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VARIETAL VARIABILITY OF LESS GROWN MINTS: INFLUENCE ON SELECTED ANTIOXIDANTS

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ABSTRACT

Genus *Mentha* belongs among the important part of spice, aromatic and medical plants. It includes a large amount of varieties and forms which are not spread generally because of low knowledgeability about possibilities of individual varieties using and also about their high content of bioactive substances. Five less-known varieties and forms (*Mentha* x *piperita* var. ´Danica´, *Mentha* x *piperita* var. ´Chocolate´, *Mentha arvensis* f. Banana, *Mentha sp*. f. Mojito, *Mentha sp*. f. White Grape) with different aroma and plant habitus were chosen to estimate the content of essential oils, chlorophyll *a*, chlorophyll *b* and carotenoids. From obtained results varietal variability of individual varieties and forms have been specified and compared with commonly used *Mentha* x *piperita*. The content of essential oils was estimated by steam distillation. The pigment content was determined by spectrophotometric measurement of absorbance at a wavelength of 649 nm (chlorophyll *a*), 665 nm (chlorophyll *b*) and 450 nm (carotenoids). The results showed that ´Danica´ and ´Chocolate´ varieties have reached the highest amount of essential oils. Most of the chlorophyll *a*, *b* and carotenoids content reached commonly used *Mentha* x *piperita*, but there wasn´t found significant difference when compared with ´Danica´ and ´Chocolate´. The effect of varietal variability on the content of qualitative characteristics in the case of essential oils, chlorophyll *a* and *b* was confirmed according to used statistical analyse (p <0.05). The varietal variability of tested mint forms and varieties on carotenoids content was not confirmed.

Keywords: mint; essential oils; carotenoids; chlorophyll

INTRODUCTION

Mentha x *piperita* L. and *Mentha arvensis* L. are perennial plants belonging to *Lamiaceae* family, originating from Europe but spread around the world and cultivated in many different climates **(Heydari et al., 2018)**. Mint essential oils have long been used in various forms such as in management of plant pathogens and insect pests, in traditional medicine as well as in culinary and cosmetics **(Singh and Pandey, 2018)**.

The essential oils are included in the group of terpenes; these are volatile substances soluble in ethanol. The quality and quantity of essential oils is influenced by the environment, the growing methods, the way of harvesting, the post-harvest processing of plants and the way of essential oils obtaining **(Šalamon, 2015)**. The use of essential oils is extremely diverse depending on the source, quality, extraction procedure, etc. Essential oils have proven industrial applications in the manufacture of perfumes, cosmetics, soaps, shampoos, or cleaning gels. Another interesting aspect of these oils is their potential as therapeutic agens in aromatherapy or as active principles or excipients of medicine **(Tančinová et al., 2018)**. Another significant application of essential oils is in the agrofood industry, both for producing beverages and for flavoring foods **(Ríos, 2016)**.

Chlorophyll as a photosynthetic pigment occurs in green plants in two forms: chlorophyll *a* and chlorophyll *b*, bound on a protein as chromoprotein. The ratio of chlorophyll *a* to chlorophyll *b* is usually 3:1 **(Hudec et al., 2002)**. The basic structure of the chlorophyll molecule is the porphyrin ring, which is formed by four heterocyclic nuclei with 10 double bonds and a centrally bonded magnesium atom **(Kleňová and Turianica, 2010)**.

Chlorophylls are fitted and accompanied by carotenoids in chloroplasts, on tylacoid membranes. They are naturally decomposed simultaneously with the breakdown of chloroplast membranes at the end of activity of green plant parts **(Ivanišová, 2014)**. Many studies support that chlorophylls and its derivatives have antioxidant properties **(İnanç, 2011)**. Chlorophyll as well as carotenoids, has the ability to react with singlet oxygen and it is acting as an extinguisher of free radicals as an antioxidant **(Kleňová and Turianica, 2010)**.

The main compounds with the antioxidant properties in mints are phenols, ascorbic acid and carotenoids **(Capecka, Mareczek and Leja, 2005)**. Based on the results of a number of clinical and epidemiological studies there was confirmed the antioxidant effect of carotenoids in the prevention and treatment of certain types of cancer, cardiovascular and ocular diseases and photosensitivity disorders **(Fiedor and Burda, 2014)**.

At the present many experts have agreed on that the antioxidants taken in their natural state have greater efficacy and usability than their equivalent amounts taken in pure form as a food supplement **(Chrpová, 2010)**.

