



Evaluation of bioactive compounds in leaf and root of five beetroot varieties

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ABSTRACT

Natural compounds are essential in modern nutrition for preventing chronic diseases in the human body. The bioactive substances in 5 beetroot cultivars' leaves and roots were evaluated in the experiment, providing an answer to which stage (60th, 85th, and 105th) of the vegetation is most suitable for fresh consumption. The results show that the polyphenol, flavonoid, and FRAP levels in the leaf and root samples varied throughout the vegetation period. The 60-day old leaf samples of 'Liberio' had the highest total polyphenol content. The flavonoid content showed a similar trend. Furthermore, the highest FRAP value was found in leaf samples from 'Liberio' and 'Pablo F1' that were 60 days old.

In 85-day-old plants, the polyphenol content of the leaves was significantly reduced.

On day 85, however, a value was slightly higher in the roots. These findings support the nutritional value of the 85-day-old beetroot (leaves and 3–5 cm diameter roots).

At the 85-day stage, the leaf and root had a relatively low nitrate content (10–70 mg/kg).

However, higher nitrate content was detected at the end of the growing period (105 days). The experiment demonstrated that beet leaves can be used as a component of fresh salads on the 60th day of the growing season because the amount of bioactive substances (polyphenols, flavanoids) is highest at that time.

By the 85th day of growth, the root had grown to 4–5 cm in diameter, and the tissue structure of the leaves is edible (it can be eaten raw), making it ideal for batch sales. By the end of the growing season (day 105), the root has been strengthened, and its bioactive substances have been reduced by up to 1/3 compared to the previous state (85 days), resulting in a decrease in the amount of bioactive substances.

1. Introduction

Red beetroot is one of the top ten vegetable species in several countries. Its edible roots contain 12–20% dry matter, with 4–12% sugar, 1.5% protein, and 0.8% fiber [1–3]. The importance of evaluating bioactive compounds in raw materials synthesised in various parts of the plant has grown in the food industry because they have a positive impact on human health. As a result, it can play an important role in balancing the physiological processes of the human organism (homeostasis).

Furthermore, natural food additives, such as phenolic compounds, can affect food quality and stability [4]. Several studies have discovered that beetroot juice and extract are used in traditional medicine, as well as food coloring and cosmetic additives [5].

These findings have focused on beetroot studies on betalains, which are N-containing water-soluble plant pigments [6]. Recently, a group of

researchers has begun to investigate oxidative stress and its impact on the human body. Their findings highlight the wide range of biologically active substances found in beetroot varieties [7]. The primary betalain groups are red-violet betacyanins and yellow betaxanthins, both of which have excellent antimicrobial and antiviral properties [8]. Furthermore, these materials have the potential to inhibit tumor cell proliferation [9]. There are some contradictory claims about the role of nitrate in the human diet. For a long time, beetroot's nitrate content was a negative quality parameter. However, beetroot extracts are marketed as a performance-enhancing nutrition supplement, according to the last statements.

Its active ingredient is inorganic nitrate, which bacteria in the organism convert to nitric oxide (NO). Furthermore, several studies [10–12] found that increased NO levels improve athletes' muscle efficiency and fatigue resistance [7]. In addition, researchers [13,14]

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discovered that the nitrate content of beetroot extract can lower blood pressure and thus help to prevent hypertension and cardiovascular disease [15].

Despite the positive effect, excessive beetroot consumption can cause increased endogenous nitrosation and lead to carcinogenic metabolites [16]. Additionally, nitrate can react with the amine content of meat (after digestion), producing nitrosamines, which are mutagenic compounds [17]. Furthermore, nitrate can react with haemoglobin to form methaemoglobin, which is incapable of transporting oxygen in the blood [18]. Endogenous N-nitroso compound (NOS) formation and its effect on the human organism are currently being debated, necessitating more comprehensive research [16]. The WHO's acceptable daily intake (ADI) for nitrate is 3.7 mg/kg body weight per day [19].

The flavonoids are byproducts of metabolism. Their accumulation begins directly beneath the skin of fruits and vegetables and progresses to the fruit's interior. A variety of genetic and environmental factors influence the synthesis of bioactive substances. For example, sunlight can increase synthesis in the plant, whereas processing technology (boiling, peeling), the presence of oxygen, and an acidic medium can reduce it by half [20]. The polyphenol content is primarily influenced by the species and variety. Other factors, such as soil compounds, growing conditions, ripening state, and storage conditions, also have a significant impact [21,22].

