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Pigments in mint leaves and stems

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Abstract. Mint is the genus belonging to the *Labiatae* family and includes a huge diversity of varieties with different sensory properties. An important quality parameter is its colour, and the compounds responsible for it are pigments such as chlorophyll *a* and *b*, carotenoids etc. The aim of the current research was to determine the pigment content in the leaves and the stems of different mint varieties grown in Latvia. Mint of nine varieties (*Mentha suaveolens* 'Apple mint', *Mentha suaveolens* 'Variegata', *Mentha spicata* 'Marokko', *Mentha piperita* 'Swiss', *Mentha piperita* 'Granada', *Mentha piperita f. citrate* 'Grapefruit', *Mentha piperita* 'Chocolate', *Mentha piperita* 'Almira', *Mentha piperita* 'Bavarian') collected in Latvia was analysed. Chlorophyll *a*, chlorophyll *b* and carotenoids were determined spectrophotometrically in the acetone extracts of fresh leaves and stems at various wavelength 470, 645 and 662 nm. To analyse a relationship between chlorophyll *a* and *b* in the leaves and the stems a calculation of ratio *a*/*b* was performed. Additionally the colour of samples was measured in CIE L*a*b* system. For the statistical analysis – linear correlation, analysis of variance, hierarchical cluster analysis was performed. The differences were considered significant at $P < 0.05$. Among studied mint leaves the highest content of chlorophyll *a* and *b*, carotenoids was determined in the 'Bavarian' mint. The colour component L* value for variety 'Bavarian' leaves was one of the lowest among studied samples (showing a darker colour intensity). The stems of the mint variety 'Bavarian' had a high content of chlorophyll especially chlorophyll *b*. The highest content of carotenoids was determined in *Mentha spicata* 'Marokko'. Analysing a relationship between the colour components L*a*b* and the content of pigments no significant correlations were determined.

Key words: mint varieties; chlorophyll *a*; chlorophyll *b*; colour.

INTRODUCTION

Peppermint (*Mentha*×*piperita* L.), belonging to the *Labiatae* family, is a large family of annual or perennial, herbaceous plants of 30–100 cm hight, which is cultivated in temperate climates, in America, Europe and Asia (Arslan et al., 2010). Peppermint (*Mentha piperita* L.) is a natural hybrid of water mint (*Mentha aquatica* L.) and spearmint (*Mentha spicata* L.) and is cultivated globally for its use as a flavouring in foods and also in some shampoos and soaps (Tarhan et al., 2010). Mint is one of the most important and common flavours in the world coming after vanilla and citrus flavours (Arslan et al., 2010). Peppermint oil and some of its constituents is known for antimicrobial and antioxidant properties. Peppermint is one of the most widely consumed single ingredients in herbal teas, and the essential oil of peppermint is used in traditional medicine (Lv et al., 2012). Peppermint has significant antimicrobial and antiviral activities, strong antioxidant and antitumor actions, and exhibits some antiallergenic potential (Skalicka-Woźniak & Walasek, 2014).

Among the major components found in peppermint leaves are fatty acids such as linoleic, linolenic, and palmitic acid. A variety of volatile compounds, mainly menthol, menthone and isomenthone have also been identified along with β-carotene, chlorophyll, α- and γ-tocopherols and ascorbic acid (Figueroa Pérez et al., 2014).