Scientific hypothesis

According to different aroma and plant habitus (colour of the leaves) there is prediction of different content of essential oils and pigments (chlorophyll *a*, *b* and carotenoids) in selected mint varieties and forms.

MATERIAL AND METHODOLOGY

Small plot field experiment with mint was conducted in area of the Department of Vegetable production, SUA Nitra, in 2016 and 2017. Before planting the plot preparing was done according to the cultivation technology of mint species, and on the basis of the agrochemical analysis of the soil, the land was fertilized with a dose of nitrogen fertilizer DASA in the amount of 0.44 kg 20 m^2 on 5th of May 2016. Nonwoven fabric was applied on 9th of May 2019. From each variety 10 plants in a spacing 0.4 x 0.4 m were planted. Selected range of *Mentha* spp. was purchased through the Lumigreen internet store, which has the widest spectrum of the mint genus at Slovak market. After the planting, nitrogen in the form of ammonia (LAD) was applied at 0.64 kg.20 m^{-2} in two dosages in 2016 and in 0.50 kg.20 m^{-2} in two dosages in 2017. Additional irrigation was used as required. Pesticides during the vegetation period (both years) were not applied because the plants were not infected with diseases and pests and also the content of selected antioxidants was monitored.

Soil – climatic conditions

The plot area is situated in a very warm agro climatic area based on climatic conditions, which is characterized by warm lowland climate with long to very long, warm and dry summer, mild, dry to very dry winter with duration of snow cover from 30 to 40 days a year. The average annual rainfall in the area is 500 – 600 mm **(Hreško, Pucherová and Baláž, 2006)**. The average annual temperature varies between 9 – 10 °C. Average July temperatures are 18 °C to 20.5 °C and average January temperatures are -1 °C to -3° C. The wind prevails in the northwest; less frequent winds are east, northeast and west. Southwest, south and southeast winds are the least common **(Špánik, Repa a Šiška, 2002)**.

Characteristics of selected less grown mint varieties and forms

Mentha arvensis f. Banana (Figure 1) – A fast growing low variety comes from France. Light green leaves are small, oblong-ovate to elliptic, with sparsely saw – leaves edge, covered with simple trichomes. It blooms from June to September with purple flowers. It grows to a height of 45cm (60cm). It requires humid, permeable soil, sunny position, well tolerates by also half-shade, it is also suitable for growing in containers. It has a characteristic banana menthol flavour.

Mentha x piperita var. 'Danica'(Figure 2) – The ´Danica´ variety is 80 cm tall. The leaves are oblong-elliptical to lanceolate, toothed, 4 – 8 cm long and 2.5 – 3.5 cm broad, green to red-green. The flowers are 3 – 5 mm long, lilac, or pale violet, produced in clusters on tall, branched, tapering spikes; flowering from July till September. It spreads via rhizomes to form clonal colonies.

Mentha x piperita var. ´Chocolate´ (Figure 3) – mint variety grows up to 30 – 60 cm high. The leaves are shaggy, opposed, elongated, egg-shaped, lanceolate, with a slightly arched edge, bronze-green. The stem is reddish-brown. The cup is a cylindrical purple pike on the top of the stem. It blooms from June to September. It requires moist, nutritious and permeable soil, sunny position.

Mentha sp. f. Mojito (Figure 4) – The variety comes from Cuba, brought to Europe in 2006. It creates upright stems 45 – 60 cm high. The leaves are opposed, seated or short stems, pale green, markedly wrinkled, egg-shaped, with a cut base, the edge of the leaf blade is sharply arched. The flowers are white in colour; they have a conical to cylindrical shape. It blooms from July to September. It requires light, aerated and humus soil, plenty of soil moisture. Full Sun to Partial Shade. It is the main ingredient of the alcoholic beverage 'Mojito'.

Mentha sp. f. White Grape (Figure 5) – It is a fast growing variety with size up to 50 – 90 cm. The stems are red in the lower part. The leaves are opposed, on short stalks, dark green, wide to oval, markedly wrinkled, with petiole edges, cheeks and ruby covered with trichomes. The flowers are conical of pale violet colour. It blooms from July to September. Plants grow in moist, permeable, nutritious soils, in direct sunlight or in half-shade.

Harvesting and post-harvest treatment

The harvest of the mint was carried out twice a season for estimation of quantitative characteristics (yields). For qualitative parameters were used plants from second harvest in 31th of August in 2016, and 10th of August in 2017. The harvest was carried out mechanically (by knife); the whole plants were cut 10 cm above the surface of soil. After harvest each variety was prepared for analysis according to the chosen methodology **Hegedűsová, Mezeyová and Andrejiová (2015)**. The process of determination of photosynthetically active pigments and essential oils was carried out in the laboratories of the Research Institute AgroBioTech SUA in Nitra and of Department of Vegetable production, FHLE, SUA in Nitra.