Furthermore, stress during the vegetation period (e.g., sunburn, UV-light, pathogen organism) affects the synthesis of these secondary metabolic products [23]. The growing method has an impact on polyphenol content. According to Asami and colleagues [21] discovered that bio-production products have higher polyphenol content than conventional or hydroculture growing. It is explained by the rapid utilization of mineral elements in fertilizers in plants, which accelerates the assimilation of the product while delaying secondary product synthesis (e.g., polyphenols). Additionally, using regular plant protection against fungi and diseases causes less stress during the growing period, resulting in less synthesis of materials (e.g., polyphenols) for the protection system [24].

Apart from the root, the beetroot leaf is used in the food industry and gastronomy as a fresh salad component. Thus being the primary reason for studying bioactive compounds, polyphenol synthesis, and the factors that can influence it, such as growing method (length of growing period) and genetic background (different varieties). The purpose of this study was to determine the polyphenol and flavonoid content in more plant parts to evaluate the chemical composition of different beetroot varieties and their effect on antioxidant potential (leaves and roots). Furthermore, the nitrate content of different varieties was determined at various stages of the growing season. Furthermore, the experiment's main goal was to highlight the leaf and root of beetroot as an excellent raw vegetable for fresh consumption. At the same time, to highlight the appropriate variety and harvesting stage (development) to prepare a healthy product with high bioactive compounds.

Table 1

The evaluated beetroot genotypes' names, origins, and root types (Debrecen, 2016).

Variety	Breeder/distributor	Origin	Root type
'Akela'	Rijk Zwaan	NL	spherical
'Bonel'	Nickerson Zwaan	NL	spherical
'Larka'	Rijk Zwaan	NL	spherical
'Libero'	Rijk Zwaan	NL	spherical
'Pablo F ₁ '	Bejo Zaden	NL	spherical

NL – Netherlands.

2. Materials and methods

2.1. Experimental setup

The open-field experiment with five beetroot genotypes (Table 1) was carried out at the University of Debrecen, Hungary's Farm and Regional Research Institute, Botanical and Exhibition Garden. The experimental design was randomised and replicated four times for each genotype. The sowing took place on March 30, 2016, with a row distance of 35 cm. During the vegetation period, the following cultivation methods were used: soil loosening, nutrient supply, mechanical and chemical weed control, plant protection, and drip irrigation.

2.2. The soil's quality

The experiment was conducted on lowland chernozem soil and was evaluated for the quality parameters listed in Table 2.

The pH, total water-soluble salt, humus, and available nutrients all contributed to soil quality.

According to Table 2, the experimental area was suitable for beetroot production.

2.3. Temperature and precipitation each day

The Agrometeorological and Agro-ecological Monitoring Centre measured the daily minimum and maximum temperatures, as well as the natural precipitation. The temperature ranges were ideal for growing beetroot (Fig. 1).

During the first period of vegetation, the daily temperature fluctuation was around 20 °C. Because beetroot is a cold-resistant species, it did not cause damage during production. In the second half of May, the daily temperature rarely fell below 25 °C. This environment was ideal for the synthesis of bioactive compounds.

The daily precipitation during the vegetation is depicted in Fig. 2. However, because the total amount of precipitation (approx.188 mm) was not distributed uniformly, drip irrigation with a 20–25 mm water dose was used on occasion to ensure proper development.

2.4. Quality parameters were sampled and analyzed

Fresh tablebeet consumption is becoming increasingly important. The leaves are tender, fresh, and have intense photosynthesis on the 60th day of vegetation. However, because the roots are still very small (1–2 cm) at this time, they provide no economic benefit. By the 85th day of the growing season, both parts of the plant (leaves and roots) can be used effectively, because the roots have grown to a diameter of 4–5 cm, making them suitable for batch sales.

By the end of the growing season (day 105), the leaves had aged and the petiole had become stronger, making them unfit for fresh consumption. This explains why, on the 105th of vegetation, only the roots were evaluated for bioactive substances. The samples were collected on the 60th (May 27), 85th (June 23), and 105th (July 13) days of the

Table 2

The experimental field's chemical analysis (Debrecen, 2016).

