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BIOCHEMICAL AND MINERAL EVALUATION OF FOUR EXOTIC BEETROOT VARIETIES GROWN IN BANGLADESH

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ABSTRACT

In Bangladesh beetroot is getting attention in recent years due to its high food value and potential industrial use. The present study was conducted to assess the food value in terms of biochemical and mineral nutritional constituents of four exotic beetroot varieties (viz. Chioggia, Detroit Dark Red, Early Wonder Tall Top, and Red Ace) of Bangladesh. The pH, brix and dry matter (DM%) levels of the four beetroot varieties ranges between 5.52 to 6.51, 8.9 to 10.5, and 8.8 to 11.0%, respectively; and Detroit Dark Red had the highest DM%. the protein, fibre, ash, and total sugar contents were also significantly varied in the four varieties and the values ranged between 10.80-12.50, 5.10-6.20, 7.65-8.86, 2.62-6.06%DM, respectively. The vitamin C was found highest in Chioggia (6.20 mg/100g). Among the micro and macronutrient elements tested, all parameters were significantly varied and Detroit Dark Red contained the highest amount of N (1904 mg 100 g^{-1} DM), Fe (53 mg 100 g^{-1} DM), Zn (26 mg 100 g^{-1} DM) and Mn (17 mg 100 g^{-1} DM); while Chioggia contained the highest amount of K (3331 mg 100 g^{-1} DM), Ca (151 mg 100 g^{-1} DM) and B (21 mg 100 g^{-1} DM). Total anthocyanin and total phenolic contents also varied among the varieties and the highest values were observed in Chioggia and Early Wonder Tall Top, respectively. Based on the observed parameters, Chioggia and Detroit Dark Red seemed to be the better performers among the four beetroot cultivars.

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INTRODUCTION

Red beetroot (*Beta vulgaris* L.) is a traditional and popular vegetable that is consumed worldwide (Ceclu *et al*., 2020). In recent years, beetroot demand has been created both among consumers and producers of Bangladesh due to its high nutritional values. Moreover, diet concern people are attracted to beetroot as it contains high amounts of bioactive compounds mineral elements, and vitamins (Ceclu *et al*., 2020). Throughout the world, it is used predominantly for consumed either fresh or after fermentation, or after thermal processing (Clifford *et al*., 2015 and Sawicki *et al*., 2018). Apart from that, beetroot is used as an additive or food colorant in the food industry, as it improves the red colour of various food products (Chhikara *et al*., 2019). Beetroot is among the ten vegetables with the highest antioxidant activity and phenolic compound content (Chawla *et al*., 2016). Other compounds present in this vegetable are fiber, proteins, and sugars with low energetic values (Kale *et al*., 2018). Additionally, red beetroot includes crucial minerals that are beneficial to human health, including iron, zinc, phosphorus, potassium, calcium, and manganese (Chhikara *et al*., 2019). However, the chemical composition and nutritional value of red beetroot depends on the variety, the growth conditions, and the anatomical part of the plant (Sawicki *et al*., 2016). In recent years many exotic beetroot varieties are getting introduced in Bangladesh. However, limited information is available on how the biochemical and nutritional values appear in the acclimatized beetroot varieties in Bangladesh. This information will help the end users for its potential usage in the food industries. Therefore, the aim of the study was to establish the mineral and proximate compositions and investigate the bioactive compounds of four red beetroot varieties grown in Bangladesh.

MATERIALS AND METHODS

Materials

The following red beetroot (*Beta vulgaris* L.) varieties were selected for the investigations: 'Chioggia', 'Detroit Dark Red', 'Early Wonder Tall Top', and 'Red Ace'. Using standard agronomic practices, all these beetroot varieties were cultivated in 2021 at a farm in the Gozaria, Munshiganj. The beetroot seeds were sown in the middle of May. They were harvested at maturity during the first week of October.

Total soluble solids and dry matter analysis

Dry matter and total soluble solids were analysed in fresh beetroot samples. Dry matter was determined by drying samples at 105◦C to a constant weight (LST ISO 751: 2000). The amount of total soluble solids was measured using the refractometry method with a digital pocket refractometer (Atago, Kobe, Japan) (Latimer, 2016). The results were expressed as Brix.

Sample preparation for biochemical and mineral analysis

The fresh beetroots were washed and carefully peeled manually with a standard hand-held vegetable peeler and the beetroot flesh was separated and cut into slices with a thickness of approximately 1 cm. The sliced beetroot flesh samples were oven-dried at a temperature of 60◦C for 12 h (KD-400, NUVE, Turkey) and then pulverized in a laboratory mill (IKA-A11B, Germany). The obtained powder was packed in plastic bags and kept in the dark until analyses were carried out.

