSION

The results of the analysis of ascorbic acid content in the selected basil varieties are shown in Table 2. The ascorbic acid contents of basil ranged from 34.3 to 222.0 mg/kg f. w. The lowest content of ascorbic acid throughout the period 2015–2016 was found in the variety 'Mánes', and the highest was measured in the variety 'Ohře'. Ascorbic acid content was always higher in pot culture 1, but decreased progressively over the growing seasons under investigation. The effects of year, cultivation season and variety on ascorbic acid content were found to be statistically significant. The findings show that the highest content of ascorbic acid in basil cultivated in a greenhouse is achieved in the early spring months (time for sowing: February–March) and at the harvest approximately eight weeks after sowing. Abundant resources usually do not have a significant amount of the daily dose, because it is eaten only occasionally and in small amounts (Velíšek 2009). According to Holm (1999), fresh basil contains a large amount of ascorbic acid. Dumbrava et al. (2012) determined the ascorbic acid content in basil and rosemary leaves. They found that the basil leaves are richer in vitamin C (271 mg/kg f. w.) than rosemary leaves (185 mg/kg f. w.). Martyniak-Przebyszewska and Wojciechowski (2004) recorded lower concentrations of ascorbic acid in basil (119 mg/kg f. w.). Vábková and Neugebauerová (2009) investigated the content of ascorbic acid in different varieties of basil. The amount of ascorbic acid was highest in the 'Ohře' variety (271.7 mg/kg f. w.). The lowest ascorbic acid content was found in the 'Lettuce Leaf' variety (150.1 mg/kg f. w.). Our results are similar to those described by the studies cited above. Dzida (2010) reported that 'Kasia', a Polish variety of basil, accumulated more ascorbic acid (265 mg/kg f. w.) than the 'Wala' variety (204 mg/kg f. w.).

The results of the analysis of nitrate content in the selected basil varieties are shown in Table 3. The content of nitrates in basil varied depending

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Table 2. Ascorbic acid content in basil (mg/kg, f. w.)

Year/Variety	2015			2016		
	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
Ohře	222.0^{bB}	100.1^{aA}	189.1^{bAB}	85.4^{abA}	83.5^{bA}	103.5^{bA}
Lettuce Leaf	139.4^{abA}	110.9^{aA}	93.3^{abA}	78.5^{abA}	59.1^{abA}	47.2^{abA}
Purple Opaal	166.9^{abA}	121.8^{aA}	141.7^{abA}	108.1^{bA}	79.2^{abA}	85.4^{bA}
Dark Green	164.0^{abB}	70.0^{aA}	149.6^{abAB}	72.9^{abA}	60.3^{abA}	58.6^{abA}
Mammolo Genovese	140.0^{abB}	72.3^{aA}	112.9^{abAB}	54.4^{abA}	50.6^{abA}	54.0^{abA}
Mánes	104.7^{aA}	56.2^{aA}	74.3^{aA}	48.7^{aA}	38.9^{aA}	34.3^{aA}
Red Rubin	111.0^{aA}	95.8^{aA}	79.9^{abA}	74.2^{abA}	58.5^{abA}	76.5^{abA}

PC 1 – pot culture 1; PC 2 – pot culture 2; PC 3 – pot culture 3; means with the same lowercase letters in a column and uppercase letters in a row (pot culture 1, 2, 3 = 2015 × 2016) do not differ at 5% significance in the Kruskal-Wallis test

on the year, the pot culture and variety. The content of nitrates ranged from 237.7 to 4280.6 mg/kg f. w. The 'Purple Opaal' and 'Red Rubin' varieties had higher nitrate contents. Purple leaves have a higher tendency to accumulate nitrates. This is caused by the predominance of anthocyanin colorants in the plastids (Wojciechowska et al. 2000).