Plant pigment is a generic expression used to designate a large number of coloured molecules. On the basis of their chemical structure, they can be classed into 5 families i.e. tetrapyrroles (e.g. chlorophyll), carotenoids (e.g. β -carotene), phenolic compounds (e.g. teaflavin) and N-heterocyclic compounds (e.g. betalains) (Schoefs, 2002). Appreciable amounts of carotenoids are present in fresh tea leaves, but this value is greatly decreased during tea processing, leading to various degradation products (Ravichandran, 2002). In a Japanese study on green teas there were detected 38 carotenoids (6 of them were unknown) (Suzuki & Shioi, 2003). Among these tea pigments, pheophytins a and b were abundant, followed by chlorophylls *a* and *b*, and carotenoids such as β-carotene and lutein in lower concentrations. All these pigments exhibited significant antioxidant activities against hydroperoxide generation, in the order chlorophyll *a* > lutein > pheophytin a > chlorophyll *b* > β-carotene > pheophytin b (Loranty et al., 2010). Chlorophylls and carotenoids are frequent organic food components because they are naturally present in plants, giving their specific colouration. *In vivo*, these pigments play key roles in photosynthesis (Schoefs, 2002). Chlorophyll is the principal photoreceptor in photosynthesis, the light-driven process by which carbon dioxide is fixed to yield carbohydrates and oxygen. While carotenoids are a class of natural fat-soluble pigments found principally in plants, algae and photosynthetic bacteria, where they play a critical role in the photosynthetic process (Ong &Tee, 1992) and also protect chlorophyll from a photooxidative destruction (Siefermann-Harms, 1987; Giri et al., 2013). An important quality parameter of peppermint is its colour, and the compounds responsible for it are pigments such as chlorophyll *a* and *b*, carotenoids etc. There are only few papers about pigments in herbs and teas (Loranty et al., 2010).

The aim of the current research was to determine the pigment content in the leaves and the stems of different mint varieties grown in Latvia.

MATERIALS AND METHODS

Plant materials

Mint samples were grown in Latvia, Jelgava region (latitude – 56° 35’ N; longitude – 23° 17’ E) and harvested in September 2014. In experiments 9 mint varieties – *Mentha suaveolens* ‘Apple mint’, *Mentha suaveolens* ‘Variegata’, *Mentha spicata* ‘Marokko’, *Mentha piperita* ‘Swiss’, *Mentha piperita* ‘Granada’, *Mentha piperita f. citrate* ‘Grapefruit’, *Mentha piperita* ‘Chocolate’, *Mentha piperita* ‘Almira’, *Mentha piperita* ‘Bavarian’ were collected and stored at 4 ± 1 °C in polyethylene bags until analysis (less than 24 hours).

Determination of chlorophyll *a*, chlorophyll *b* and carotenoids

A 0.4 g sample of plant material was homogenized, extracted with 10 ml of acetone in a conical flask with a magnetic stirrer at 700 rpm for 15 minutes at room temperature. The supernatant was separated and the extraction was repeated. The extraction process was done in triplicate. The solution was analysed for chlorophyll *a* (Ch *a*), chlorophyll *b* (Ch *b*) and carotenoids content by a spectrophotometer JENWAY 6300 at various wavelengths 470, 645 and 662 nm using a glass cuvette. The equations used for the quantification are given in Table 1. Results were expressed as mg g^{-1} of plant material.

Table 1. The equations for calculation of chlorophyll *a*, chlorophyll *b* and the total carotenoids content (Sumanta et al., 2014)

Parameter, units	Equations*
Chlorophyll *a,* mg ml^{-1}	$C_{Cha} = 12.25\ A_{662} - 279\ A_{645}$
Chlorophyll *b,* mg ml^{-1}	$C_{Chb} = 21.5\ A_{645} - 5.1\ A_{662}$
Total chlorophyll, mg ml^{-1}	$C_{Cht} = C_{Cha} + C_{Chb}$
Ratio between chlorophyll *a* and *b*	$R\ a/b = \frac{C_{Cha}}{C_{Chb}}$
Total carotenoids, mg ml^{-1}	$C_{Ca} = \frac{1000 A_{470} - 1.82\ C_{Cha} - 85.02\ C_{Chb}}{198}$

* In column Equations following abbreviations are used: C_{Cha} – concentration of chlorophyll *a* in extract; C_{Chb} – concentration of chlorophyll *b* in extract; C_{Cht} – concentration of total chlorophyll in extract; C_{Cca} – concentration of carotenoids in extract; A_{662} –absorbance of the extract at wavelength 662 nm; A_{645} – absorbance of the extract at wavelength 645 nm; A_{470} – absorbance of the extract at wavelength 470 nm.

