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Betaxanthin Profiling in *Beta vulgaris* Leaves and *Gymnocalycium mihanovichii* Grafted Cacti: A Comprehensive Study

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This study was focused on the identification and quantification of betaxanthins using high-performance liquid chromatography with a diode array detector coupled to electrospray ionisation mass spectrometry (HPLC-DAD-ESI-MS) in leaves of various cultivars of *Beta vulgaris* (beet) and *Gymnocalycium mihanovichii* grafted cacti. In *G. mihanovichii* grafted cacti, four betaxanthins, namely histidine-Bx, histamine-Bx, serine-Bx, and proline-Bx, were tentatively identified in the yellow, orange, pink, and red varieties, with contents ranging from 0.09 to 1.55 mg/kg fresh weight (FW). Betaxanthins were not detected in the green cactus. Histidine-Bx was the prevailing betaxanthin compound in the majority of cultivars. Fifteen betaxanthins were successfully identified in the leaves of five *B. vulgaris* cultivars (cv.): Snow Ball, Boldor, Cylindra, Rhubarb, and Round Dark Red. Leaves of yellow beet (cv. Boldor) had the highest total betaxanthin content (20.4 mg/kg FW), while white beet (cv. Snow Ball) had the lowest one (3.43 mg/kg FW). The leaves of red cultivars had comparable betaxanthin contents, ranging from 13.4 to 18.8 mg/kg FW, similarly to the yellow cultivar, indicating their potential as valuable sources of betaxanthins. There was no single dominant betaxanthin in *B. vulgaris* leaves. The leaves of *B. vulgaris* were found to be a richer source of betaxanthin than the grafted cactus *G. mihanovichii*.

Key words: beet, betalains, colorants, grafted cactus, LC-MS, secondary metabolites

INTRODUCTION

Nowadays, as society's interest in healthy nutrition continues to grow, food producers are actively searching for safe alternatives to synthetic food dyes, which frequently raise concerns regarding potential adverse health effects. Natural plant-based pigments are emerging as highly appealing options, not only for the absence of their harmful effects but also for their valuable health-promoting properties. Betalains, anthocyanins, carotenoids, and chlorophyll represent four essential groups of plant pigments abundantly found in nature, playing a significant role in maintaining an excellent overall state of health [Cai *et al*., 2005a; Manzoor *et al*., 2021; Stintzing *et al*., 2002].

Betalains, constituting a group of secondary metabolites found in plants of the Caryophyllales order, exhibit structural diversity, enabling their categorization into two groups: red-violet betacyanins and yellow-orange betaxanthins [Wybraniec *et al*., 2010]. They exert a significant influence on human health due to their various bioactivities, including antioxidant, antibacterial, anticancer, antiviral, and anti-inflammatory potential [Naseer *et al*., 2019]. Betacyanins derive from betanidin, an imine adduct of betalamic acid and *cyclo*-3,4-dihydroxyphenylalanine (*cyclo*-DOPA). Conversely, betaxanthins can be synthesized through the condensation of amino acids or amines with betalamic acid [Gengatharan *et al*., 2015]. It is worth highlighting that

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betaxanthins can function as a valuable dietary source of essential amino acids [Cai *et al*., 2001].

The only commercial source of betalains is the root of red beet (*Beta vulgaris* L.), which provides pigments in various shades of red and purple betacyanins as well as yellow and orange betaxanthins [Stintzing *et al*., 2002]. Currently, there are only few other known sources of betaxanthins, including the roots of yellow varieties of *B. vulgaris* [Spórna-Kucab *et al*., 2023], flowers of *Portulaca grandiflora* Hook. [Spórna-Kucab *et al*., 2022], fruits of *Hylocereus polyrhizus* [Wybraniec *et al*., 2009], yellow fruits of *Stenocereus pruinosus* [Sandate-Flores *et al*., 2020], yellow pulp of *Opuntia ficus-indica* [Fernández-López *et al*., 2018], tuber skin of *Ullucus tuberosus* Caldas [Mosquera *et al*., 2020], pure yellow genotype of *Celosia argentea* var. *plumosa* [Cai *et al*., 2005b], orange-red genotype of *Celosia argentea* var. *cristata* [Cai *et al*., 2005b], and leaves of *Amaranthus tricolor* L. [Cai *et al*., 2005b]. However, there are promising alternative sources of betaxanthins that have not been studied yet, such as the leaves *of B. vulgaris*.

B. vulgaris roots, commonly known as beetroots, rightfully earn the title of a superfood due to their numerous health benefits and their rich content of biologically active compounds, including betaxanthins, betacyanins, carotenoids, and flavonoids [Bangar *et al*., 2022; Székely & Máté, 2022]. Their bioactive compounds have been demonstrated capable of inhibiting the growth of specific types of cancer cells, scavenging free radicals, mititgating harmful cholesterol levels in the bloodstream, and alleviating inflammation within the body [Bangar *et al*., 2022; Székely & Máté, 2022]. Undoubtedly, one of the most significant advantages of beetroot is the ease of its cultivation, widespread availability, and affordability. Moreover, beet leaves, often considered as mere waste, are increasingly becoming a staple in our diets, as they constitute a valuable source of the above-mentioned bioactive compounds [Székely & Máté, 2022].

Cacti are ornamental plants with a perennial nature, characterized by succulent stems and slow growth rates. They are known for their exceptional ability to survive in dry conditions, displaying a wide variety of shapes and sizes [Perumal *et al*., 2019].