Determination of quantitative and qualitative parameters

Estimation of essential oils content

In the dried drug the essential oils content was estimated by the distillation method by using distilled apparatus according to **(Hegedűsová, Mezeyová and Andrejiová, 2015)**.

Estimation of chlorophyll a and chlorophyll b content

The chlorophyll *a* and chlorophyll *b* were determined spectrophotometrically (Spektralquant PHARO 100) laterally in the acetone extract on the wavelengths 649 nm and 665 nm in homogenisated fresh plant (150 – 200 g) **(Hegedűsová, Mezeyová and Andrejiová, 2015)**. Number of analysed samples for average content of chlorophyll *a* and *b* was 10 in case of each variety.

Estimation of total carotenoids content

The extraction of samples was done at the Laboratory of Beverages, AgroBioTech Research Center, Slovak University of Agriculture (SUA) in Nitra. The content of total carotenoids was estimated by spectrophotometric measurement of substances absorbance in petroleum ether extract on spectrophotometer PHARO 100 at 450 nm wavelengths **(Hegedüsová, Mezeyová and Andrejiová, 2015)**.

Statistic analysis

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

RESULTS AND DISCUSSION

The content of essential oils was ranged from 0.67 ±0.30 (White Grape) to 3.10 ±0.39 mL.100 g^{-1} (´Danica´) in average from both tested years according to Table 1. The varietal variability was confirmed according to used statistical analyzes (p <0.05) between tested mint varieties, when F. Banana (0.86 ±0.05 mL.100 g^{-1}) and White Grape had the lowest content of the essential oils and ´Danica´ reached the highest value. Generally used *Mentha* x *piperita* reached higher value of essential oils in comparison with f. Banana and f. White Grape. The other varieties ´Chocolate´, ´Danica´ and f. Mojito were richer in essential oils, what was also statistically confirmed. Varietal variability was confirmed also by **Boukhebti et al. (2011)** where *Mentha spicata* had 1% off essential oils and *Mentha pulegium* 0.87%. Similarly the highest content of essential oils was found in *Mentha spicata* L. ‘Moroccan’(0.82%) and *Mentha piperita* L. ‘Glacialis’ leaves, while the least in *Mentha suaveolens* Ehrh. ‘Variegata’ (0.07%) **Tarasevičienė et al. (2019)**. **Hussain et al. (2010)** determined that amount of essential oil depended on species and cultivation time of mint. The authors found that amount of essential oil in *M. arvensis* was 17.0 g $kg^{−1}$, *M. piperita* 12.2 g $kg^{−1}$, *M. longifolia* 10.8 g $kg^{−1}$ and *M. spicata* 12.0 g $kg^{−1}$ in summer grown plants, respectively 9.20, 10.5, 7.00 and 9.50 g $kg^{−1}$ in the winter crops. The influence of climatic condition, cultivation and soil composition was obvious in our results, when significant difference was found between tested years in essential oils content in tested varieties at p <0.05 (Table 1).

According to Table 2 the chlorophyll *a* content estimated in fresh matter ranged in average from 84.74 ±4.49 mg.100 g^{-1} in case of f. Banana to 125.54 ±0.48 mg.100 g^{-1} (*Mentha* x *Piperita*). Second light coloured variety f. Mochito had also low level of chlorophyll *a* content with reached value 87.92 mg.100 g^{-1}. Generally used *Mentha* x *Piperita* reached highest values in comparison with all tested forms and varieties, the significant difference was confirmed (p <0.05) with f. Mojito, f. White Grape and f. Banana. Also **Straumite, Kruma and Galoburda (2015)** monitored the effect of varietal variability on the plant pigment content in leaves and stems of nine different species and mint varieties. The highest chlorophyll *a* content in leaves was reached by *M.* x *piperita* L. var. ´Bavarian´ (0.849 mg.g^{-1} of fresh matter) and the lowest *M.* x *piperita* L. var. ´Almira´ (0.321 mg.g^{-1}), with difference of 164%. The ´Chocolate´ mint variety contained 0.361 mg.g^{-1} (36.1 mg.100g^{-1}) of chl *a* in fresh matter. Because of differences in comparison with our results it is needed to say, that the chlorophyll content in fresh plant matter varies depending on the place of cultivation, its climatic conditions, the soil composition, the date of cutting, etc. **(Bohn et al., 2006)**. It is obvious f. e. on the statistically confirmed influence of the year on chlorophyll *a* according to values of our trial (Table 2). **Tewari et al. (2012)** tested the chlorophyll *a* content in case of *Ocimum kilimandscharicum*, where 96.57 mg.100 g^{-1} was measured what is comparable with the our results of light mints like f. Banana or f. Mochito.