A significant impact of the cultivar on the total nitrogen content was recorded in the study of Dzida (2010). The experiments conducted by Nurzynska-Wierdak et al. (2011) also revealed a relationship between the cultivar and nitrate content in the plant.

The nitrate content of culinary herbs is an important point to consider in greenhouse production. The potential health hazards of nitrates are well described. According to Telesiński et al. (2013), the nitrate content of basil can be as high as 9,950 mg/kg f. w. The chemical analyses of Majkowska-Gadomska, et al. (2015) indicated that lemon basil plants had the highest nitrate content (1,904 mg/kg f. w.). In the studies performed by Vábková and Neugebauerová (2011), nitrate levels in dill ranged from 252 to 617 mg/kg f. w. In kale, nitrate levels were significantly higher in plants obtained in the first harvest than from those harvested at later dates. These levels varied from 1,643 to 1,730 mg/kg f. w., on average, depending on the year of the investigation and the cultivar (Korus, Lisiewska, 2009). Jaworska (2005) noted a much higher content of 2,804 mg/kg f. w. in New Zealand spinach.

The mean I_{AN} (Table 4) was found to range from 0.016 to 0.629. Neutral values were found in pot culture 3 in 2015 (for the 'Ohře' and 'Dark Green' varieties). Low values of this ratio were determined in the other varieties. The higher the value of this index, the more potent the action of the ascorbic acid and the less harmful the effects of plant nitrates for the human body. Zahradníková (2011) reported that the harvest period had an effect on the value of I_{AN} in watercress which was cultivated hydroponically.

Table 3. Nitrate content in basil (mg/kg, f. w.)

Year/Variety	2015			2016		
	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
Ohře	1,790.9^{a}	899.3^{a}	361.8^{ab}	3,263.7^{a}	1,758.7^{a}	1,490.5^{a}
Lettuce Leaf	1,631.8^{a}	1,223.1^{a}	654.9^{ab}	3,307.7^{a}	1,915.2^{a}	1,408.4^{a}
Purple Opaal	1,925.8^{a}	1,627.8^{a}	748.2^{ab}	3,888.0^{a}	2,412.4^{a}	2,143.1^{a}
Dark Green	1,348.1^{a}	903.3^{a}	237.7^{a}	2,854.5^{a}	1,866.4^{a}	1,061.3^{a}
Mammolo Genovese	1,811.4^{a}	1,033.7^{a}	294.9^{a}	3,489.3^{a}	1,926.2^{a}	1,205.8^{a}
Mánes	1,924.9^{a}	1,036.9^{a}	381.6^{ab}	3,137.1^{a}	2,207.5^{a}	1,400.1^{a}
Red Rubin	4,280.6^{a}	2,583.8^{a}	2,235.7^{b}	3,495.4^{a}	2,856.3^{a}	2,124.6^{a}

for explanation see Table 2

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Table 4. Ascorbate-nitrate index (I_{AN}) in different basil varieties

Year/Variety	2015			2016		
	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
Ohře	0.124	0.111	0.523	0.026	0.047	0.069
Lettuce Leaf	0.085	0.091	0.142	0.024	0.031	0.034
Purple Opaal	0.087	0.075	0.189	0.028	0.033	0.040
Dark Green	0.122	0.078	0.629	0.026	0.032	0.055
Mammolo Genovese	0.077	0.070	0.383	0.016	0.026	0.045
Mánes	0.054	0.054	0.195	0.016	0.018	0.024
Red Rubin	0.026	0.037	0.036	0.021	0.020	0.036

values of below 0.5 are considered risky; values of between 0.5 and 1.0 are considered moderately safe; values marked by grey colour are neutral

CONCLUSION

Our findings show that the content of ascorbic acid is dependent on the variety. The highest content was exhibited by the 'Ohře' variety, while the lowest values were determined in the 'Mánes' variety. Ascorbic acid content was also influenced by the cultivation season. The first harvest in each year showed the highest values, with levels decreasing thereafter.