Within a year, betalains derived from beetroot are capable of meeting up to 10% of the global demand for food pigments [Manchali *et al*., 2012; Sadowska-Bartosz & Bartosz, 2021]. The food coloring market is flourishing at an annual growth rate of 4.6%, with estimates projecting it to reach a global market value of 2.3 billion dollars [Prajapati & Jadeja, 2022]. However, cacti, particularly the grafted *G. mihanovichii*, can also function as a source of betacyanins. Utilizing these plants as an alternative betalain source can broaden the spectrum of colorants and pigments [Belhadj Slimen *et al*., 2017]. *G. mihanovichii* grafted cactus has played a notable role in the subtropical regions of South and North America, being responsible for an impressive percentage of cactus production, reaching up to 70% [Belhadj Slimen *et al*., 2017]. This high percentage constituted a significant portion of exported specimens, primarily focused on the markets of the Netherlands and the United States due to their unique colors and forms, which are highly valued as ornamental potted plants. Currently, they do not have significant applications in the food, medical, or industrial sectors. Nevertheless, it is worth noting that the potential use of this cactus as a source of yellow-orange betaxanthins could greatly expand its export opportunities in the food and medical markets [Manchali *et al*., 2012; Sadowska-Bartosz & Bartosz, 2021].

The presented research involved the chromatographic analysis of betaxanthin profiles of the leaves of *B. vulgaris* and grafted cacti of *G. mihanovichii*, utilizing the high-performance liquid chromatography with a diode array detector coupled to electrospray ionisation mass spectrometry (HPLC-DAD-ESI-MS) technique. The main objective of this study was to investigate alternative natural sources of food colorants, betaxanthins. It is important to note that similar studies have not been conducted to date. Previous investigations primarily focused on the betaxanthin profiles of *Beta vulgaris* L., *Chenopodium formosanum*, *Opuntia ficus-indica* L., and *Portulaca grandiflora* Hook. [Gamba *et al.,* 2021; Kugler *et al.,* 2004; Otálora *et al*., 2020; Spórna-Kucab *et al*., 2023, 2022; Xie & Chen, 2021].

MATERIALS AND METHODS

Reagents and reference compounds

Acetone and formic acid purchased from Avantor Performance Materials Poland S.A. (Gliwice, Poland) were used for the extraction process. The solvents used were of analytical grade. Liquid chromatography-mass spectrometry (LC-MS) grade methanol and formic acid with a minimum purity of 98% were acquired from Sigma-Aldrich (St. Louis, MO, United States). Deionized water, obtained through a Milli-Q purification system (Merck, Darmstadt, Germany), was utilized in the experiments.

Plant material and extraction process

Plant material from five different cultivars of *B. vulgaris* (Snow Ball, Boldor, Cylindra, Rhubarb, and Round Dark Red) and five varieties of *G. mihanovichii* grafted cacti (orange, green, yellow, red, and pink) was used in the study, as illustrated in Figure 1.

The beet seeds were purchased from the company W. Legutko, located in Jutrosin, Poland. *B. vulgaris* plants were cultivated in the botanical garden of the University of Agriculture in Cracow, Poland, from June to September 2022 by a unit specializing in plant cultivation. Orange, green, and yellow cacti were imported from a Dutch plantation, whereas the red and pink varieties originated from a Polish company named Tomaszewski in Warsaw. After harvesting, the leaves of beets, as well as the upper colored parts of the cacti, were washed, weighed, and directly subjected to the extraction process.

The leaves of beets as well as the upper peeled parts of cacti were individually blended in a household blender. Appropriate extraction procedures were employed to extract betaxanthins from 100 g of beets and cacti. The beets were extracted using the maceration method [Celli & Brooks, 2017], utilizing 200 mL of a 50% (*v/v*) aqueous acetone solution. In contrast, the cacti underwent extraction using 300 mL of a 50% (*v/v*) aqueous

Figure 1. *Beta vulgaris* of selected cultivars - Boldor and Snow Ball (**A**) and *Gymnocalycium mihanovichii* grafted cacti in colors: yellow, green, orange, pink, and red (**B**).

acetone solution enriched with 1% formic acid. The entire extraction process lasted for 90 min, maintaining ambient temperature and ensuring darkness. Upon completing the extraction, the obtained extracts were filtered under reduced pressure to eliminate potential impurities, then evaporated using a vacuum evaporator (Hei-VAP Advantage, Heidolph, Germany) at 25°C, and lyophilized in a freeze dryer (Christ, Osterode am Harz, Germany). After lyophilization, the resulting extracts were weighed. The obtained crude extracts, following appropriate preparation, were employed for analysis using UV-Vis spectroscopy and LC-MS techniques.

Spectrophotometric quantification of total betaxanthins

A quantitative analysis of the total betaxanthin content was conducted using spectrophotometry with the Tecan Infinite 200 microplate reader (Grödig/Salzburg, Austria) [Stintzing *et al*., 2003]. Triple measurements of absorption were performed for the extracts from both beets and cacti, dissolved in water, with each sample having a volume of 200 µL. Spectrophotometric measurements were carried out in the range of 350 to 750 nm with a 1 nm step at a temperature of 25°C. Total betaxanthin content (BC) was calculated for absorbance measured at 474 nm (λ_{max}) according to Equation (1):

$$BC = \frac{A \times DF \times MW \times 1{,}000}{\varepsilon \times l} \quad (1)$$

where: A is the absorbance, DF is the dilution factor, l is the path length (0.53 cm) of the microplate, ε is the molar absorption coefficient for betaxanthin (4.80×10^4 $cm^{-1}M^{-1}$), and MW is molecular weight (339 g/mol) [Stintzing *et al*., 2002]. Results were expressed in mg of pigment *per* 100 g of dry extract (DE) and in mg of betaxanthins *per* kg of fresh weight (FW) of plant material.

