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QUALITATIVE PARAMETERS OF NON-TRADITIONAL TYPES OF VEGETABLES - DETERMINATION OF NITRATES AND ASCORBIC ACID CONTENT

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ABSTRACT

The main aim of this study was to determine selected quality indicators of non-traditional types of leafy vegetables. Mizuna (*Brassica rapa japonica*), Chinese mustard (*Brassica juncea*), edible chrysanthemum (*Chrysanthemum coronarium*) and arugula (*Eruca sativa*) belonged among the selected species of vegetables. During the one-year experiment, spring and autumn sowing was carried out for these species of vegetables. The measured quality parameters were the content of nitrates and ascorbic acid. Sampling was done in the morning and in the laboratory, the samples were further processed according to the type of determination. To determine the content of nitrates and ascorbic acid, leaves were removed from plants. The filtrate from the leaves was then prepared. Determination of nitrates and ascorbic acid was carried out using a special test strip and device Rqflex plus 10. The results of measurement of both sowing varieties were compared. Total nitrate content was higher up to 22% in plants sown in the autumn except edible chrysanthemum (*Chrysanthemum coronarium*). The highest content was recorded in arugula (*Eruca sativa*), which was recently implemented to the studies of the European Union and for which there were set the limits of nitrates. Overall, the nitrate content ranged from 221 to 334 ppm in spring varieties and from 249 to 384 mg/kg in autumn varieties. Ascorbic acid content was very high in Chinese mustard (*Brassica juncea*), edible chrysanthemum (*Chrysanthemum coronarium*) and arugula (*Eruca sativa*) in both spring and autumn varieties. Values of ascorbic acid ranged from 839 in autumn sowing up to 2909 mg/kg in spring sowing. These non-traditional types of leafy vegetables could be included among the other importants sources of vitamin C in the future.

Keywords: ascorbic acid; nitrate; mizuna; Chinese mustard; edible chrysanthemum; rocket

INTRODUCTION

Leafy vegetables contain many nutritionally valuable substances but also compounds that negatively affect human metabolism. Leafy vegetables are crops in which the whole leaves or their parts are eaten **(Richardson, 2012)**, mostly fresh, heat unprocessed. Many species of leafy vegetables are grown throughout the world and thrive in different climatic regions. Generically, leafy vegetables varied greatly and the most of types can be grown during the whole year. The main parts are leaves which contain large amount of various substances. Highly valued is particlarly vitamin C and A as well as E, H, K and the B group. Significant is also the mineral content, primarily K, Fe, Ca, and Zn **(Pokluda, 2006; Açıkgöz 2012; Fujime, 2012)**. New, non-traditional kinds of vegetables come to the Czech Republic mainly from Western Europe and in recent years also from the Orient.

Mizuna (*Brassica rapa japonica*) is an annual plant, native to Asia which has at least 16 known varieties. However it thrives well even in temperate climates and tolerates frost down to -10 °C. The leaves have high content of vitamin C, potassium and calcium and are used particularly for preparing of fresh salads, cooking or pickling. Chinese mustard (*Brassica juncea*) is an annual, the longest known and most widely used of oriental vegetables in Europe. The leaves are rich in vitamin C, they have dietetic attributes and to a certain extent they are germicidal. For its pungent flavour it is most commonly used for preparing of fresh salads. Chinese mustard can withstands winter temperatures up to -10 °C, as well as mizuna **(Pekárková, 2002; Bhandari and Kwak, 2015)**. Edible chrysanthemum (*Chrysanthemum coronarium*) is an annual plant which is used especially in Asian cuisine. Young stems and leaves which contain a large amount of vitamins, fiber, flavonoids, calcium and potassium are used for the culinary processing. Unlike the mizuna and Chinese mustard, this vegetable is sensitive to low temperatures and thus at temperatures around zero its above-ground parts get frosted and lose their palatability **(Kopec, 2010)**. Arugula (*Eruca sativa*) is an annual, originally weedy plant, which has a place of origin in the Mediterranean and Asia. Thanks to its pungent taste it is used for preparing of salads. It contains lots of vitamin C and at higher doses of nitrogen fertilization also nitrate content. This vegetable is very resistant to drought and in winter can withstand temperatures up to -4 °C **(Miyazawa et al., 2002; Warwick, 2011)**.

Nitrates are natural metabolites of plants **(Pekárková, 2002)** which accumulate mainly in the leaves, stems and roots, and at higher concentrations they adversely affect the human organism. Therefore it is important to eliminate their content by providing of sufficient amount of light and moisture to the plants and reduce fertilization with high doses of nitrogenous fertilizers **(Hlušek, 2004)**. Intake of ascorbic acid, pectins, fiber and certain minerals, which significantly suppress the conversion of nitrate to N-nitroso compounds, is also favourable **(Šrot, 2005)**. Nitrates which are received in the usual amount together with food are not dangerous for humans **(Velíšek and Hajšlová, 2009)**. Crops harvested in cooler conditions and grown in northern areas have higher nitrate content than summer varieties grown in southern areas **(Weigthman et al., 2006; Prugar et al., 2008)**. The highest nitrate content have leafy vegetables **(EFSA, 2008; Prugar et al., 2008)**, where amount can be up to 6000 mg NO_3/kg **(EUR-Lex, 2015)**. The determination of nitrates is performed by colorimetry, using ion-selective electrode **(Prugar and Prugarová, 1985)**, HPLC/IC, enzymatically catalyzed reaction of the aqueous extract of the sample, continuous flow **(ČSN, 1998)** or by reflectometry **(Merck, 2013)**.