Analyzed parameter (unit of measurement)	Amount
pH (KCl)	6.83
Plasticity index of Arany K _A	38
Total water-soluble solids (%)	0.04
CaCO ₃ (%)	0.99
Humus (%)	2.91
AL-soluble P ₂ O ₅ (mg/kg)	481
AL-soluble K ₂ O (mg/kg)	309
KCl-soluble NO ₃ ⁻ + NO ₂ ⁻ - Nitrogen (mg/kg)	2.16

Source: Agricultural Laboratory Centre, University of Debrecen. The values are expressed in air-dry matter.

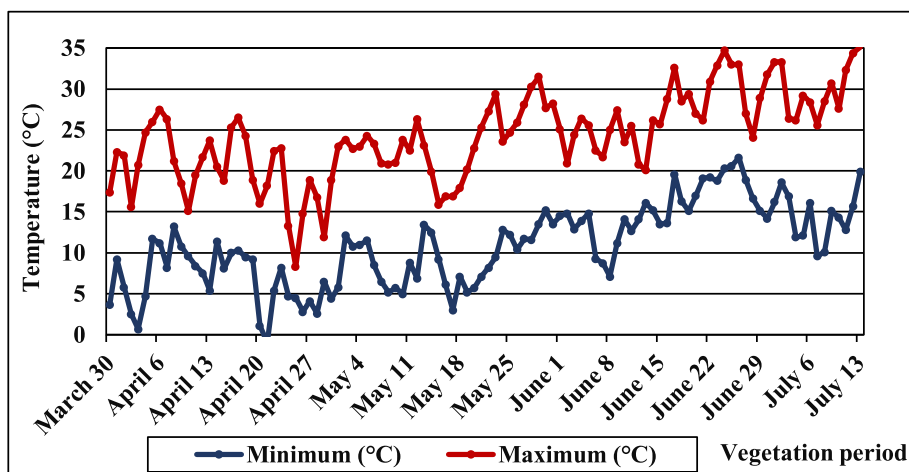


Fig. 1. Daily minimum and maximum temperature (°C) (Debrecen, 2016)

Source: Agrometeorological and Agro-ecological Monitoring Centre, Institutes for Agricultural Research and Educational Farm, Farm and Regional Research Institute, University of Debrecen.

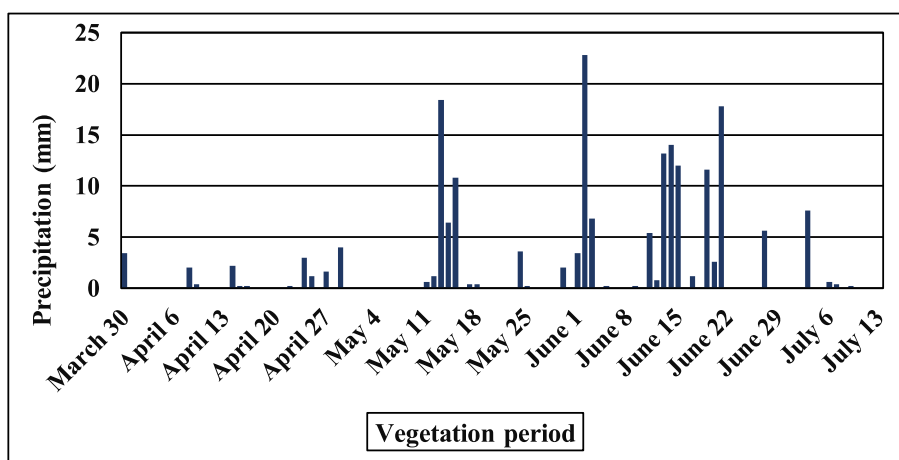


Fig. 2. The amount of precipitation that fell during the vegetation period (Debrecen, 2016) Source: Agrometeorological and Agro-ecological Monitoring Centre, Institutes for Agricultural Research and Educational Farm, Farm and Regional Research Institute, University of Debrecen.

vegetation period.

Ten representative plants from each genotype were chosen for sampling from four randomised plots. The measurements were taken at the Agricultural Laboratory Centre, University of Debrecen, and they are as follows.

Total dry matter content (%) – drying at 105 °C until mass consistency was achieved.

Total polyphenol content (mg GAE/100 g) – in the leaf and root samples, the total polyphenol content (mg GAE/100 g) was determined by colorimetric method [25]. A spectrophotometer (Lambda 25 UV/Vis, PerkinElmer, USA) was used to take the measurements. The Folin-Ciocalteu phenol reagent was used, and the samples were measured at a wavelength of 765 nm. A standard curve plotted with a known concentration of gallic acid was used to calculate the polyphenol concentration. The results were given in milligrams of gallic acid equivalent (GAE) per 100 g fresh weight.