Colour analysis of mint leaves

Colour of mint was measured in CIE L*a*b* colour system using a colorimeter *ColorTec PCM* (Accuracy Microsensors Inc., USA). Before the measurement, the colorimeter was calibrated using a white reference tile and a light trap (black tile). Ten random leaves were measured and the mean values were reported for each sample. In colour measurement, CIE L*a*b* coordinates show the degree of brightness (L), the degree of redness (+a), or greenness (–a), and the degree of yellowness (+b), or blueness (–b), respectively (Tarhan et al., 2010).

Statistical analysis

The results (mean, standard deviation, *P* value) were processed by mathematical and statistical methods. For the statistical analysis - linear correlation, analysis of variance, hierarchical cluster analysis was performed. Significance was defined at $P < 0.05$. Hierarchical cluster analysis was performed to distinguish similar or close species. The method used was linkage between groups. The distances between samples were calculated using square Euclidean distances. As the pre-treatment of data, transformation of values of variables (average zero and standard deviation 1) called *Z* scores was carried out. The dendrogram similarity scales generated by the SPSS program ranged from zero (greater similarity) to 25 (lower similarity).

RESULTS AND DISCUSSION

Chlorophyll a and chlorophyll b analysis

Chlorophyll *a* and b differed between all the tested mint samples (Table 2). Other scientists also reported that changes in the colour and the content of chlorophylls were related to the genotype but not to the growing conditions (Bekhradi et al., 2015). The highest content of chlorophyll *a*, chlorophyll *b* and total chlorophyll in the 'Bavarian' mint leaves was observed 0.849, 0.179 and 1.028 mg g^{-1} respectively.

Table 2. Chlorophyll in mint leaves and stems

Plant material		Chlorophyll, mg g^{-1}			Ratio $a\ b^{-1}$
		Ch *a*	Ch *b*	Ch total	
Mentha suaveolens 'Apple mint'	Leaves	0.525 ± 0.001	0.071 ± 0.001	0.596	7.41
	Stems	0.042 ± 0.002	0.054 ± 0.006	0.097	0.78
Mentha suaveolens 'Variegata'	Leaves	0.351 ± 0.006	0.075 ± 0.002	0.427	4.67
	Stems	0.077 ± 0.004	0.068 ± 0.002	0.145	1.12
Mentha spicata 'Marokko'	Leaves	0.582 ± 0.009	0.153 ± 0.004	0.736	3.79
	Stems	0.050 ± 0.001	0.105 ± 0.009	0.155	0.48
Mentha piperita 'Swiss'	Leaves	0.507 ± 0.021	0.066 ± 0.001	0.574	7.64
	Stems	0.084 ± 0.002	0.074 ± 0.005	0.158	1.13
Mentha piperita 'Granada'	Leaves	0.518 ± 0.022	0.087 ± 0.001	0.606	5.94
	Stems	0.106 ± 0.001	0.093 ± 0.004	0.199	1.14
Mentha piperita f. citrate 'Grapefruit'	Leaves	0.519 ± 0.013	0.073 ± 0.005	0.592	7.14
	Stems	0.063 ± 0.002	0.091 ± 0.008	0.154	0.70
Mentha piperita 'Chocolate'	Leaves	0.361 ± 0.013	0.076 ± 0.003	0.437	4.75
	Stems	0.077 ± 0.001	0.078 ± 0.006	0.155	0.99
Mentha piperita 'Almira'	Leaves	0.321 ± 0.005	0.072 ± 0.001	0.393	4.49
	Stems	0.108 ± 0.007	0.073 ± 0.002	0.181	1.48
Mentha piperita 'Bavarian'	Leaves	0.849 ± 0.020	0.179 ± 0.008	1.028	4.74
	Stems	0.107 ± 0.004	0.157 ± 0.006	0.264	0.68