The chlorophyll *b* content estimated in fresh matter moved in order 41.86 ±1.39 mg.100 g^{-1} (f. Banana) <47.63 ±2.25 mg.100 g^{-1} (f. Mojito) <53.32 ± 3.54 mg.100 g^{-1} (f. White Grape) <59.97 ± 15.95 mg.100 g^{-1} (´Danica´) <62.62 ±5.48 mg.100 g^{-1} (´Chocolate´) <to 67.40 ±10.51 mg.100 g^{-1} (*Menth*a x *Piperita*) according to Table 3. The difference in varietal variability of tested forms and varieties was statistically confirmed at p <0.05. **Grzeszczuk and Jadczak (2009)** estimated the biological value of fresh matter in five different mint varieties and species, with the highest chl *b* content in case of *M. aquatica* L. – 519.14 mg.kg^{-1} (51.914 mg.100g^{-1}) and the lowest in *M.* x *piperita* L. var. citrata Ehrh. with 325.55 mg.kg^{-1}, (32.56 mg.100g^{-1}). According to **Tarasevičienė et al. (2019)** the amount of chlorophyll *b* in *Mentha piperita* ‘Glacialis’ was 6.6-times higher than in *Mentha piperita* ‘Swiss’. Total amount of chlorophylls in leaves was the highest in *Mentha piperita* ‘Glacialis’ (0.376 ±0.014 mg.g $^{-1}$ FW) and *Mentha suaveolens* ‘Variegata’ (0.307 ±0.011 mg.g^{-1} FW), while in *Mentha piperita* ‘Swiss’ the lowest (0.057 ±0.002 mg.g^{-1} FW).

According to Table 4 total carotenoids content moved in 2 – years average from 5.80 ±5.54 mg.100 g^{-1} FM (f. Banana) to 10.38 ±5.16 mg.100 g^{-1} FM (f. Danica).

Table 1 The content of the essential oils in selected mint genus representatives [mL.100 g^{-1} DM]*.

variety/form	2016^{A}	2017^{B}	average
´Danica´	2.83 ±0.25	3.37 ±0.10	3.10 ±0.39^{d}
´Chocolate´	2.73 ±0.25	2.74 ±0.16	2.73 ±0.01^{cd}
f. Banana	0.83 ±0.11	0.90 ±0.01	0.86 ±0.05^{a}
f. White Grape	0.45 ±0.21	0.88 ±0.03	0.67 ±0.30^{a}
f. Mojito	1.68 ±0.04	3.14 ±0.03	2.41 ±1.04^{c}
Mentha* x *Piperita	1.75 ±0.14	1.27 ±0.06	1.51 ±0.34^{b}

Note: a, b, A, B – Different letters in the upper index represent a statistically proven difference (p <0.05, LSD test, ANOVA), Statgraphic XVII.
*average ± standard deviation, DM = dry matter.

Table 2 The content of the chlorophyll *a* in selected mint genus representatives [mg.100 g^{-1} FM]*.

variety/form	2016^{A}	2017^{B}	average
´Danica´	143.12 ±15.40	82.00 ±5.66	112.56 ±43.22^{bc}
´Chocolate´	128.14 ±9.74	103.29 ±2.59	115.71 ±17.57^{bc}
f. Banana	87.91 ±1.60	81.56 ±1.09	84.74 ±4.49^{a}
f. White Grape	112.32 ±1.04	92.00 ±4.28	102.16 ±14.37^{ab}
f. Mojito	91.71 ±4.44	84.14 ±4.93	87.92 ±5.36^{a}
Mentha* x *Piperita	125.88 ±19.46	125.21 ±5.41	125.54 ±0.48^{c}

Note: a, b, A, B – Different letters in the upper index represent a statistically proven difference (p <0.05, LSD test, ANOVA), Statgraphic XVII.
*average ± standard deviation, FM = fresh matter.