Flavonoid content (mg CE/100 g) – it was determined using a spectrophotometer and a colorimetric method described by Kim et al. [26]. The results were expressed in milligrams of catechin equivalent (CE) per 100 g fresh weight.

Ferric Reducing Antioxidant Power (FRAP) – the total antioxidant capacity was determined using the Benzie and Strain [27] method with some modifications [28]. The results were expressed in milligrams of

vitamin C equivalent per 100 g of fresh weight (mg vitamin C equivalent/100 g).

Nitrate content (mg/kg) – nitrate was determined using the CON-TIFLOW method developed by the Hungarian Standards Institute [29]. The results were expressed in milligrams per 100 g of fresh weight.

2.5. Data examination

SPSS was used to organize and analyze the data (IBM, USA). The ANOVA test was performed in the same program. Duncan's multiple comparison test was used as a post hoc test. The findings were presented as mean standard deviation. Pearson's correlation was also calculated between the parameters.

3. Results and discussion

3.1. Total amount of dry matter

One of the most important parameters of raw vegetables was their dry matter content. Its concentration varies depending on the part of the plant; however, the root has a higher dry matter content than the leaf (Fig. 3). Thus, consuming leaves at an early stage of development (60 days old) is recommended because later in the vegetation period

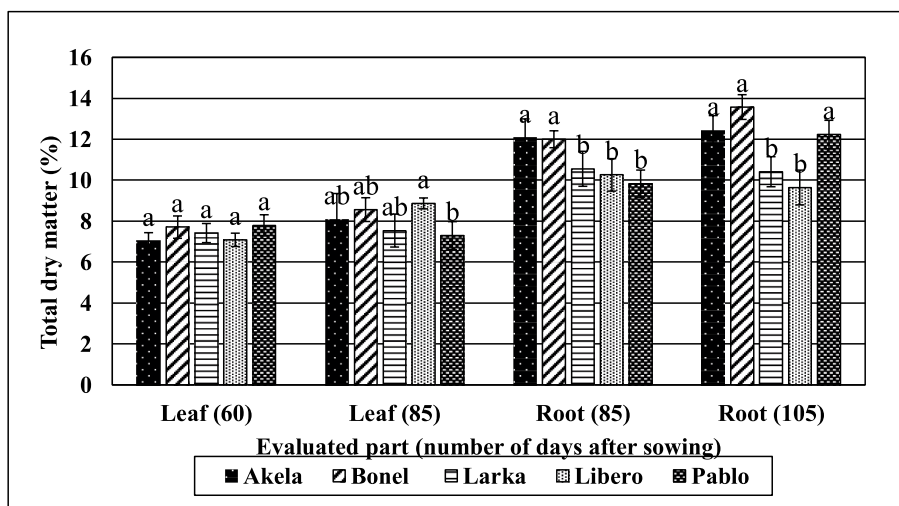


Fig. 3. Total dry matter content (%) of beetroot leaf and root at various stages According to Duncan’s multiple range test, values with the same letter within the column are not significantly different from each other (p = 0.05).

becomes rather old with several symptoms of disease. The dry matter content of the leaf was approximately 7% in the mean of the varieties at this stage. However, by the 85th day, this value had risen. A similar pattern was observed in root samples, which increased by the end of the vegetation period (105-day).

Previous experiments demonstrated that the dry matter content of the root could reach 16% by the second cultivation, with harvest in October [30]. As a result, it emphasizes that spring sowing is preferred for fresh and nutritious raw material suitable for fresh consumption.

On the 60th day of the vegetation period, there was no significant difference in dry matter content between genotypes (leaf). The ‘Akela’ and ‘Bonel’ genotypes had the highest dry matter content in the root samples (85- and 105-day).

3.2. Total polyphenol content

Polyphenols play an important role in reducing free radicals in the human body. As a result, leaf and root samples were collected on various days throughout the vegetation period (Fig. 4).

