Less-known Leaf Vegetables Grown in Slovak Republic Conditions: New Sources of Antioxidants

Menej známe druhy listovej zeleniny pestované na území Slovenskej Republiky: Nové zdroje antioxidantov

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Abstract

The goal of this study was to determine the content of bioactive substances (chlorophyll, carotenoids, vitamin C) in less-known leafy vegetable species, such as Chinese mustard (*Brassica juncea* (L.) Czern. Et Coss.), mibuna (*Brassica rapa* ssp. *japonica* Makino) and mizuna (*Brassica rapa* ssp. *nipposinica* (Bailey) Hanelt). Within study, two cultivars of Chinese mustard were tested, concretely cv. Ruby Streaks (purple leaves) and cv. Spicy Green (green leaves). The field experiment was realised on the land of Slovak University of Agriculture in Nitra in 2014 and 2015. The highest content of chlorophyll a and chlorophyll b, as the biologically most active chlorophyll types, was found in mibuna leaves. The Chinese mustard cv. Ruby Streaks was shown as the richest source of total carotenoids. The highest vitamin C content was found in leaves of Chinese mustard cv. Spicy Green. Compared to the typical leafy species like spinach, all tested less-known vegetable species were indicated as the comparable sources of chlorophyll. The content of other tested substances, such as carotenoids and vitamin C, was even higher than results presented in studies of several authors with spinach.

Keywords: *Brassicaceae*, carotenoids, chlorophyll, vitamin C

Abstrakt

Cieľom štúdie bolo sledovať obsah bioaktívnych látok (chlorofyl, karotenoidy, vitamín C) u vybraných menej známych druhoch listovej zeleniny, konkrétne v listoch horčice čínskej (*Brassica juncea* (L.) Czern. Et Coss.), mibuny (*Brassica rapa* ssp. *japonica*

Makino) and mizuny (*Brassica rapa* ssp. *nipposinica* (Bailey) Hanelt). V rámci pokusu boli zaradené dve odrody horčice čínskej, a to Ruby Streaks (purpurové listy) a Spicy Green (zelené listy). Poľný pokus bol realizovaný v areáli Slovenskej poľnohospodárskej univerzity v Nitre v rokoch 2014 a 2015. Najvyšší obsah chlorofylu *a* a chlorofylu *b*, ktoré sú biologicky najaktívnejšími zložkami chlorofylu, bol zistený v listoch mibuny. Najbohatším zdrojom karotenoidov boli listy odrody horčice čínskej Ruby Streaks. Najvyšší obsah vitamínu C bol stanovený v listoch odrody horčice čínskej Spicy Green. Všetky sledované menej známe druhy listovej zeleniny sa preukázali ako významné zdroje chlorofylu, pričom jeho obsah bol porovnateľný s typickým druhom listovej zeleniny ako je špenát. Obsah karotenoidov a vitamínu C bol dokonca vyšší v porovnaní s jeho hodnotami v špenáte, ktoré boli prezentované v štúdiách viacerých autorov.

Kľúčové slová: *Brassicaceae*, chlorofyl, karotenoidy, vitamín C

Introduction

The group of leafy vegetables includes wide spectrum of species such as spinach, lettuce, Chinese cabbage, endives etc. (Uher et al., 2009). The ratio of leafy vegetable species on the total vegetable production was 1.58% in 2014 in Slovakia. The total production of all leafy vegetables was 5 166 tones at grown area of 600 hectares. The most grown species in Slovakia was head lettuce and spinach which presented nearly total production of leafy vegetables (Meravá, 2015). According to Petříková et al. (2012), besides commonly grown leafy species (lettuce, spinach, Chinese cabbage, Pak Choi etc.), various less-known leafy vegetables are popular in abroad, such as Chinese mustard, mibuna, mizuna, rocket or celtuce. Phillips and Rix (1995) indicate that origin region of these species is mainly Asia where they are grown in large areas.