HPLC-DAD-ESI-MS analysis of betaxanthins

Before analysis, all samples were carefully diluted in demineralized water (15 mg *per* 500 µL of water) and briefly centrifuged at 3,000×g for 5 min using a centrifuge (Hermle Z323K, Gosheim, Germany). The betaxanthin profiles in beets and cacti were determined using the HPLC-DAD-ESI-MS technique (LCMS-8030 system, Shimadzu, Kyoto, Japan). The LC-MS system included a precise SIL-20ACXR autosampler, an efficient degasser, and a binary pump LC-20ADXR Nexera. Samples were separated using a Kinetex C18 chromatographic column (Phenomenex, Torrance, CA, United States) with dimensions of 100 mm length × 4.6 mm i.d., containing 5.0 µm particles and protected by a 4 mm length × 2 mm i.d. guard column of the same material (Phenomenex). The column temperature was maintained constant at 40°C. Analyses were carried out using a two-component gradient. The mobile phase consisted of methanol (A) and 2% formic acid in water (B). The flow rate was 0.5 mL/min, and 10 µL of the sample was injected for analysis. The solvent gradient system for extracts was as follows: 1% A in B at 0 min, gradient to 11% A in B at 12.0 min, 60% A in B at 24 min, and then gradient to 90% A in B at 24.01 min. UV/Vis spectra were collected using a DAD detector model SPD-M20A (Shimadzu).

In ESI-MS analyses conducted in the positive electrospray ionization mode, the capillary voltage was set at 4.5 kV, and the capillary temperature was maintained at 250°C. ESI-MS data were recorded in the scan mode with *m/z* ranging from 100 to 2,000 Da and the selected ion monitoring (SIM). LabSolution software version 5.91 SP1 (Shimadzu) was used for data acquisition in the HPLC-DAD-ESI-MS configuration.

Reference standards from *B. vulgaris* cv. Chrobry [Spórna-Kucab *et al*., 2023] and *P. grandiflora* Hook. extracts [Spórna-Kucab *et al*., 2022], which contained previously identified betaxanthins, were used to identify individual betaxanthins in the extracts.

The quantitative analysis of individual betaxanthins was carried out by determining peak areas from MS chromatograms of *B. vulgaris* and *G. mihanovichii* extracts. The total betaxanthin content in the examined extracts was previously determined through spectrophotometric method. All samples were analyzed in three independent LC-MS runs.

Statistical analysis

The data were presented as mean and standard deviation (SD) based on three independent analyses. Statistical analysis was conducted using Statistica software version 7.1 (StatSoft, TIBCO Software Inc., Palo Alto, CA, United States), employing one-way analysis of variance (ANOVA) and the Tukey post hoc test, with a significance level of α set at 0.05. *p*-Values below 0.05 were considered statistically significant. The statistical analysis was performed separately for *B. vulgaris* leaves and *G. mihanovichii* grafted cacti.

RESULTS AND DISCUSSION

Betaxanthins in *G. mihanovichii* grafted cacti

Studies on betalain profiles in their numerous sources demonstrate that betacyanins were often found in conjunction with betaxanthins [Cai *et al*., 2005a; Otálora *et al*., 2020; Spórna-Kucab *et al*., 2013, 2018, 2022, 2023; Wybraniec *et al*., 2010; Xie & Chen, 2021]. One of the sources of betacyanins are cacti. Previously, a total of 32 different betacyanins were identified in the red variety of *Gymnocalycium mihanovichii* cv. Hibotan scions [Wybraniec *et al*., 2010]. However, there is a lack of information regarding the presence of individual betaxanthins. Interestingly, according to a previous source [Wybraniec *et al*., 2010], the yellow-orange color is not attributed to the presence of betaxanthins but rather to the synthesis of carotenoids in cacti. In the mentioned study, no betaxanthins were detected in any of the analyzed violet, pink, and red cacti. Here, based on chromatographic, spectrophotometric, and mass-spectrometric data (Table 1), the presence of four polar betaxanthins was indicated in yellow, orange, red and pink varieties of *G. mihanovichii* grafted cacti: histidine-Bx (**1**), histamine-Bx (**2**), serine-Bx (**3**), and proline-Bx (**11**). The chemical structures of compounds **1** (predominant), **2** and **11** are shown in Figure 2. All the betaxanthins were solely identified in the red cactus variety, while they were absent in the green variety. Notably, the yellow and orange varieties lacked histamine-Bx (**2**), while the pink variety contained only histidine-Bx (**1**) and proline-Bx (**11**) (Figure 3). The samples displayed a range of total betaxanthin contents, varying from 0.09 to 1.55 mg/kg FW. The highest levels were determined in the red cactus (1.55 mg/kg FW) followed by the pink variety (1.29 mg/kg FW). Subsequently, the orange and yellow varieties had lower contents (0.22 and 0.09 mg/kg FW, respectively) (Table 2).

The total betaxanthin content determined in *G. mihanovichii* grafted cacti was lower than that of the yellow pulp of the *Opuntia ficus-indica* fruits, which was 275 mg/kg FW [Fernández-López *et al*., 2018]. Some similarities with *G. mihanovichii* grafted cacti were observed regarding the betaxanthin profile of the yellow

Table 1. Chromatographic, spectrophotometric, and mass-spectrometric data of the analyzed betaxanthins of leaf extracts of white, yellow and red *Beta vulgaris* cv. Snow Ball, Boldor, Cylinder, Rhubarb, and Round Dark Red, as well as *Gymnocalycium mihanovichii* grafted cactus (orange, green, yellow, red, and pink) extracts.