Other authors reported chlorophyll content in dry tea of *M. piperita* 75.2 µg g^{-1} (Loranty et al., 2010), but there was not more detailed description available about analysed *Mentha* species. For the fresh samples similar results were obtained for dill that contains 144 mg 100 g^{-1} chlorophyll (Lisiewska et al., 2004). The major chlorophylls in plants include chlorophyll *a* and chlorophyll *b*, which are usually present at a ratio of 3 (Chen & Chen, 1993). In the tested mint leaves a ratio between chlorophyll *a* and chlorophyll *b* ranged from 3.79–7.64, meaning that chlorophyll *a* is the main form of chlorophyll in the leaves. In *M. officinalis* tea significant levels of chlorophyll *b* were also present (72.9 µg g^{-1} dry tea from Tetley) and intra-species variation in chlorophyll *b* levels was seen to occur depending on the source company of tea (Loranty et al., 2010). In the stems chlorophyll content was significantly lower than in the leaves.

In the stems a ratio between chlorophyll *a* and chlorophyll *b* was lower and ranged from 0.48–1.48, meaning that chlorophyll *b* is more significant pigment in the stems. Analysing relationships between the composition of chlorophylls in the leaves and the stems several trends were observed. A strong correlation between the content of chlorophyll *b* in the tested leaves and stems was observed (r = 0.89), whereas for chlorophyll *a* such tendency was not observed (r = 0.06).

Carotenoids analysis

The basic pigments of green plants are chlorophylls, always accompanied by carotenoids. The highest content of carotenoids was observed in *Mentha spicata* ‘Marokko’ stems and leaves, 16.9 and 10.3 mg 100 g^{-1} respectively (Fig.1). According to various authors the content of carotenoids ranges from 7.2 to 36.4 mg per 100 g of fresh matter of leafy vegetables (Ben-Amotz & Fischler, 1998; Kmiecik & Lisiewska, 1999). Carotenoids content in other herbs like dills are significantly higher 30.3 mg 100 g^{-1} (Lisiewska et al., 2004)

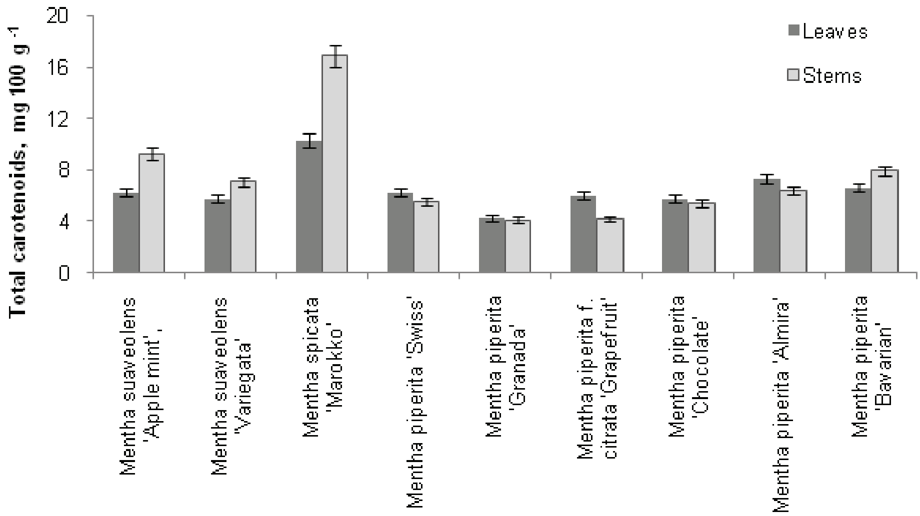

Figure1. Carotenoid content in mint leaves and stems.

Carotenoid content differed between all the tested mint samples. In leafy *Lactuca sativa* salads also large differences were observed in carotenoids concentrations among the different cultivars with an almost identical phenotype (Reif et al., 2013). In part of samples significantly higher ($P < 0.05$) concentration of carotenoids in the stems was observed (*Mentha suaveolens* ‘Apple mint’ *Mentha suaveolens* ‘Variegata’ *Mentha spicata* ‘Marokko’ *Mentha piperita* ‘Bavarian’). Significantly higher ($P < 0.05$) content of carotenoids in the leaves only in *Mentha piperita* f. citrata ’Grapefruit’ was determined. For other samples differences between the leaves and the stems were not significant ($P > 0.05$).