Chinese mustard, also known as Indian mustard, leaf mustard or mustard green, is a species of *Brassicaceae* family. Its primary centre of origin is central Asia (northwest India). Secondary centres are central and western China, eastern India through Iran to the Near East. The main growing countries are Japan, China, India, Nepal, Pakistan, Bangladesh, as well as southern Russia (Kumar et al., 2011). Its edible part is rosette of light-green leaves, sometimes with purple or reddish colour shade. Leaves have a slightly pungent taste which intensity increases within vegetation period (Pekárková, 2014). Typical taste of mustard leaves are caused by presence of sulphur containing substances known as glucosinolates. In addition, *Brassica juncea* is known as a good source of several other bioactive phyto-chemical compounds, including vitamin C, flavonoids, phenolic compounds, chlorophyll or carotenoids (Cartea et al., 2011; Duma et al., 2014; Gupta et al., 2012).

Mizuna and mibuna are vegetable species typical in Japan cuisine. Difference between mentioned species is mainly from botanically-morphological aspect. The leaf blade of mizuna is deeply cut; on the contrary, mibuna has leaves with non-lobed blade (Petříková et al., 2012). Both plants form compact rosette of leaves known for relatively high content of various health-promoting substances, such as chlorophyll,

carotenoids, vitamin C or several mineral nutrients (Duma et al., 2014; Kudrnáčová, Kouřimská, 2015; Martinéz-Sánchez et al., 2008).

Leafy vegetables, as well as other green vegetables, are rich source of chlorophyll which is able to detoxify human body by absorption of various toxic compounds (Žnidarčič et al., 2011). In addition, it improves organism hyperaemia and wound healing. The chlorophyll is also a good source of magnesium important for muscle activity and balancing with stress situation (Levent İnanç, 2011).

Carotenoids are group of pigments soluble in fats having an important role in human body from aspect of its prevention against various serious diseases (Riccioni, 2009). Carotenoids are very effective antioxidant substances because of their activity against free radicals dangerous for human body (Gülçin, 2012). According to Olives-Barba et al. (2006), higher carotenoid intake helps to decrease of several cancer type risk (stomach, colon or larynx), bone calcification, eye degeneration, neurotic or cardiovascular diseases.

Due to its properties, vitamin C is characterized as very effective antioxidant. The human body is not able to synthesize vitamin C, thus, it must be ingested in the food form (Šlosár et al., 2013). The vitamin C plays important role in immune system, stimulation of leucocytes to the increased bacteria degradation, secretion of antibodies and increase of body resistance to the coldness (Hacişevki, 2009). Feiz and Mobarhan (2002) indicate that intake of sufficient vitamin C amount affect against *Helicobacter pylori* bacteria considered as important risk factor in stomach cancer formation. According to the Iqbal et al. (2004), vitamin C helps to prevent human organism by elimination of nitrosamine formation which descend from nitrates contained in many food sources.

Materials and methods

Experimental locality

The field experiment with less-known leafy vegetables was realised on land of the Slovak University of Agriculture in Nitra in 2014 and 2015. The experimental area is situated at an absolute altitude of 144 m above sea level. The climate of experimental area is characterized by warm and dry summer and slightly warm, dry or very dry winter. According to the climatic normal 1951-2000 for Nitra, annual mean temperature is 9.9 °C and mean rainfall total is 548 mm (Šlosár and Uher, 2013). Within experimental period, the average month air temperature was 11.9 °C in 2014 and 11.5 °C in 2015. The rainfall total was 590 mm in 2014 and 418.2 mm in 2015. The soil type in experimental area is classified as Hortic Calcaric Fluvisol (Polláková, Šimanský, 2015).

Organisation of experiment

Short characteristics of tested less-known leafy vegetable species are mentioned in the Table 1. All species belongs to the *Brassicaceae* family. Seeds of Chinese mustard (both cultivars) were come from company Botanical Interests Colorado (USA). Mizuna and mibuna seeds were purchased in company SEMO a. s. Smržice from Czech Republic.

All species were grown from direct sowing realised on 28th August 2014 and 27th August 2015. All species were grown in 3 rows (replications) with length of 2.0 m. Inter-row distances was 0.15 m. Within growing, plants were covering by white non-woven textile and regularly irrigated. The harvest of particular examined species was realised on 10th October 2014 and 8th October 2015.

Determination of examined qualitative parameters

The chlorophyll and carotenoid content was determinated spectrophotometrically according to Hegedüsová et al. (2007). The vitamin C content were measured chromatographically (HPLC) according to the method of Stan et al. (2014) at the certified laboratory of Regional Public Health Authority in Nitra.