Based on the results of the experiment, it is possible to conclude that

the 85-day-old root contained more polyphenols than the same-age leaf. This amount in the root, however, decreased at the end of the vegetation period. This decrease can also be explained by the higher temperature (30 °C) at this time of year, which may have weakened the assimilation processes. As a result, the younger raw material is more valuable than the mature root (105-day old). The amount was found to be higher in the leaf samples that were 60 days old. These findings show that eating beetroot leaves as a fresh salad at this stage of growth (60 days) is highly recommended. Furthermore, ‘Libero’ and ‘Pablo F1’ outperformed the others in terms of polyphenol content in the leaves. The polyphenol content of 85-day-old root samples was higher than that of 105-day-old root samples. It is well known that pigment content decreases significantly by the end of the growing season (summer) due to the unfavorable effect of higher temperatures on its accumulation (30). The amount of coloured material can influence the total polyphenol content of the root. Čanadanović-Brunet et al. [31] obtained a higher value (218.3 mg GAE/g dry extract) in a water extract from beetroot pomace. Among vegetables, artichoke, parsley, and Brussel sprouts had the highest polyphenol content (>250 mg GAE/100 g) [32]. In other studies, the total polyphenol content of the root was found to be relatively high,

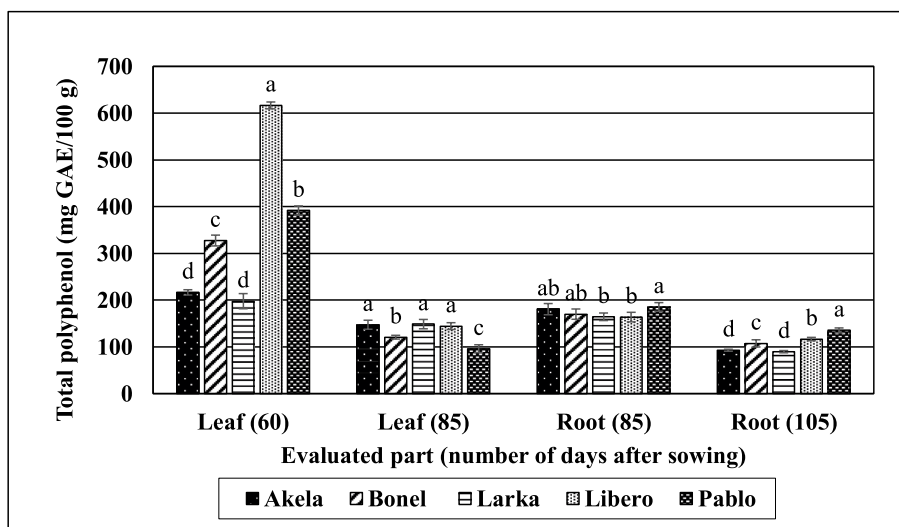


Fig. 4. Total polyphenol content of beetroot leaf and root (mg GAE/100 g) According to Duncan’s multiple range test, values with the same letter within the column are not significantly different from each other (p = 0.05). GAE – gallic acid equivalent.

ranging from 820.10 to 1280.56 mg/kg FW in different beetroot varieties [3]. Ninfali and Angelino [33] found similar results (720–1276 mg/kg FW) in their study of different beetroot varieties.

As a result, it is recommended to harvest beetroot from spring sowing before the end of the growing season (day 60 or 85) for fresh consumption, because the amount of bioactive substances in the root decreases significantly by the end of the growing season.

3.3. Flavonoid content

Flavonoids are polyphenolic compounds that are yellow in color resulting from their synthesis being intensified by sunlight. It is the primary reason why partial shade is unfavorable for the production of leafy vegetables. Furthermore, the presence of oxygen and the thick peeling of the root can reduce the flavonoid content [20]. Even though, research has shown that beetroot leaves are an excellent source of phenolic compounds and have significant antioxidant activity [34]. A similar trend was discovered in the flavonoid content, as well as in the total polyphenol content (Fig. 5).

By the end of the vegetation period (105th day), this component had decreased in the root. On the 85th day, it was approximately 30–50 mg CE/100 g in the root and approximately 20 mg CE/100 g in the leaf. The amount of these compounds decreases as the leaves age (from 60 to 85 days). The negative trend can be explained by increased temperature effects, which are similar to polyphenol content development.

The 85-day old roots of ‘Akela’ and ‘Pablo F₁’ had the highest flavonoid content among the genotypes. However, the content of the 60-day-old leaves was higher than that of the 85-day-old leaves. During the development stages, the chemical constituents of the leaves change.

Biondo et al. [34] discovered a similar tendency of phenolic content in 60-, 80-, and 100-day old leaves in natura based on fresh weight in their study.