The content of nitrates in basil is determined by plant metabolism in response to greenhouse conditions. The content of nitrates was relatively high in the selected basil varieties (especially 'Purple Opaal' and 'Red Rubin'). On the other hand, most of the cultivated varieties of basil grown under greenhouse conditions showed values that were below the recommended limit for nitrates in foodstuffs. Low values for the ascorbate-nitrate index in basil does not adversely affect human health, as the amount which is consumed is low and is used solely to improve the taste.

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References

Arya S.P., Mahajan M., Jain P. (2000): Non-spectrophotometric methods for the determination of vitamin C. Analytica Chimica Acta, 417: 1–14.

Buwalda F., Warmenhoven M. (1999): Growth-limiting phosphate nutrition suppresses nitrate accumulation in greenhouse lettuce. Journal of Experimental Botany, 50: 813–821.

Chanwitheesuk A., Teerawutgulrag A., Rakariyatham N. (2005): Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. Food Chemistry, 92: 491–497.

Dumbrava D.G., Moldovan C., Raba D.N., Popa M.V. (2012): Vitamin C, chlorophylls, carotenoids and xanthophylls content in some basil (*Ocimum basilicum* L.) and rosemary (*Rosmarinus officinalis* L.) leaves extracts. Journal of Agroalimentary Processes and Technologies, 18: 253–258.

Dzida K. (2010): Biological value and Essential oil content in sweet basil (*Ocimum basilicum* L.) herb depending on calcium carbonate dose and cultivar. Acta Scientiarum Polonorum, Hortorum Cultus, 9: 153–161.

Dzida K. (2011): Influence of nitrogen nutrition and cultivar on quality of sweet basil herbs. Annales UMCS sec. DDD XXIV, 3: 125–132.

European Commission Food Science and Techniques (1997): Opinions of the scientific committee for food on: Nitrates and Nitrite.

Favell D.J. (1998): A comparison of the vitamin C content of fresh and frozen vegetables. Food Chemistry, 62: 59–64.

Franke A. A., Custer L. J., Arakaki C., Murphy S.P. (2004): Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. Journal of Food Composition and Analysis, 17: 1–35.

Fraszcak B., Gasecka M., Golcz A., Zawirska-WojtasiakR. (2015): The chemical composition of lemon balm and basil plants grown under different light conditions. Acta Scientiarum Polonorum, Hortorum Cultus, 14: 93–104.

Freire C.M.M., Marques M.O.M., Costa M. (2006): Effects of seasonal variation on the central nervous system activity of *Ocimum gratissimum* L. essential oil. Journal of Ethnopharmacology, 105: 161–166.

Gruda N. (2005): Impact of Environmental Factors on Product Quality of Greenhouse Vegetables for Fresh Consumption. Critical Reviews in Plant Sciences, 24: 227–247.

doi: 10.17221/216/2016-HORTSCI

Harris J. R. (1996): Subcellular Biochemistry, Ascorbic Acid: Biochemistry and Biomedical Cell Biology. Vol. 25. New York: Plenum Press.

Holm Y. (1999): Bioactivity of basil. In: Hiltunem R., Holm Y. (ed.) Basil: the genus *Ocimum*. Amsterdam: Harwood Academic.

Javorský P., Krečmer F. (1987): Chemické rozbory v zemědělských laboratořích 2. Praha, Ministerstvo zemědělství a výživy ČSR.

Jaworska G. (2005): Content of nitrates, nitrites, and oxalates in New Zealand spinach. Food Chemistry, 89: 235–242.

Korus A., Lisiewska Z. (2009): Effect of cultivar and harvest date of kale (*Brassica oleracea* L. var. *acephala*) on content of nitrogen compounds. Polish Journal of Environtal Studies, 18: 235–241.

Lachman J., Pivec V., Orsák M. (1997): Askorbát-nitrátový a vláknino-nitrátový index-faktory charakterizující kvalitu zeleniny. Chemické listy, 91: 708–709.