No.	Betaxanthin	Trivial name	t_R (min)	λ_{max} (nm)	*m/z* $[M+H]^+$
1	Histidine-Bx	Muscaaurin VII	4.5	470	349
2	Histamine-Bx		5.7	470	305
3	Serine-Bx		6.1	469	299
4	Glutamine-Bx	Vulgaxanthin I	6.2	467	340
5	Ornithine-Bx		6.7	465	326
6	Ethanolamine-Bx		7.0	454	255
7	Lysine-Bx		7.2	458	340
8	Glutamic acid-Bx	Vulgaxanthin II	8.4	469	341
9	Alanine-Bx		10.5	466	283
10	γ-Aminobutyric acid-Bx		12.2	454	297
11	Proline-Bx	Indicaxanthin	13.5	477	309
12	Valine-Bx		19.0	469	311
13	3-Methoxytyramine-Bx		19.6	471	361
14	Isoleucine-Bx	Isovulgaxanthin IV	21.0	469	325
15	Leucine-Bx	Vulgaxanthin IV	21.3	469	325
16	Tryptophan-Bx		22.0	473	398

Bx, betaxanthins; t_R, retention time; λ_{max}, absorption maximum wavelength; *m/z*, mass-to-charge ratio.

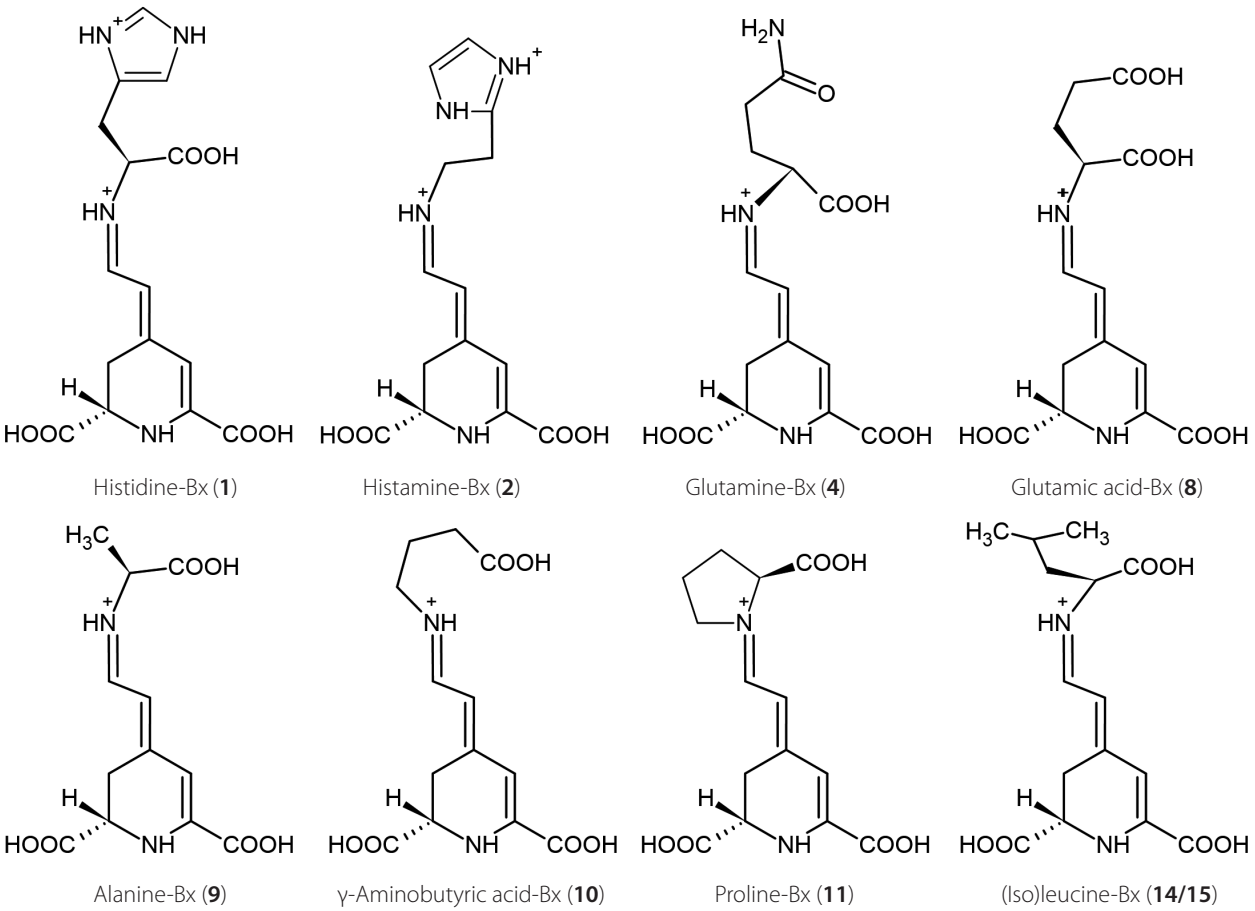

Figure 2. Chemical structures of predominant betaxanthins detected in the leaves of *Beta vulgaris* (compounds **2, 4, 8, 9, 10, 11, 14**, and **15**) and *Gymnocalycium. mihanovichii* grafted cacti (compound **1**).

O. ficus-indica fruits [Fernández-López *et al*., 2018]. Importantly, two specific compounds **1** and **11** were found in both profiles. However, in the case of *O. ficus-indica* fruits, compound **11** prevailed as the dominant pigment. In turn, compound **1** prevailed in *G. mihanovichii* grafted cacti scions, particularly in the orange, red, and pink varieties (Table 2). Additional betaxanthins, namely glutamine-Bx (**4**) and 5-methionine-betaxanthin, were detected in the yellow *O. ficus-indica* [Fernández-López *et al*., 2018], in contrast to their absence in *G. mihanovichii* grafted cacti. In turn, Kugler *et al*. [2007] analyzed the betaxanthin profile of *O. ficus-indica* cv. Gialla and identified a total of 13 distinct betaxanthins. Proline-Bx (**11**) was reported as the major betaxanthin. However, in this complex profile, histamine-Bx (**2**) was absent, in contrast to betaxanthin profile of the red variety of *G. mihanovichii* grafted cacti analyzed in our study (Figure 3, Table 2). Similarly, betaxanthin **2** and two other compounds, histidine-Bx (**1**) and serine-Bx (**3**), were not identified in the betaxanthin profile of *O. dillenii* [Betancourt *et al*., 2017].