As a result, it can be concluded that these bioactive compounds are more abundant in younger leaves (60th day of growing season), which are more nutritious and better suited for fresh consumption. Beetroot harvested on the 85th day of the growing season is better for fresh consumption, but the flavonoid content in the leaves is slightly lower than on the 60th day. The value measured in the root on the 85th day of the growing season, on the other hand, can be up to ten times higher than on the 105th day.

3.4. FRAP (Ferric Reducing Antioxidant Power)

Bioactive materials derived from natural sources would be ideal for scavenging free radicals in the human body. It is the primary reason for supplementing food with enough health-promoting constituents to prevent disease development. Because of its betalain content, beetroot is used as an antioxidant. Their ability to scavenge free radicals, these compounds and other phenolic substances can reduce oxidative damage to lipids and improve the antioxidant status of the human organism, thereby preventing cancer and cardiovascular disease [35].

Wruss et al. [7] discovered a connection between FRAP (Ferric Reducing Antioxidant Power) and betalain content. They concluded that the antioxidant capacity of beetroot juice varies significantly between varieties. The ‘Mona Lisa’ variety had the highest antioxidant capacity. Czapski et al. [36] used their experiments to support a highly significant correlation between antioxidant capacity and beetroot red pigments (betacyanins).

The evaluation of total polyphenol and flavonoid contents revealed a similar tendency for FRAP values in different growing stages (Fig. 6). The highest concentration was found in 60-day-old leaf samples. Among the genotypes, ‘Liberio’ had more than 3000 mg vitamin

C equivalent/100 g. By the 85th day of vegetation, the FRAP values of the leaves had decreased. As a result, the value of the older leaves was only one-third of what it was at the earlier stage.

Measurements confirmed that the evolution of phenolic compounds and antioxidant activity levels differ during the vegetation period, which can be explained by environmental factors. Extreme environmental conditions, according to Bulbovas et al. [37], can increase the concentration of reactive oxygen species and antioxidant activity. However, in the mean of genotypes, the FRAP value was higher in the 85-day old root than at the end of the growing period (105th day). This finding supports the consumption of young root and leaf (as a whole) in fresh form; otherwise, heat-sensitive materials can degrade till the end of the vegetation period. Among the genotypes, ‘Akela’ and ‘Pablo F₁’ had the highest FRAP values in the root (more than 1600 mg vitamin C equiv./100 g).

The relationship between the FRAP value, flavonoid content, and total polyphenol content was evaluated using Pearson’s correlation (Table 3).

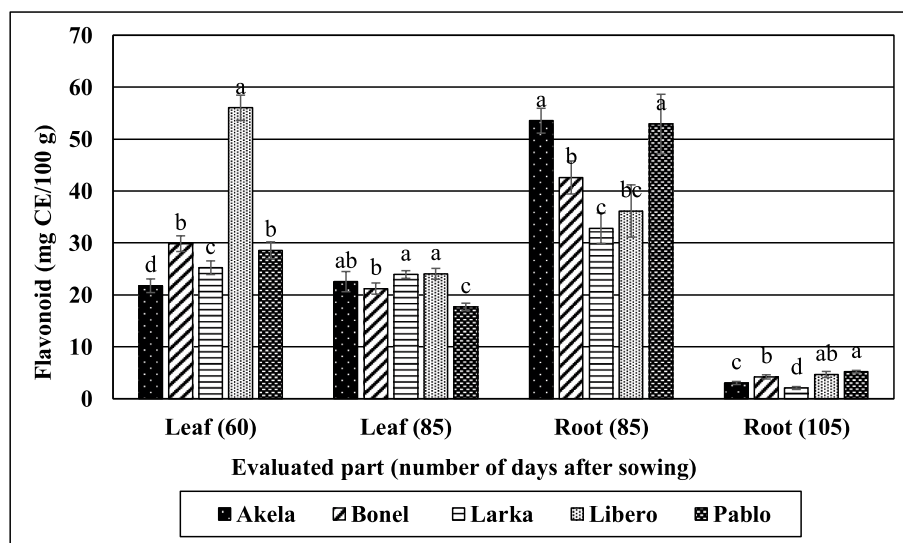


Fig. 5. Flavonoid content of beetroot leaf and root (mg CE/100 g)

According to Duncan’s multiple range test, values with the same letter within the column are not significantly different from each other ($p = 0.05$). CE – catechin equivalent.