The amount of fiber from dried beetroot flesh and skin was determined by the Association of Official Agricultural Chemists (AOAC) official methods, the amount of ash was determined by combustion at 550◦C [(71/250/EEC). p. 22], and the amount of proteins was determined by the Kjeldahl method (DK-20 & UDK-129, VELP Scientifica, Italy). The total sugar content was established based on the Luff Schoorl method [(71/250/EEC). p. 22]. The results of the total sugar content were recalculated in fresh matter (FM).

Before elemental analysis, dried beetroot samples were treated with hydrogen peroxide and nitric acid at 120◦C until digestion was complete to mineralize them (DK-20, VELP Scientifica, Italy). A total of 0.5 g of beetroot sample was mixed with known quantities of nitric acid and hydrogen peroxide and then heated. After digestion, the resulting solution was diluted to 50ml with deionized water. The elements of calcium (Ca), potassium (K), phosphorus (P), iron (Fe), zinc (Zn), manganese (Mn), and boron (B) were measured in dried beetroot flesh. UV-VIS Spectrophotometric method was employed for P and B determination (PG-80 UV-VIS Spectrophotometer, UK); while Ca, Fe, Zn, and Mn were analysed by Atomic Absorption Spectrophotometer (AA-7000, Shimadzu, Japan). Flame photometer (PFP7, Jenway, UK) was used for K analysis. The appropriate standards for each mineral were made within the concentration range of the mineral elements in the beetroot samples. Standard blank solutions were prepared in the same conditions as the samples. The amount of N (nitrogen) was determined using the Kjeldahl method (DK-20 & UDK-129, VELP Scientifica, Italy). All chemical analyses were performed in triplicate. The results for N, Ca, K, and P were expressed in mg 100 g^{-1} DM, while the results for Fe, Zn, B, and Mn were expressed in mg kg^{-1} DM.

Sample preparation for bioactive compounds determination

The 1cm thick fresh beetroot flesh samples were frozen at −20◦C until lyophilisation. Using a lab mill (IKA-A11B, Germany), the lyophilized beetroot flesh samples were ground into a fine powder. The obtained powder was vacuum-packed and kept until analyses were carried out.

By using the Folin–Ciocalteu spectrophotometric method, as reported by Tamil-selvi et al. (Tamil-selvi *et al*., 2012), the total phenolic amount of freeze-dried beetroot was determined. A total of 0.1 g of freeze-dried beetroot sample was mixed with 10 mL of 70% ethanol and extracted for 30 min in an ultrasonic laboratory bath (Branson Ultrasonic Bath-230, HACH, Germany). After that, the extract was centrifuged at 3000 rpm for 30 min. The obtained extract was then combined with 1 mL of sodium carbonate (20%), 0.2 mL of the Folin–Ciocalteu reagent, and 5 mL of pure water. A spectrophotometer (PG-80 UV-VIS Spectrophotometer, UK) was used to measure the absorbance at 760 nm after 30 min of incubation at 20◦C in the dark. The total phenolic content was calculated with the calibration curve using gallic acid equivalent standards. The results for the total phenolic content were expressed as mg of gallic acid equivalent (GAE) per g of DM.

The total anthocyanin amount of freeze-dried beetroot flesh samples was determined using the pH differential method with a spectrophotometer (PG-80 UV-VIS Spectrophotometer, UK) (Tonutare *et al.,* 2014). A total of 0.1 g of beetroot sample was dissolved in 10 mL of solvent (85 % ethanol + 015% 1 M HCl). The obtained extract was extracted in an ultrasonic laboratory bath (Branson Ultrasonic Bath-230, HACH, Germany) for 10 min, then centrifuged at 3500 rpm for 10 min. After that, 9 mL of pH 1.0 buffer solution were combined with 1 mL of the filtered extract; the same steps were repeated by dilution with 9 mL of pH 4.5 buffer solution. The absorbance at 510 nm and 700 nm was measured after 30 min of incubation at room temperature. For quantification of total anthocyanins, the molecular weights (449 g/mol) and molar extinction coefficients (26,900 $M^{-1}cm^{-1}$) were applied. The results were given in mg of cyanidin-3-glucoside per kg of DM.

Statistical and multivariate analysis

A one-way analysis of variance (ANOVA) method was used to statistically process all data. Fisher's LSD (least significant difference) test ($p < 0.05$) was performed to determine the statistical significance of the differences between the means. The software program, Mini-TAB 17.0, was employed to analyse the data. The beetroot samples were classified based on their biochemical and mineral compositions, as well as their bioactive substances using principal components analysis utilizing the same software.