Table 1. Characteristics of tested less-known leafy vegetable species

Table 1. Charakteristika skúmaných menej známych druhov listovej zeleniny

Common name	Latin name	Cultivar
Chinese mustard	*Brassica juncea* (L.) Czern. Et Coss.	Spicy Green
Chinese mustard	*Brassica juncea* (L.) Czern. Et Coss.	Ruby Streaks
Mibuna	*Brassica rapa* ssp. *japonica* Makino	-
Mizuna	*Brassica rapa* ssp. *nipposinica* (Bailey) Hanelt	-

Statistical analysis

A statistical analysis was performed using Statgraphic Centurion XVII (StatPoint Inc. USA). Obtained results were evaluated by analysis of variance (ANOVA) and average values were tested by Tukey HSD test performed at the significance level of 95%.

Results and discussion

Chlorophyll content

According to Milenković et al. (2012), chlorophyll is consisted from several compounds but its biologically most active substances are chlorophyll *a* (*Chl a*) and chlorophyll *b* (*Chl b*). Within experiment, average content of *Chl a* was varied from 367.84 mg*kg^{-1} to 443.99 mg*kg^{-1} of fresh weight (f. w.). The average content of *Chl b* was ranged from 152.64 mg*kg^{-1} to 192.38 mg*kg^{-1} f. w. Among tested species, mibuna was shown as the richest source of both chlorophyll types. On the contrary, the lowest content of *Chl a* and *Chl b* was found in leaves of mizuna. The variance analysis of experimental results revealed statistically significant difference in

chlorophyll content among particular tested leafy species. Difference between Chinese mustard cultivars was evaluated as statistically non-significant (Table 2).

Table 2. Chlorophyll content in less-known leafy vegetable species (mg*kg^{-1} fresh weight)

Table 2. Obsah chlorofylu v menej známych druhoch listovej zeleniny (mg*kg^{-1} čerstvej hmoty)

Species	Chlorophyll *a*			Chlorophyll *b*		
	2014	2015	Average	2014	2015	Average
Chin. mustard SG[1]	403.70	446.48	425.09[b]	180.03	185.15	182.59[b]
S. D.[3]	±1.99	±1.03		±1.74	±3.15	
Chin. mustard RS[2]	421.70	431.53	426.62[b]	181.62	197.95	189.78[bc]
S. D.	±1.25	±1.19		±0.96	±2.89	
Mibuna	432.99	455.00	443.99[c]	181.70	203.07	192.38[c]
S. D.	±3.18	±1.34		±1.42	±2.25	
Mizuna	347.28	388.40	367.84[a]	143.76	161.52	152.64[a]
S. D.	±0.75	±1.88		±0.86	±2.69	

[1] Chinese mustard cultivar Spicy Green, [2] Chinese mustard cultivar Ruby Streak, [3] Standard deviation (number = 9)

Values in the same column with different letters are significantly different at ($P < 0.05$)

[1] Horčica čínska - odroda Spicy Green, [2] Horčica čínska - odroda Ruby Streak, [3] Štandardná odchýlka (počet = 9)

Rozdielne hodnoty v stĺpcoch, označené malými písmenami v hornom indexe, vyjadrujú štatisticky preukázaný rozdiel ($P < 0,05$, LSD test, ANOVA (Statgraphic XVII)).

Compared to experimental results, Duma et al. (2014) found higher content of *Chl a* in mustard leaves (687 mg*kg^{-1} f. w.). On the contrary, *Chl b* content was lower in comparison with realised experiment (155 mg*kg^{-1} f. w.). Pokluda (2007) state that chlorophyll content in Chinese mustard leaves is expressively depending on the cultivar. The content of *Chl a* was varied in the range from 2072 mg*kg^{-1} to 3426 mg*kg^{-1} f. w. The *Chl b* content was ranged from 1001 mg*kg^{-1} to 1203 mg*kg^{-1} f. w. Author found several fold higher content of both chlorophyll substances in Chinese mustard leaves compared to obtained experimental results. Banerjee et al. (2012) examined total chlorophyll content (*Chl a* + *Chl b*) in leaves of several mustard

cultivars. Its value was varied from 426 mg*kg^{-1} to 756 mg*kg^{-1} f. w. (average = 654 mg*kg^{-1}) what is similar to experimental results obtained in this study.