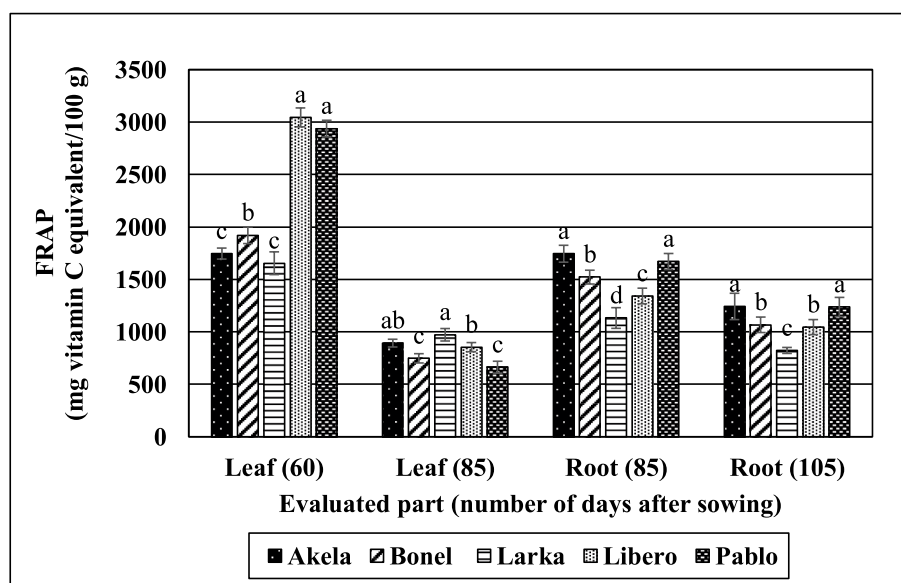


Fig. 6. FRAP value of beetroot leaf and root (mg vitamin C equivalent/100 g product) According to Duncan's multiple range test, values with the same letter within the column are not significantly different from each other ($p = 0.05$).

Table 3
Correlation of bioactive compounds observed in leaves and roots ($n = 15$).

		Total polyphenol	Flavonoid	FRAP
Leaf (60)	Total polyphenol	1	0.935**	0.879**
	Flavonoid		1	0.725**
	FRAP			1
Leaf (85)	Total polyphenol	1	0.882**	0.809**
	Flavonoid		1	0.831**
	FRAP			1
Root (85)	Total polyphenol	1	0.873**	0.638*
	Flavonoid		1	0.876**
	FRAP			1
Root (105)	Total polyphenol	1	0.848**	0.384
	Flavonoid		1	0.586*
	FRAP			1

A strong correlation ($>r = 0.900$) was found between the flavonoid and total polyphenol content of the leaf on the 60th day of the growing period. A similar correlation ($r = 0.879$) was found between total polyphenol and FRAP value. It is important to note that by the 85th day of the vegetation, there was a strong correlation between bioactive compounds (total polyphenol, flavonoid) and FRAP value in leaf and root samples. The high content of bioactive materials and the strong correlation between these compounds and the FRAP value confirmed the fresh consumption of younger leaves.

However, at the end of the growing period (105 days), the relationship between the root and the FRAP value is weaker for the flavonoid and total polyphenol content ($r = 0.586$ and $r = 0.384$, respectively).

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

3.5. The nitrate content

It is well understood that the nitrate-accumulating ability of root vegetables is primarily due to the relatively high ratio of the root's xylem. Grzebelus and Baranski [38] studied 40 beetroot varieties and discovered that the nitrate content of the root ranged from 600 to 1400 mg NO_3^-/kg fresh weight. Fig. 7 illustrates the nitrate content measurement data.

In the mean of genotypes, significant differences in nitrate content of leaf and root were discovered. The highest concentration was found in the root during harvest.

When compared to other genotypes ('Akela,' 'Bonel,' and 'Pablo F₁'), the 'Larka' and 'Libero' had higher nitrate content in the root. This

parameter was less than 80 mg/kg for all genotypes on the 85th day of vegetation. The nitrate content of the leaves was less than 20 mg/kg at the same growth stage, which can be attributed to the intense sunlight, which increased nitrate assimilation in organic products. The leaves had a slightly higher nitrate content at 60 days of development. The highest value, however, was only around 20 mg/kg. As a result, it is safe to say that the young leaves are ideal for a fresh salad.