RESULTS AND DISCUSSION

Brix, dry matter and pH

The proximate composition results for the flesh of four beetroot varieties are reported in Table 1. One of the most relevant components of raw vegetables is dry matter. Its amount varies depending on variety, cultivation method and year, as well as the part of the plant (Yasaminshirazi et al., 2020). The findings of the present study suggest that pH level differs from range 5.52-6.51, where 'Chioggia' is less acidic (6.51), while, Detroit Dark Red is more acidic (5.52) compared to other varieties. On the other hand, Detroit Dark Red had the highest amount of TSS, which is (10.50◦Brix) and the Red Ace had the lowest amount of TSS (8.90◦Brix), compared to four varieties beetroot. Dry matter content is one of the most relevant components of raw vegetables. According to the literature, the amounts of dry matter widely from 8.80-11.0% (Yasaminshirazi *et al*., 2020). DM% varies depending on variety, cultivation method and year, as well as the part of the plant (Yasaminshirazi *et al*., 2020). The findings of the present study suggest that genotype differences significantly influenced the dry matter amount of the tested samples. The 'Detroit Dark Red' had the highest amounts of this component (10.50◦Brix) among genotypes. According to the literature, the amounts of dry matter and TSS for the whole beetroot varied widely from 11.0–18.1% (Yasaminshirazi *et al*., 2020) and from 8.90–10.50 ◦Brix (Šlosár *et al*., 2020).

Table 1. Status of pH, brix and dry matter contents of four beetroot varieties of Bangladesh

Variety	pH		Brix		Dry Matter (%)	
	Mean	SEM	Mean	SEM	Mean	SEM
Chioggia	6.51a	0.01	10.00b	0.06	8.80d	0.06
Detroit Dark Red	5.52d	0.01	10.50a	0.12	11.00a	0.06
Early Wonder Tall Top	5.62c	0.00	10.00b	0.06	10.40b	0.12
Red Ace	6.24b	0.01	8.90c	0.06	9.44c	0.01
p value	0.000		0.000		0.000	

Table 2. Biochemical constituents (protein, fiber, ash, total sugar and vitamin C) in different beetroot **varieties**

Variety	Protein (% DM)		Fiber		Ash		Total Sugars		Vit C (mg/100g)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Chioggia	10.80c	0.06	6.20a	0.06	8.66b	0.01	4.93b	0.09	6.20a	0.03
Detroit Dark Red	12.50a	0.12	5.40b	0.12	8.05c	0.01	6.06a	0.01	3.89b	0.03
Early Wonder Tall Top	11.47b	0.30	5.10b	0.12	7.65d	0.01	2.65c	0.01	6.09a	0.05
Red Ace	11.73a	0.18	6.10a	0.06	8.86a	0.01	2.62c	0.01	3.62c	0.06
p value	0.001		0.000		0.000		0.000		0.000	

Proximate composition and mineral elements

Mineral elements in plants and humans play a relevant role in many physicochemical processes and can affect overall health. Humans require not only proteins, lipids, carbohydrates, and vitamins, but also require some essential nutrients, such as minerals. Therefore, the nutritive value of vegetables such as beetroot is based on the amount of minerals, which are necessary for a healthy body (Ekholm et al. 2007 and Petek *et al*., 2012); however, there is lack of information in the literature on the mineral element amounts in beetroot under Bangladesh context. According to the literature, the amount of minerals in beetroot is affected by the variety, weather conditions, soil nutrient amount, fertilizer use, and harvest maturity state (Petek *et al*., 2019 and Nizioł *et al*., 2016). The number of macro-elements in the four tested red beetroot cultivars is shown in Table 2, 3 and 4.

A one-way ANOVA indicated significant variations in the amounts of macro-elements such as K, Ca, and N, depending on the beetroot variety. In Table 2, DM of four varieties beetroot indicates the variation among protein percentages, which is 10.80-12.50. Meanwhile, Detroit Dark Red is rich in protein (12.50 % DM), while Chioggia is in lower range (10.80 % DM).