The highest total betaxanthin content of *G. mihanovichii* grafted cacti found in the red variety (Table 2) was consistent with a previous study of 35 different cactus varieties, which showed that red varieties generally had the highest content of betaxanthins [Pérez-Loredo *et al*., 2016]. Interestingly, similar results were obtained in the case of beetroots, where red cultivars contained higher amounts of betaxanthins compared to the white and yellow varieties [Spórna-Kucab *et al*., 2023]. The total betaxanthin content in the red *G. mihanovichii* grafted cacti of 1.55 mg/kg FW (Table 2) was higher than that determined in green fruits of *O. ficus-indica* originating from the United States and reaching 1.7 mg/kg DW [Pérez-Loredo *et al*., 2016]. Here, analyses of the green variety of *G. mihanovichii* grafted cacti failed to identify any betaxanthins (Table 2).

The highest total content of betaxanthins in the *G. mihanovichii* grafted cacti extract was determined in the red variety, amounting to 19.5 mg/100 g DE, followed by the pink variety at 13.9 mg/100 g DE, then the orange variety at 5.3 mg/100 g DE, and finally the yellow variety at 1.4 mg/100 g DE. The compound profile of *G. mihanovichii* grafted cacti is not complex. Therefore, the isolation of histidine-Bx (**1**) from the extract of red and pink *G. mihanovichii* grafted cacti is indeed achievable.

Betaxanthins in *B. vulgaris* leaves

For the first time, the identification of betaxanthins has been accomplished in the leaves of *B. vulgaris* cv. Snow Ball, Boldor, Cylindra, Rhubarb Chard, and Round Dark Red. Analysis conducted by utilizing the HPLC-DAD-ESI-MS technique enabled a tentative identification of 15 betaxanthins: histamine-Bx (**2**), serine-Bx (**3**), glutamine-Bx (**4**), ornithine-Bx (**5**), ethanolamine-Bx (**6**),

Table 2. The content of total betaxanthins and individual betaxanthins in extracts from the leaves of five cultivars of *Beta vulgaris* and five varieties of *Gymnocalycium mihanovichii* grafted cactus (mg/100 g dry extract, DE) and the total betaxantin content in fresh weight (FW) of beet leaves and grafted cacti, respectively, (mg/kg FW), determined by HPLC-DAD-ESI-MS.

No.	Betaxanthin	*Beta vulgaris*					*Gymnocalycium mihanovichii*				
		Snow Ball	Boldor	Cylindra	Rhubarb	Round Dark Red	Orange	Green	Yellow	Red	Pink
1	Histidine-Bx	–	–	–	–	–	2.76 ± 0.20^{C}	–	0.99 ± 0.06^{D}	16.1 ± 1.5^{A}	12.5 ± 1.1^{B}
2	Histamine-Bx	14.7 ± 1.3^{b}	0.51 ± 0.03^{e}	19.5 ± 1.3^{a}	3.14 ± 0.20^{d}	5.89 ± 0.47^{c}	–	–	–	0.44±0.03	–
3	Serine-Bx	0.81 ± 0.06^{b}	–	–	0.62 ± 0.04^{c}	1.47 ± 0.10^{a}	2.40 ± 0.18^{A}	–	0.32 ± 0.03^{C}	2.33 ± 0.16^{B}	–
4	Glutamine-Bx	–	25.7 ± 2.3^{a}	–	2.68 ± 0.17^{b}	–	–	–	–	–	–
5	Ornithine-Bx	7.25 ± 0.75^{b}	5.96 ± 0.39^{c}	11.1 ± 0.9^{a}	2.86 ± 0.24^{e}	5.22 ± 0.39^{d}	–	–	–	–	–
6	Ethanolamine-Bx	–	8.28 ± 0.63^{a}	–	1.87 ± 0.17^{c}	6.76 ± 0.55^{b}	–	–	–	–	–
7	Lysine-Bx	–	3.75 ± 0.28^{a}	–	1.24 ± 0.12^{b}	1.13 ± 0.07^{b}	–	–	–	–	–
8	Glutamic acid-Bx	–	24.9 ± 2.1^{a}	18.0 ± 1.2^{b}	1.51 ± 0.13^{d}	8.39 ± 0.67^{c}	–	–	–	–	–
9	Alanine-Bx	–	11.6 ± 0.9^{c}	17.5 ± 1.1^{a}	15.9 ± 1.4^{b}	4.64 ± 0.34^{d}	–	–	–	–	–
10	γ-Aminobutyric acid-Bx	–	42.7 ± 3.6^{b}	32.2 ± 2.8^{c}	26.4 ± 2.3^{d}	44.8 ± 3.2^{a}	–	–	–	–	–
11	Proline-Bx	–	15.5 ± 0.9^{a}	13.3 ± 0.8^{c}	14.6 ± 1.1^{b}	7.32 ± 0.6^{d}	0.12 ± 0.01^{C}		0.04 ± 0.003^{D}	0.55 ± 0.04^{B}	1.36 ± 0.12^{A}
12	Valine-Bx	–	9.34 ± 0.70^{a}	5.57 ± 0.40^{c}	3.14 ± 0.24^{d}	6.07 ± 0.47^{b}	–	–	–	–	–
13	3-Methoxytyramine-Bx	–	3.07 ± 0.21^{a}	1.14 ± 0.08^{c}	2.02 ± 0.14^{b}	0.71 ± 0.09^{d}	–	–	–	–	–
14	Isoleucine-Bx	15.4 ± 1.3^{b}	2.31 ± 0.17^{e}	19.6 ± 1.6^{a}	2.99 ± 0.22^{d}	10.5 ± 0.7^{c}	–	–	–	–	–
15	Leucine-Bx	8.71 ± 0.69^{b}	5.06 ± 0.47^{d}	17.6 ± 1.3^{a}	3.54 ± 0.29^{e}	8.13 ± 0.65^{c}	–	–	–	–	–
16	Tryptophan-Bx	1.11 ± 0.09^{d}	2.62 ± 0.21^{b}	2.94 ± 0.22^{a}	2.99 ± 0.27^{a}	1.54 ± 0.10^{c}	–	–	–	–	–
Total content in extract (mg/100 g DE)		**48.0 ± 4.0^{e}**	**161.2 ± 14.0^{a}**	**158.5 ± 9.8^{b}**	**85.5 ± 5.5^{d}**	**112.5 ± 10.7^{c}**	**5.29 ± 0.50^{C}**		**1.35 ± 0.11^{D}**	**19.5 ± 1.3^{A}**	**13.9 ± 1.1^{B}**
Total content in FW (mg/kg FW)		**3.43 ± 0.35^{e}**	**20.4 ± 1.7^{a}**	**18.8 ± 1.3^{b}**	**15.4 ± 1.1^{c}**	**13.4 ± 0.9^{d}**	**0.22 ± 0.02^{C}**		**0.09 ± 0.01^{D}**	**1.55 ± 0.12^{A}**	**1.29 ± 0.08^{B}**