Colour analysis of mint leaves

Colour parameters of the mint samples presented the significant differences between species (Table 3). *Mentha suaveolens* ‘Apple mint’ and *Mentha piperita* ‘Swiss’ mint is lighter (higher L* value) more green (a* parameter is more negative) and more yellow (higher value parameter b*) compared to other samples. The darkest mint samples were *Mentha piperita* ‘Granada’, *Mentha piperita* ‘Almira’, *Mentha piperita* ‘Bavarian’ (lower L* parameter).

Table 3. Colour characteristics of the mint samples

Plant material	Colour parameter values		
	L*	a*	b*
Mentha suaveolens 'Apple mint'	39.44 ± 3.59	-10.70 ± 0.54	18.27 ± 0.73
Mentha suaveolens 'Variegata'	38.60 ± 2.97	-9.50 ± 0.68	16.03 ± 0.70
Mentha spicata 'Marokko'	38.33 ± 2.13	-8.10 ± 0.40	13.68 ± 0.85
Mentha piperita 'Swiss'	40.04 ± 2.86	-9.12 ± 0.83	18.62 ± 0.93
Mentha piperita 'Granada'	32.36 ± 1.80	-3.99 ± 0.29	9.13 ± 0.38
Mentha piperita f. *citrata*'Grapefruit'	36.88 ± 2.84	-8.92 ± 0.59	14.96 ± 1.07
Mentha piperita 'Chocolate'	35.23 ± 2.94	-5.96 ± 0.24	10.61 ± 0.62
Mentha piperita 'Almira'	32.80 ± 1.49	-8.91 ± 0.47	18.47 ± 1.09
Mentha piperita 'Bavarian'	32.44 ± 1.35	-4.93 ± 0.38	12.50 ± 0.66

Correlation analysis was performed to determine the relationship between colour parameters and pigment concentration. Carotenoids are in colour range from yellow to dark red. In mint there was weak (r = 0.25) correlation between the content of carotenoids and colour b* parameter. There was also weak negative correlation between the lightness and the concentration of chlorophyll *a*, chlorophyll *b* and total chlorophyll, similarly also between the a* colour parameter and the chlorophyll content.

Hierarchical cluster analyses

Hierarchical cluster analysis was applied to a data set of three variables (chlorophyll *a*, chlorophyll *b*, and carotenoids) and nine mint species. According to the hierarchical cluster analysis, mints can be grouped as follows:

- the cluster A – *Mentha spicata* 'Marokko' with the highest content of carotenoids and also high content of chlorophyll *a*, chlorophyll *b*;
- the cluster B – *Mentha piperita* 'Bavarian' with the highest content of chlorophyll *a*, chlorophyll *b*, and average content of carotenoids;
- the cluster C – all other studied mint samples.

Additionally hierarchical cluster analysis was applied to a data set of colour parameters (L*, a*, b*) and according to these parameters mints can be classified in three clusters:

- the cluster A – *Mentha piperita* 'Almira' with darker colour and more yellow and green colour intensity;
- the cluster B – *Mentha piperita* 'Granada', *Mentha piperita* 'Chocolate', *Mentha piperita* 'Bavarian' with darker colour and less intense yellow and green colour;
- the cluster C – all other studied mint samples.

CONCLUSIONS

The content of pigments and the colour of analysed mint species differed significantly. The highest content of chlorophyll *a*, chlorophyll *b* and total chlorophyll in *Mentha piperita* 'Bavarian' mint leaves was observed, whereas *Mentha spicata* 'Marokko' has the highest content of carotenoids. There was not established correlation between the concentration of pigments and the parameters characterizing colour.

Hierarchical cluster analysis can be used to differentiate the mint species according to the pigment concentration and the colour parameters.

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