The amount of P was significantly affected by beetroot. Fiber and ash did not show that much variation among four varieties as it ranges between 5.10-6.20% DM and 8.86-7.65% DM, respectively. The varieties of beetroot showed some significant differences between total sugar content and level of vitamin C, ranges between 2.62-6.06% FM and 3.62-6.20 mg/100g, respectively. The results, as supplied in Table 3 indicate macro elements of beetroot variety. K shows less difference among samples (ranges between 3331 and 2901 mg 100 g^{-1} DM). Moreover, Chioggia contained the highest amount of K (EU Regulation no.1169/2011, 2022). Our study indicated that an average N amount of Detroit Dark Red' is 1904 mg 100 g^{-1} DM, which is significantly higher than other varieties and Red Ace is 1506 mg 100 g^{-1} DM, which is significantly shown in the last position. A comparison of the amount of P between all types of beetroot indicated less significance as it ranges 212-325 mg 100 g^{-1} DM. Chioggia had a higher amount of Ca (151mg 100 g^{-1} DM); Early Wonder Tall Top and Red Ace have the lowest amount of Ca with no significant difference between them.

The RDA of K is 800 mg per day (EU Regulation no.1169/2011, 2022). The intake of 100 g per day of the investigated beetroot skin powders supplies between 47.6% and 56.3% of the RDA for Ca, while 100 g of the flesh powders supplies only 15.0–18.8% of the RDA for Ca (Šeremet *et al*., 2022) also indicates that the main macro-element in red beetroot was K (3331 mg 100 $^{g-1}$ DM), followed by a lower quantity of P (212 mg 100 $^{g-1}$ DM) and Ca (151 mg 100 $^{g-1}$ DM) (Shuaibu *et al*., 2021).

Table 3. Amounts macro-nutrient elements (mg 100 g^{-1} DM) in four beetroot varieties of Bangladesh

Variety	Potassium (K)		Nitrogen (N)		Phosphorus (P)		Calcium (Ca)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Chioggia	3331a	24	1720c	59	212c	10	151a	14
Detroit Dark Red	3283b	47	1904a	98	311b	24	141b	9
Early Wonder Tall Top	3000c	59	1772b	78	325a	13	120c	18
Red Ace	2901d	48	1506d	91	219c	21	126c	20
p value	0.000		0.000		0.000		0.000	

Table 4. Amounts micro-nutrient elements (mg 100 g^{-1} DM) in four beetroot varieties of Bangladesh

Variety	Iron (Fe)		Zinc (Zn)		Boron (B)		Manganese (Mn)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Chioggia	46c	5	24c	2	21a	1	15c	2
Detroit Dark Red	53a	8	26a	7	19b	5	17a	4
Early Wonder Tall Top	49b	6	22d	6	19b	4	12d	2
Red Ace	36d	4	25b	9	20b	8	16b	3
p value	0.000		0.000		0.000		0.000	

The number of microelements in the flesh of beetroot is summarized in Table 4. The statistical analysis revealed significant variations in the amounts of Fe, Zn, and B, and Mn depending on the beetroot variety. The dominant microelements in the investigated beetroots were Fe. The amount of this element varied widely in the beetroot. Fe varies from (36-53)mg kg^{-1} DM and Detroit Dark Red contain higher levels of Fe considering other variety, which is 53 mg kg^{-1} DM; meanwhile, comparing to other types, Red Ace shows less Fe level (36 mg kg^{-1} DM), respectively. Zn, B and Mn have no significant changes among samples, rather than other elements. However, Detroit Dark Red is rich in both Zn (26 mg kg^{-1} DM) and Mn (17 mg kg^{-1} DM); while, Early Wonder Tall Top required the less amount both in Zn (22mg kg^{-1} DM) and Mn (12 mg kg^{-1} DM), respectively.

Bioactive compounds

Phenolic compounds are the secondary metabolites of plants and contribute to the plant's growth, pigmentation, and reproduction, as well as affecting the sensorial attributes (flavour, taste, and colour) and functional properties of plant food products (Zhang *et al*., 2016 and Ribas-Agustí *et al*., 2022). The total phenolic and total anthocyanins of four red beetroot types are presented in Figure 1.

A statistical analysis showed a significant impact of beetroot varities investigated bioactive compounds. Among the flesh samples, Chioggia had the greatest total phenolic content (19.5mg g^{-1} DM) (Zin *et al*., 2022), which reported that the total phenolic content values in the flesh extract was 12.7 mg GAE g^{-1} DM, respectively. (Kujala *et al*., 2001) evaluated red beetroot skin extracts prepared by different extraction methods and solvents and found that the total phenolic content in them was lower and varied from 17.4 to 24.1 mg GAE g^{-1} DM. Carrillo et al. (Carrillo *et al*., 2019) detected that the total phenolic content for the whole beetroot grown under conventional and organic conditions ranged from 5.6–19.1 mg 100 g^{-1} DM. Data collected in the scientific literature show that the total phenolic content in red beetroot is quite variable and may be influenced by many factors. The quantities of phenolic compounds vary even among plants of the same species due to the differences in various aspects of the plants, such as different types, growing conditions, developmental stage of the plant, maturity, and analysed plant part, as well as pre-harvest factors (Vaitkeviciene et al. 2022; Kavalcová *et al*., 2015).