In experiment, the content of two main chlorophyll compounds (Chl *a* + Chl *b*) in leaves of mibuna (636.37 mg*kg^{-1} f. w.) and mizuna (520.48 mg*kg^{-1} f. w.) was 4-5 fold higher than it was presented in research work of Artemyeva and Solovyeva (2006). The chlorophyll content in mibuna and mizuna leaves was also examined in experiment realised by Kalisz et al. (2012) who determined several fold lower values of both chlorophyll types (*Chl a* and *Chl b*) compared to results gained in realised experiment. Similarly to this study, authors found higher chlorophyll content in mibuna in comparison with mizuna leaves. This fact is also in consistency with study of Świąder and Radzanowska, 2006).

Andrejiová and Mendelová (2012) found that content of *Chl a* in spinach, as traditional leafy vegetable, was 477.4 mg*kg^{-1} f. w. (average value of 5 cultivars). The average value of Chl *b* content was 856.6 mg*kg^{-1} f. w. According to these facts, it is possible to state that less-known leafy vegetable species are also a good source of chlorophyll as important bioactive substance.

Total carotenoid content

The carotenoid group is one of the most important classes of plant pigments which play a crucial role in defining the quality parameters of fruit and vegetables (Eldahshan and Singab 2013). Van den Berg et al. (2000) indicate that besides β-carotene, which is the best-known carotenoid, this group includes α-carotene, lycopene, lutein, zeaxanthin and cryptoxanthin. The largest contribution of vitamin A intake comes from provitamin A - carotenoids in plant food which may contribute up to 82% of total vitamin A intake. Thus, the vegetables, including leafy species, are good sources of carotenoids.

Within experiment, average total carotenoid content in less-known vegetables species was ranged from 296.08 mg*kg^{-1} to 372.75 mg*kg^{-1} f. w. The richest source of carotenoids was Chinese mustard cv. Ruby Streaks with red-black or purple leaf foliage. On the other side, the lowest carotenoid content was found in mizuna leaves. Statistical analysis showed statistically significant difference among Chinese mustard cv. Ruby Streak and other tested species. Other differences were evaluated as statistically non-significant (Table 3).

Regarding to the carotenoid content in leaves of *Brassica juncea*, obtained results are similar with experiment results presented in study of Kumar et al. (2013). Dependent on the cultivar and cultivation period, the total carotenoid in mentioned study was varied from 260 mg*kg^{-1} to 490 mg*kg^{-1} f. w. The higher content of total carotenoid was presented in study of Pokluda (2007) in study with 13 cultivars of Chinese mustard. Author found carotenoid content variation in the range from 527 mg*kg^{-1} to 899 mg*kg^{-1} f. w. (average = 668.8 mg*kg^{-1}). Similarly, higher carotenoid content was also found by Kapoor et al. (2014) in experiment with *Brassica juncea*.

Artemyeva and Solovyeva (2006) detected lower carotenoid content in mibuna and mizuna leaves as it was found in this study, concretely about 30% (mibuna) and 36% (mizuna). Kalisz et al. (2012) found markedly lower content of carotenoids in mibuna (15.8 mg*kg^{-1} f. w.) and especiall in mizuna (4.95 mg*kg^{-1} f. w.). Świąder and

Radzanowska (2006) detected higher carotenoid content in mibuna in comparison with mizuna leaves how it was also found in realised experiment.

According to Bunea et al. (2008), total carotenoid content in typical leafy vegetable species like spinach was ranged from 119.0 mg*kg^{-1} to 125.6 mg*kg^{-1} f. w. in dependency on its determination method. These values are markedly lower compared to all less-known leafy vegetable species tested in realised experiment.

Table 3. Total carotenoid and vitamin C content in less-known leafy vegetable species (mg*kg^{-1} fresh weight)

Table 3. Obsah celkových karotenoidov a vitamínu C v menej známych druhoch listovej zeleniny (mg*kg^{-1} čerstvej hmoty)

Species	Total carotenoids			Vitamin C		
	2014	2015	Average	2014	2015	Average
Chin. mustard SG[1]	215.77	416.15	315.96[a]	792.44	834.46	813.45[c]
S. D.[3]	±2.41	±3.32		±5.52	±3.25	
Chin. mustard RS[2]	288.33	457.17	372.75[b]	679.00	824.92	751.96[c]
S. D.	±3.78	±4.93		±7.17	±1.74	
Mibuna	268.28	382.55	325.41[a]	313.19	402.97	443.35[b]
S. D.	±1.37	±3.99		±7.03	±2.65	
Mizuna	275.59	334.57	296.08[a]	299.53	400.50	346.51[a]
S. D.	±3.65	±4.25		±5.70	±3.31	