Ascorbic acid known as vitamin C is synthesized by all autotrophic plants **(Velíšek and Cejpek, 2008)**. It helps the absorption of iron, preventing LDL oxidation, supports the immune system of humans and inhibits the formation of nitrosamines **(Brambilla and Martelli, 2007; Begum et al., 2009)**. The main source are citrus furits, another important source are then a leafy and brassicaceous vegetables **(Lee and Kader, 2000; Fořt, 2011)**, which cover about 30 – 40% of the daily intake of vitamin C **(Mindell and Mundisová, 2010)**. There are considerable losses of vitamin C during post-harvest handling, storage and heat treatment **(Lee and Kader, 2000; Toledo et al., 2003)**, which may be reduced by using of crops in the fresh state, by limiting the contact of vegetables with air and by freezing. The determination is performed by colorimetry **(Velíšek and Cejpek, 2008)**, spectrophotometrically **(Valášek and Rop, 2007)**, plarographically **(Arya et al., 2000)**, chromatographically **(Škrovánková et al., 2007)** or by reflectometry.

Given the increasing popularity and occurence of lesser-known varieties of leafy vegetables in our market was the main aim of this study assessment of selected quality indicators (content of nitrates and ascorbic acid) in mizuna (*Brassica rapa japonica*), Chinese mustard (*Brassica juncea*), edible chrysanthemum (*Chrysanthemum coronarium*) and aurgula (*Eruca sativa*).

MATERIAL AND METHODOLOGY

The seeds of mizuna (*Brassica rapa japonica*), Chinese mustard (*Brassica juncea*), edible chrysanthemum (*Chrysanthemum coronarium*) and arugula (*Eruca sativa*) originated from the SEMO company were used for this research. Half of the seeds were used for srping sowing (April) and the other half of seeeds was kept in dry place for the autumn sowing (August). Plants were seeded at 40 m line in 5 cm spacing between the individual seeds and 40 cm between rows. All plants were maintained by weeding, they were watered three times a week and they were not fertilized during the whole growing.

Four plants from each species and always from every quarter row were taken in the process of sampling. The plants were further processed according to the type of determination in the laboratory. Morning sampling for nitrate determination is very important because their content in plants change significantly during the day. Especially in the warm season, nitrate reductase activity grow in plants, thereby the content of accumulated nitrates ís on the decrease **(Weightman et al., 2006)**. Plants were harvested in the morning for the determination of nitrate and ascorbic acid content.

The leaves from all plant species were removed for the determination of nitrates and a representative sample was created from these leaves. There were took 4 x 10 g of material. The sample was weighed into a 200 ml volumetric flask with a widened neck, supplemented with distilled water to 2/3 of the content of the flask and heated in a water bath at approximately 100 °C. After removing the sample was allowed to cool down, the flask was filled with distilled water to 200 ml and filtered. The filtrate was poured into a 50 mL beaker, to which was subsequently inserted a special test strip for the determination of nitrate on RQflex plus 10, which was set to Nitrate Test with a measuring range of 5 – 225 $mg.L^{-1}$. The strip was left in the beaker for 10 seconds and then lightly wiped dry and inserted into the apparatus, which assessed the nitrate content in $mg.L^{-1}$. According to the scope of the results there was made calibration solution of $NaNO_3$, which was measured using test strips on the device RQflex plus 10. There was created calibration curve from the measured values.

For the determination of ascorbic acid content was prepared 3% solution of 1 L metaphosphoric acid for acid extraction. The leaves from freshly harvested plants were collected, and a mixed sample was made from them. For determination was used 4 x 5 g of sample. The sample was picked and weighed into a 200 mL beaker, by pipette was added 50 mL of metaphosphoric acid, and the mixture was homogenized by mixer for 1 minute. The extract was filtered through paper and the filtrate was poured into a 50 ml beaker. Into a beaker were then inserted a special test strip for the determination of ascorbic acid on the RQflex plus 10. The device was set to the Test for the determination of ascorbic acid content with a measuring range of 25 – 450 $mg.L^{-1}$. The strip was left in the beaker for 5 seconds, then wiped dry and placed into a meter, which provided the ascorbic acid content in mg/l. According to extent, there were created calibration solutions of ascorbic acid of known concentration. From the measured values was made calibration curve.