Majkowska-Gadomska J., Mikulewic E., Dobrowolski A., Dziedzic A. (2015): Ecotype and foliar fertilization with florovit affect herbage yield and quality of greenhouse-grown basil (*Ocimumbasilicum* L.). Journal of Agricultural Science, 7: 195–200.

Martyniak-Przebyzewska B., Wojciechowski T. (2004): Plonowanie wybranych gatunkow roslin przyprawowych w rejonie Olsztyna. Folia Universitatis Agriculturae Stetinensis. Agricultura, 239: 245–248.

Nurzynska-Wierdak R. (2011): Yield and chemical composition of basil herb depending on cultivar and foliar feeding with nitrogen. Acta Scientiarum Polonorum, Hortorum Cultus, 10: 207–219.

Pokluda R. (2005): Stanovení askorbát-nitrátového indexu ve vybraném sortimentu zeleniny. Acta Horticulturae et Regiotecturae, 8: 52–54.

Polieteo O., Jukic M., Milos M. (2007): Chemical composition and antioxidant capacity of free volatile aglycones from basil (*Ocimum basilicum* L.) compared with its essential oil. Food Chemistry, 101: 379–385.

Saadatian M., Peyvast G.H., Olfati J.A., Pramezani-Kharazi P. (2014): Different species of basil need different ammonium to nitrate ratio in hydroponics' system. Acta Agriculturae Slovenica, 103: 223–232.

Suppakul P., Miltz J., Sonneveld K., Bigger S. W. (2003): Antimicrobial properties of basil and its possible application in food packaging. Journal of Agricultural and Food Chemistry, 51: 3197–3207.

Telesinski A., Grzeszczuk M., Jadczak D., Wysocka G., Onyszko M. (2013): Ocena zmian zawartości azotanów w wybranych ziołach przyprawowych w zależności od sposobu ich utrwalania i czasu przechowywania Żywność. Nauka. Technologia. Jakość, 5: 168–176.

Urbonavičiute A., Samuoliene G., Brazaityte A., Ulinskaite R., Jankauskiene J., Duchovskis P., Žukauskas A. (2008): The possibility to control the metabolism of green vegetables and sprouts using light emitting diode illumination. Sodininkysté ir Daržininkysté, 27: 83–92.

USDA (2016). National Nutrient Database for Standard Reference, Release 28. Basic Report 02044, Basil, fresh. Available at: https://ndb.nal.usda.gov/ndb/foods/show/293?fgcd=&manu=&lfacet=&format=&count=&max=50&offset=&sort=default&order=asc&qlookup=basil&ds= (Accessed March 10, 2016).

Vábková J., Neugebauerová J. (2009): Content of phenolic compounds and ascorbic acid in 4 Lamiaceae species at the fresh stage. In: Horna A.: Vitamins, Nutrition, Diagnostic 2009, The Abstract Book. 1st Ed. Pardubice: Univerzita Tomáše Bati ve Zlíně.

Vábková J., Neugebauerová J. (2011): Vliv způsobu pěstování na vnitřní kvalitu *Anethum graveolens* L. Úroda, LIX: 647–651.

Velíšek J., Hajšlová J. (2009): Chemie potravin 1+2. 3rd Ed. Ossis, Tábor.

WHO, FAO (2004). Vitamin and mineral requirements in human nutrition. 2nd Ed. China, WHO and FAO.

Wojciechowska R., Smoeln S., Pryzybylo J. (2000): Zawartość azotanów w różnych częściach użytkowych wybranych gatunków warzyw. Zesz. Nauk. AR w Krakowie. Sesja Naukowa, 364: 205–208.

Zahradníková H. (2011): Rychlení a možnosti využití potočnice lékařské (*Nasturtium officinale* R. Br.) [Ph.D. Thesis.] Brno, Mendel University in Brno.

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