Data are expressed as mean ± standard deviation (n=3). The superscript letters within each row (a–e or A-D) mean significant differences between results (p<0.05). The statistical analysis was conducted separately for beet leaves and grafted cacti. The assigned betaxanthin numbers correspond to those listed in Table 1. –, Not detected.

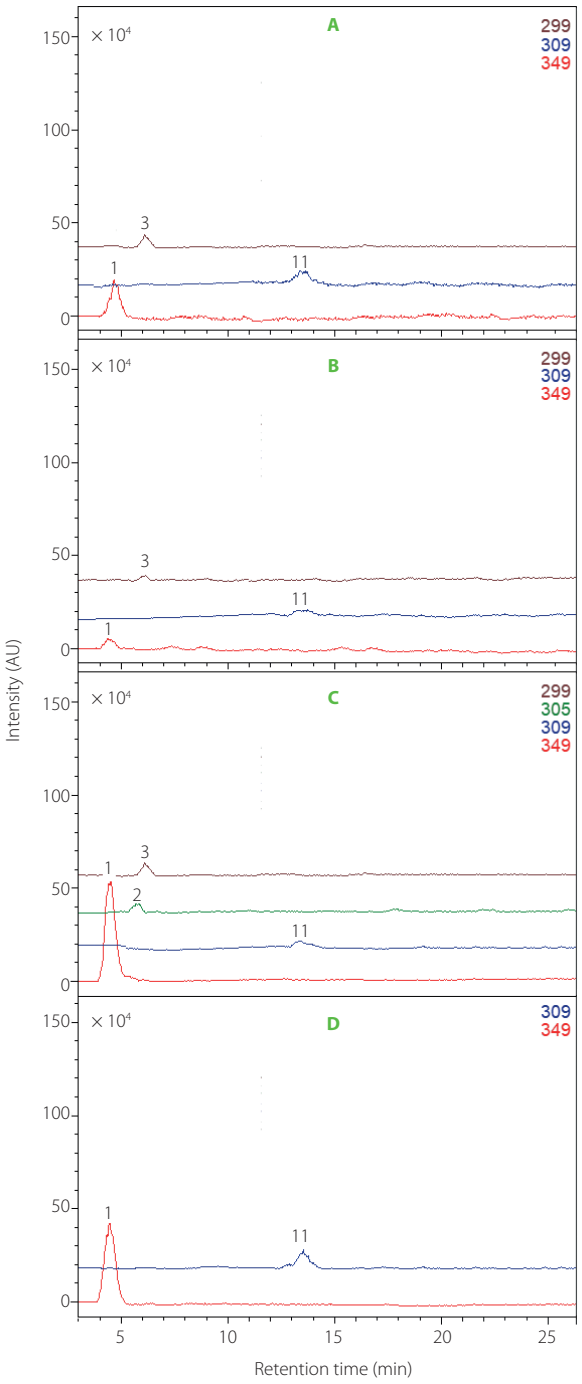

Figure 3. Chromatogram monitoring selected ions (ESI-MS) in the positive ion mode for betaxanthins from *Gymnocalycium mihanovichii* grafted cacti varieties: (**A**) orange, (**B**) yellow, (**C**) red, and (**D**) pink. The numbers and names are available in Table 1.

lysine-Bx (**7**), glutamic acid-Bx (**8**), alanine-Bx (**9**), γ-aminobutyric acid-Bx (**10**), proline-Bx (**11**), valine-Bx (**12**), 3-methoxytyramine-Bx (**13**), isoleucine-Bx (**14**), leucine-Bx (**15**), and tryptophan-Bx (**16**) (Figure 4, Table 1). The chemical structures of the dominant betaxanthins in beet leaves are shown in Figure 2. The presence of all compounds, except for histidine-Bx (**1**), was confirmed based on the root extract of *B. vulgaris* cv. Chrobry obtained in our previous study [Spórna-Kucab *et al*., 2023]. In turn, the extract from *P. grandiflora* analyzed previously [Spórna-Kucab *et al*., 2022] played a crucial role in betaxanthin **1** identification.