Table 3 The content of the chlorophyll *b* in selected mint genus representatives [mg.100 g^{-1} FM]*.

variety/form	2016^{A}	2017^{A}	average
´Danica´	71.25 ±19.38	48.70 ±3.88	59.97 ±15.95^{bcd}
´Chocolate´	66.49 ±3.34	58.74 ±1.47	62.62 ±5.48^{cd}
f. Banana	40.87 ±2.33	42.84 ±0.06	41.86 ±1.39^{a}
f. White Grape	55.82 ±0.87	50.82 ±2.34	53.32 ±3.54^{abc}
f. Mojito	46.04 ±3.95	49.22 ±3.46	47.63 ±2.25^{ab}
Mentha* x *Piperita	59.97 ±4.81	74.84 ±1.92	67.40 ±10.51^{d}

Note: a, b, A, B – Different letters in the upper index represent a statistically proven difference (p <0.05, LSD test, ANOVA), Statgraphic XVII.
*average ± standard deviation, FM = fresh matter.

Table 4 The content of the carotenoids in selected mint genus representatives [mg.100 g^{-1} FM]*.

variety/form	2016^{A}	2017^{A}	average
´Danica´	14.02 ±3.19	6.73 ±0.07	10.38 ±5.16^{a}
´Chocolate´	5.16 ±0.49	13.09 ±1.33	9.13 ±5.60^{a}
f. Banana	9.72 ±0.26	1.88 ±1.32	5.80 ±5.54^{a}
f. White Grape	13.61 ±1.23	3.16 ±1.29	8.38 ±7.39^{a}
f. Mojito	10.72 ±0.07	2.33 ±0.54	6.52 ±5.94^{a}
Mentha* x *Piperita	6.39 ±0.01	14.89 ±0.01	10.64 ±6.01^{a}

Note: a, b, A, B – Different letters in the upper index represent a statistically proven difference (p <0.05, LSD test, ANOVA), Statgraphic XVII.
*average ± standard deviation, FM = fresh matter.

Figure 1 *Mentha arvensis* f. Banana.

Figure 2 *Mentha x piperita* var. 'Danica'.

Figure 3 *Mentha* x *piperita* var. 'Chocolate'.

Figure 4 *Mentha sp*. f. Mojito.

Figure 5 *Mentha sp.* f. White Grape.

The values differed, but according to used statistical test (p <0.05) the significant varietal variability on carotenoids for tested varieties and forms wasn´t confirmed. Carotenoids content in the mint plants differed in relation to species according to **Tarasevičienė et al. (2019)** where for *Mentha piperita* 'Glacialis' leaves the amount of carotenoids was the highest (0.310 ±0.005 mg.g^{-1} FW) and in *Mentha piperita* 'Swiss' the lowest (0.053 ±0.002 mg.g^{-1} FW) with significant varietal variability at p <0.05. **Straumite, Kruma and Galoburda (2015)** on the basis of the measurements stated the highest value of carotenoids 16.9 mg.100g^{-1} in stems of *Mentha spicata* ´Marokko´ and the lowest in *Mentha* x *piperita* ´Granada´ leaves (3.8 mg.100g^{-1}). **Rubinskienė et al. (2015)** tested in addition to the content of carotenoids also the effect of different drying methods on the chemical composition and colour of leaves in two varieties of peppermint. For the ´Peppermint´ variety the total carotenoid content was 5.7 mg.100 g^{-1} of fresh matter and for the ´Krasnodarskaja´ variety 5.8 mg.100 g^{-1}. All mentioned results are similar to our estimations.

CONCLUSION

The new mint varieties are characterized by a different flavour and aroma, which is directly connected with qualitative bioactive substances, especially with the quality and quantity of essential oils. On the basis of obtained results the significant effect (p <0.05) of varietal variability was found on the essential oils content when the highest values reached *Mentha* x *piperita* var. ´Danica 'and *Mentha* x *piperita* var. ´Chocolate´. *Menta sp.* f Mojito has also reached a higher value than the commonly available *Mentha* x *piperita*, so it can be recommended for intense cultivation not only as an interesting mint in the popular drink, but also because of the high content of essential oils. In view of other the monitored parameters, *Mentha* x *piperita* reached the highest content in case of chlorophyll *a* (125.54 mg.100 g^{-1} FM) and chlorophyll *b* (67.40 mg.100 g^{-1} FM), as well as in the case of carotenoids (10.64 mg.100 g^{-1} FM). ´Danica´ and ´Chocolate´ varieties reached very similar values of chlorophyll *a*, *b* and carotenoids compared to *Mentha* x *piperita*, the differences were not statistically significant at p <0.05. In the case of f. Banana, f. White Grape and f. Mochito the significant differences have been found and they reached lower values in the contents of monitored qualitative characteristics.

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