Nifras [39] investigated the nitrate content of leaf vegetables grown organically and conventionally. The amount was found to be lower in spinach (13.5–26.2 g/g) and drumstick (*Moringa* sp.) leaf (16.2–73.4 g/g) samples than in beetroot (48.4–56.9 g/g). The conclusion was that conventionally grown samples contained more nitrates than organically grown samples. Furthermore, it is well known that nutrition and water supply have an impact on the evaluation of nitrate content. A previous experiment [40] demonstrated that a moderate nitrogen supply of soil and an equalized water supply can provide a reasonable possibility of high-quality beetroot production.

4. Conclusions

The experiment results provide information on the bioactive substance synthesis in leaf and root samples from the early sowing of five beetroot varieties during the growing season. This growing period (March 30th sowing date) allowed for the testing of the effect of a cooler spring and a warmer summer period on the 105-day long vegetation. The main goal was to highlight the consumption of fresh leaves as a salad for the supply of bioactive compounds. As a result, this raw material has the potential to increase the bioactive compound content of the human body.

Increased free radical formation in the body can result in harmful processes and risks. This being the primary reason for ingesting a large amount of natural color-containing meals into the human organism. Young beetroot leaves and roots play an important role in this process. The total polyphenol content of the 85-day old root was higher than that of the older roots. However, the polyphenol content of the leaves was slightly lower during the same growing period. The experiment demonstrated that younger (85-day) plants are more valuable for fresh consumption than older (105-day) roots. A similar pattern was observed for flavonoid content.

The FRAP method was used to assess the free radical scavenging capacity. A strong correlation was discovered between the FRAP value

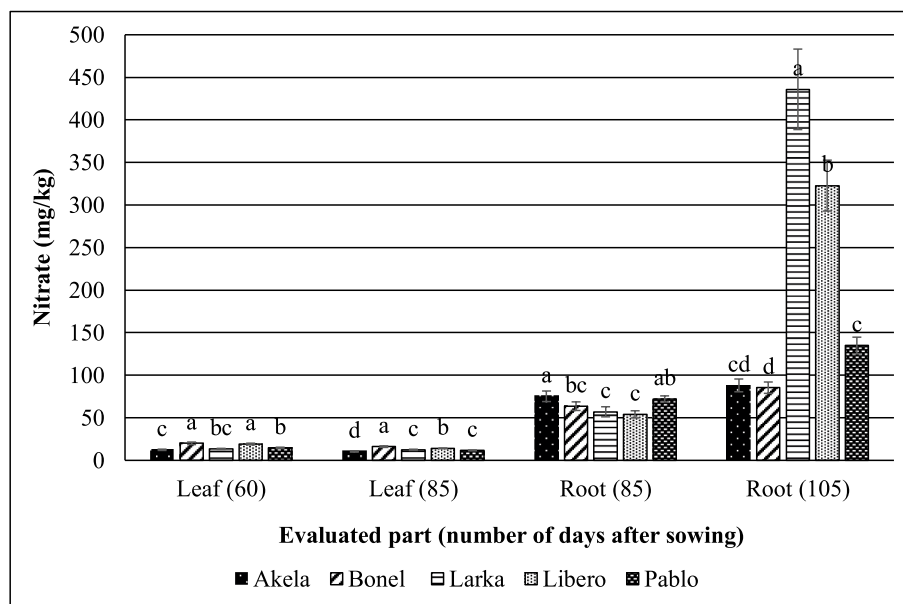


Fig. 7. Nitrate content of beetroot leaf and root (mg/kg fresh product)

According to Duncan's multiple range test, values with the same letter within the column are not significantly different from each other ($p = 0.05$).

and total polyphenol content in 85-day-old plants.

However, at the end of the growing period for the improved roots, the correlation between these parameters was weak. The increased nitrate content can reduce the quality of leaf and root vegetables. In the experiment, significant differences in the root at the end of the growing period were discovered between the varieties (105-day). When the nitrate content of the leaves (60-day) was compared to the developed roots (105-day), the young leaves contained significantly less nitrate.

Therefore it is strongly suggested that eating young leaves and roots is preferable to eating older ones.

On the 85th day of vegetation, the amount of bioactive substances in the leaf and root was highest. Thus, based on the experiment results, it can be concluded that spring beetroot production (sowing date March 30) can produce excellent raw material if the harvest time (June 23) is on the 85th day of the growing period. Plants harvested with healthy leaves and roots (diameter 4–5 cm) are suitable for fresh consumption. The tender green mass should be harvested on the 60th if only the leaf is to be used as a salad component.

Contributors

All authors have equally contributed to the article and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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