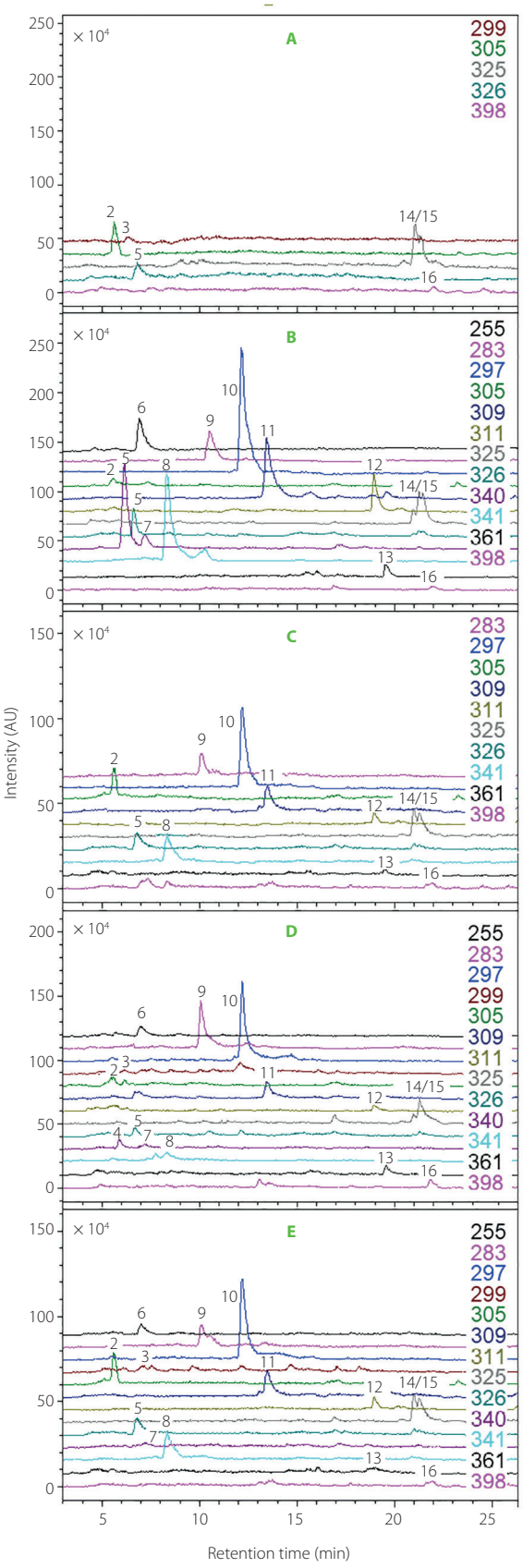

Figure 4. Chromatogram monitoring selected ions (ESI-MS) in the positive ion mode for betaxanthins from leaves of *Beta vulgaris* cultivars: (**A**) Snow Ball, (**B**) Boldor, (**C**) Cylindra, (**D**) Rhubarb and (**E**) Round Dark Red. The numbers and names are available in Table 1.

The qualitative profile of betaxanthins varied depending on the beet cultivar. Fifteen betaxanthins were identified only

in the Rhubarb cv. (Figure 4). In the other cultivars, various combinations of betaxanthins were noticeable. Remarkably, the Snow Ball cv. had the smallest diversity of betaxanthins, including 6 compounds.

Research on chemical compound profiles in different parts of a plant contributes to a more comprehensive understanding of the influence of chemical composition on plant properties and the selection of appropriate sources of specific natural compounds. The *B. vulgaris* cv. Boldor and Cylindra have already been analyzed for their betaxanthin profiles in their root systems in our previous study [Spórna-Kucab *et al*., 2023], which revealed a complex profile, including 23 betaxanthins. Additionally to most compounds indentified in *B. vulgaris* leaves in the current study, the presence of histidine-Bx (**1**), asparagine-Bx, arginine--Bx, glycine-Bx, threonine-Bx, dopa-Bx, dopamine-Bx, tyrosine-Bx, methionine-Bx, and phenylalanine-Bx was detected. It is worth noting that histamine-Bx (**2**) was not detected in the root samples. In contrast, it was present in the leaves of all *B. vulgaris* cultivars (Figure 4, Table 2). This observation is in agreement with previous research on the roots of red and yellow beet cv. Burpees Golden, as well as the leaves of Swiss chard cv. Bright Lights, which unequivocally established the presence of histamine--Bx (**2**) solely in the leaves, and its absence in the beet roots [Kugler *et al*., 2004, 2007].

Research on Swiss chard revealed the betaxanthin profile in leaves, emphasizing their potential as an equally rich source of betaxanthins [Kugler *et al*., 2004, 2007]. The presence of a total of 25 betaxanthins was established, including compounds that were not identified in the current study in the leaves of *B. vulgaris*. Specifically, these compounds were histidine-Bx (**1**), asparagine-Bx, aspartic acid-Bx, glycine-Bx, threonine-Bx, dopa-Bx, tyrosine-Bx, dopamine-Bx, methionine-Bx, tyramine--Bx, and phenylalanine-Bx. Whereas most of betaxanthins have previously been documented in Swiss chard [Kugler *et al*., 2004, 2007], ornithine-Bx (**5**) has been reported in the current study for the first time in leaves of all *B. vulgaris* cultivars. Interestingly, its presence was recently confirmed in roots of *B. vulgaris* Forono, Tytus, Ceryl, Boldor, and Chrobry cultivars [Spórna-Kucab *et al*., 2023].

The total betaxanthin content was the highest in fresh leaves of the yellow beet variety cv. Boldor (20.4 mg/kg FW), followed by the red cultivars: Cylindra, Rhubarb, and Round Dark Red (18.8 15.4, and 13.4 mg/kg FW, respectively), with the lowest value determined in the white cultivar Snow Ball (3.43 mg/kg FW). This study revealed lower total betaxanthin content in the leaves, ranging from 3.34 to 20.4 mg/kg FW, compared to 107 mg/kg FW assayed in the case of Swiss chard [Gamba *et al*., 2021; Kugler *et al*., 2007]. Here, the red beet cv. Cylindra had a similar betaxanthin content of 18.8 mg/kg FW compared to the yellow cv. Boldor which had 20.4 mg/kg FW. This may emphasize the underestimated significance of betaxanthins in red beets.