Each sample was measured four times and the results were expressed as the mean ± standard deviation. The results were then analyzed using Microsoft Excel and Statistica 12 (t-test), and compared. The obtained values of nitrate content on the device RQflex plus 10 were calculated according to calibration curve. Nitrate content determined by reflectometer in $mg.L^{-1}$ was converted to nitrate content in mg/200 mL, which took place in determining the sample and finally through the sample size was calculated nitrate content in mg/kg (ppm). The values of ascorbic acid generated by the RQflex plus 10 were calculated using the calibration curve to a more accurate value indicating the ascorbic acid content in $mg.L^{-1}$. From the found values was converted ascorbic acid content in 50 mL used in determination and through a sample weight was calculated content in mg/kg (ppm). The method is based on reflectometry, and its simplicity enables quantitative determination in the field.

RESULTS AND DISCUSSION

According to measurement results (Table 1) was found that the spring varieties contain minor amount of nitrates. This is corresponding with research of **Weightman et al. (2006)** about the favourable effect of light and heat on the reduction of nitrate content in plants. Surprisingly, the ascertained values for all crop species ranging between medium to low nitrate content, although the leafy vegetables belong to a group with a high content of nitrates. The resulting values may be influenced by the way of cultivation without fertilization using nitrogenous or other fertilizers.

Chrysanthemum, mustard and mizuna do not have yet so widespread use in Europe to make their nitrate content deal with EU legislaton. It is different e.g. in wild rocket which is becoming more popular and where the nitrate level may be up to 9300 mg/kg, such as in the study of **Santamaria (2006)**. Plants measured in this work did not exceed the limit for rucola (7000 mg/kg), nor remotely came close to this limit. It is possible to affect and significantly reduce the amount of nitrates in leafy vegetables due to climatic conditions and cultivation methods, which describe **Fontana and Nicola (2009)** in their work.

The measurement results confirm that the given types of vegetables are a good source of vitamin C (Table 2), especially arugula (*Eruca sativa*), which included nearly 3000 mg/kg. This finding not correlates with the results of **Kim and Ishii (2007)** who recorded the amount of 1420 mg of ascorbic acid per kg in leaves of arugula, and found that values are reduced by the influence of storage conditions. From the nutritional perspective it is therefore more advantageous consumption of fresh vegetables. The content of ascorbic acid in chrysanthemum, mustard and arugula was not affected by sowing period and despite the diverse values of differences in ascorbic acid content between spring and autumn sowing, both varieties seem to be a suitable source of vitamin C for human consumption. Only autumnal variation of mizuna (*Brassica rapa japonica*) showed up to two-thirds smaller amounts of vitamin C than the spring variant. These results are coincidental with measurements of **Kalisz et al. (2012)** who found that variants of mizuna sown in July had higher ascorbic acid content than those that were seeded in August. Also **Aires et al. (2011)** point out to a higher content of ascorbic acid in spring varieties for the genus

Table 1 Content of nitrates.

Plant species	**Content of nitrates in mg/kg**		
	Spring sowing	Autumn sowing	Difference
Edible chrysanthemum (*Chrysanthemum coronarium*)	309 ±43	249 ±41	60
Chinese mustard (*Brassica juncea*)	221 ±37	283 ±91	-62
Mizuna (*Brassica rapa japonica*)	221 ±38	277 ±56	-56
Arugula (*Eruca sativa*)	334 ±43	384 ±48	-50

Table 2 Content of ascorbic acid.

Plant species	Content of ascorbic acid in mg/kg		
	Spring sowing	Autumn sowing	Difference
Edible chrysanthemum (*Chrysanthemum coronarium*)	1890 ±59	1889 ±75	1
Chinese mustard (*Brassica juncea*)	2068 ±21	2047 ±29	21
Mizuna (*Brassica rapa japonica*)	2479 ±50	839 ±21	1640
Arugula (*Eruca sativa*)	2909 ±85	2717 ±66	192

Brassica in general. On the contrary **Açıkgöz (2012)** states that the vitamin C in vegetables is influenced by the seasons. He indicates in his work that the detected level of vitamin C in spring variant of mizuna (*Brassica rapa japonica*) was 702 mg/kg and in autumn variant 576 mg/kg, which correspond to our results, when ascorbic acid content was also higher in spring variant.

Ascorbic acid content detected in spring varieties ranged from 1890 to almost 3000 mg/kg which is much more than in the study of **Kopta and Pokluda (2006)**, where the measured values ranged from 590 to 720 mg/kg. Also **Bhandari and Kwak (2015)** found lower values in *Brassica* vegetables. Recorded values in both experiments may vary due to the use of different methods for the determination of ascorbic acid. In this experiment, the values were found by reflectometry, while **Kopta a Pokluda (2006)** used HPLC for determination.

Based on the one-year experiment, there was not found correlation between the observed qualitative parameters, $r = 0.361$. Except ascorbic acid content of mizuna ($p < 0.0001$) and arugula ($p = 0.0119$), there were not found any statistical significant differences between spring and autumn sowing in volumes of nitrates and ascorbic acid.

CONCLUSION

The total content of nitrates in plants was not high, neither in spring nor in autumn varieties. In both cases of sowing the greatest amount of nitrates was found in rocket (*Eruca sativa*) but even in this case the measured values were very low. Arugula is the most widely culinary used of all determined kinds and as one of the crpops mentioned above, there were set the limits for nitrates by the European Union for this plant. All types of observed vegetables showed high ascorbic acid content and thus proved to be another important source of vitamin C.

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