[1] Chinese mustard cultivar Spicy Green, [2] Chinese mustard cultivar Ruby Streak, [3] Standard deviation (number = 9)

Values in the same column with different letters are significantly different at ($P < 0.05$)

[1] Horčica čínska - odroda Spicy Green, [2] Horčica čínska - odroda Ruby Streak, [3] Štandardná odchýlka (počet = 9)

Rozdielne hodnoty v stĺpcoch, označené malými písmenami v hornom indexe, vyjadrujú štatisticky preukázaný rozdiel ($P < 0,05$, LSD test, ANOVA (Statgraphic XVII)).

Vitamin C content

Singh et al. (2007) indicate that vegetables are rich in vitamin C and its content varies in dependency on various factors, mainly species, cultivars or climatic conditions. Within realised experiment, the average content of vitamin C (2014-2015) was ranged from 346.51 mg*kg^{-1} to 813.45 mg*kg^{-1} f. w. (Table 3). The Chinese

mustard was expressed as the richest source of vitamin C among tested species. The higher content was found in its cultivar Spicy Green (green leaves) compared to the cultivar Ruby Streaks (red-black or purple leaves). Difference between mentioned cultivars of *Brassica juncea* was evaluated as statistically non-significant. Analysis of result variance for vitamin C content revealed statistically significant differences among *Brassica juncea* and mizuna/mibuna.

Duma et al. (2014) and Banerjee et al. (2012) found lower content of vitamin C in leaves of *Brassica juncea* compared to results obtained in this study. Pokluda (2007) examined effect of leaf mustard cultivar on the vitamin C content. Author presented variability of its value in the range from 327 $mg*kg^{-1}$ to 738 $mg*kg^{-1}$ f. w. Average value of vitamin C content for green-leafy cultivars was 493 $mg*kg^{-1}$. Cultivars with purple leaf blade contained 642.3 $mg*kg^{-1}$ on average. Mentioned values are markedly lower in comparison with results obtained in realised experiment. On the other side, Kudrnáčová and Kouřimská (2015) found significantly higher vitamin C content compared to this study.

Regarding to mibuna and mizuna, Artemyeva and Solovyeva (2006) found comparable value of vitamin C content in leaves of both species to results presented in this study. However, expressively lower content of vitamin C in mibuna and mizuna leaves was presented by Kalisz et al. (2012). The vitamin C content in mibuna was varied from 242.4 $mg*kg^{-1}$ to 265.8 $mg*kg^{-1}$ f. w. in dependency on the sowing date. The content of vitamin C in mizuna leaves was ranged from 175.0 $mg*kg^{-1}$ to 185.5 $mg*kg^{-1}$. On the contrary, Acikgöz (2011) found higher content of vitamin C in mizuna, concretely about 100 $mg*kg^{-1}$ f. w. compared to results in this study. Similar finding about higher vitamin C content in mizuna leaves was presented by Kopta and Pokluda (2010) or Kudrnáčová and Kouřimská (2015).

According to Petříková et al. (2012), raw spinach contains 281 mg of vitamin $C*kg^{-1}$. All less-known leafy species, tested in this study, shown higher content of vitamin C compared to spinach as typical leafy vegetable species.

Conclusions

The group of leafy vegetables is very wide from aspect of species number. Besides of typical species for Europe production, such as spinach or lettuce, other various species of less-known leafy vegetable species are included in this vegetable group which comes from Asia mostly. All species, tested in realised experiment, are less-known and grown in a small extend in Slovak Republic. The goal of this study was to determine the content of selected health-promoting bioactive substances (chlorophyll, carotenoids, vitamin C) in Chinese mustard, mibuna and mizuna leaves. All species included to this study were shown as potentially comparable sources of examined health-promoting substances in relation with typical leafy vegetable species, e. g. spinach.

Acknowledgements

This study was supported by grants of The Scientific Grant Agency (VEGA 1/0105/14) and The Culture and Education Grant Agency (KEGA 038SPU-4/2014) of the Ministry of Education, Science, Research and Sport of the Slovak Republic.

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