Dried extracts obtained from the leaves of the yellow beet cv. Boldor had the highest total betaxanthin content, reaching 161.2 mg/100 g DE. In contrast, the white cv. Snow Ball accumulated the lowest betaxanthin quantities, 48.0 mg/100 g DE. Red cultivars, on the other hand, had contents between these values (158.5, 112.5, and 85.5 mg/100 g DE for cultivars Cylindra, Round Dark Red, and Rhubarb, respectively). Betaxanthin content in dried extracts from the *B. vulgaris* roots of five cultivars (four red: Ceryl, Chrobry, Forono, and Tytus, as well as one yellow: Boldor) has been previously studied [Spórna-Kucab *et al*., 2023]. The results of that study revealed notably elevated betaxanthin content, with the highest levels noted in red beet cultivars: Ceryl, Chrobry, Forono, and Tytus (from 669 to 1231 mg/100 g DE for peel and 528 to 609 mg/100 g DE for flesh), rather than in the yellow ones (317 and 574 mg/100 g DE for flesh and peel, respectively). These findings suggest that, in contrary to the common perception that yellow beets are a rich source of betaxanthins, their value may be surpassed by those found in red cultivars. Within the leaves of *B. vulgaris*, substantial levels of betaxanthins were likewise detected in the red cultivars (Table 2).

Different pigments were found to predominate in various beet cultivars. In leaf extracts of all examined cultivars of *B. vulgaris*, γ-aminobutyric acid-Bx (**10**) was predominant, except for cv. Snow Ball. In the extract of cv. Boldor, there were notable levels of γ-aminobutyric acid-Bx (**10**) at 42.7 mg/100 g DE, glutamine-Bx (**4**) at 25.7 mg/100 g DE, and glutamic acid-Bx (**8**) at 24.9 mg/100 g DE. In the extract of cv. Rhubarb, the dominance of three pigments was noted: γ-aminobutyric acid-Bx (**10**) at 26.4 mg/100 g DE, alanine-Bx (**9**) at a level of 15.9 mg/100 g DE and proline-Bx (**11**) at a level of 14.6 mg/100 g DE. Compound **10** also dominated in the leaf extract of cv. Round Dark Red, with a quantity of 44.8 mg/100 g DE and in cv. Cylindra, with a quantity of 32.2 mg/100 g DE. Isoleucine-Bx (**14**) and histamine-Bx (**2**) were the major pigments in the extract of cv. Snow Ball, with contents of 15.4 mg/100 g DE and 14.7 mg/100 g DE, respectively. In leaf extract of cv. Cylindra, γ-aminobutyric acid-Bx (**10**) clearly dominated, reaching a level of 32.2 mg/100 g DE. In this cultivar, the following compounds stand out as well: isoleucine-Bx (**14**) with a content of 19.6 mg/100 g DE, histamine-Bx (**2**) with a content of 19.5 mg/100 g DE, glutamic acid-Bx (**8**) with a content of 18.0 mg/100 g DE, alanine-Bx (**9**) and leucine-Bx (**15**) with contents of 17.5 mg/100 g DE and 17.6 mg/100 g DE, respectively. These betaxanthins contribute unique properties to each cultivar, arousing curiosity about their potential health benefits.

Glutamine-Bx (**4**) and γ-aminobutyric acid-Bx (**10**) were predominant betaxanthins in *B. vulgaris* cv. Boldor, both in the roots [Spórna-Kucab *et al*., 2023] and in the analyzed leaf extracts. Moreover, betaxanthin **4** prevailed in all studied root extracts (Snow Ball, Boldor, Cylinder, Rhubarb, and Round Dark Red), except for the peel of yellow *B. vulgaris* (cv. Boldor) where betaxanthin **11** predominated. This underscores the significance of these compounds in the overall composition of beetroot, encompassing both its roots and leaves.

CONCLUSIONS

In conclusion, this study has provided valuable insights into the presence and distribution of betaxanthins in different varieties of *Gymnocalycium mihanovichii* grafted cacti and the leaves of *Beta vulgaris* cultivars. The research revealed distinct betaxanthin

profiles in different varieties of these plants, shedding light on their potential as sources of these pigments. In *G. mihanovichii* grafted cacti, preliminary investigations indicated the presence of four polar betaxanthins, *i.e.*, histidine-Bx (**1**), histamine-Bx (**2**), serine-Bx (**3**), and proline-Bx (**11**). The total betaxanthin contents varied among these varieties with the highest levels observed in the red one, followed by the pink, orange, and yellow ones. Betaxanthins were not identified in the green cactus variety. When comparing *G. mihanovichii* grafted cacti to other studied cacti, it becomes evident that while the betaxanthin content in *G. mihanovichii* grafted cacti was lower, its profile was unparalleled in other plants.

Betaxanthins in the leaves of various of *B. vulgaris* cultivars, including Snow Ball, Boldor, Cylindra, Rhubarb Chard, and Round Dark Red, were identified for the first time ever. The qualitative profile differed among cultivars, with the greatest diversity found in the Rhubarb cultivar. Their contents in *B. vulgaris* leaves varied among cultivars as well, with yellow cultivar Boldor exhibiting the highest total betaxanthin content. It is worth noting that the contents found in the red cultivars were also very high, confirming that the leaves of these beet cultivars are also an excellent source of betaxanthins.

In summary, this research has expanded our knowledge of betaxanthins in *G. mihanovichii* grafted cacti and leaves of *B. vulgaris*, highlighting them as sources with diverse pigment profiles.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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