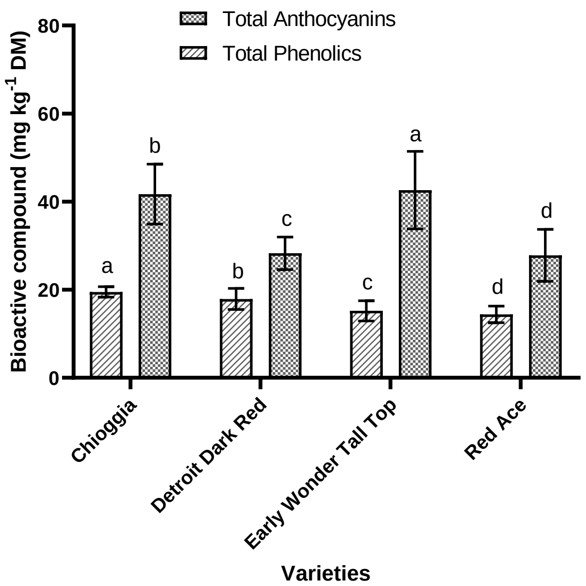

Figure 1. Bioactive compound amounts of different beetroot varieties

Anthocyanins are natural plant pigments belonging to the class of polyphenolics (Monica et al. 2018). Consequently, the highest number of total anthocyanins was recorded in Early Wonder Tall Top (42.60 mg kg^{-1}). The anthocyanins in beetroot are not well covered in the scientific literature, so it is not possible to make comparisons. Kovaroviˇc *et al*., (2017) evaluated the content of total anthocyanins in red beetroot at fresh weights and reported that the amount of this pigment in investigated beetroot samples ranged from 14.5–84.5 mg kg^{-1}. Guiné *et al*., (2018) reported that the total anthocyanins amount, which was determined using the SO_2 bleaching method and expressed as malvidin equivalents, varied from 230–770 mg kg^{-1} in fresh beetroot. The different anthocyanins extraction and analytical methods could be the causes of the differences in these investigations.

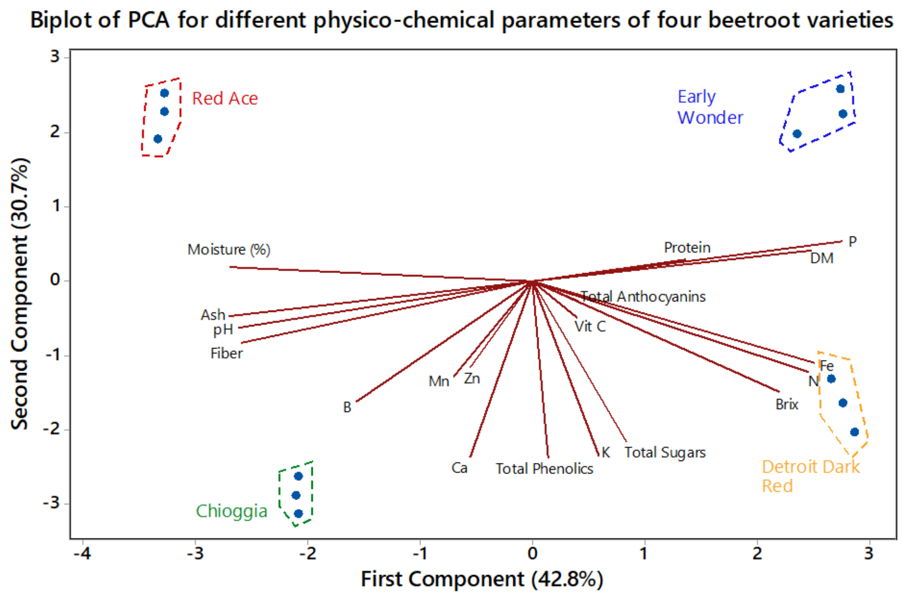

Figure 2. PCA results for the relationship between variables (pH, brix, dry matter %, moisture %, ash content, protein, fibre, vitamin C, micro and macro nutrient and bioactive compounds) of four beetroot varieties. The contribution of first component (PC1) was 42.8% and second component (PC2) was 30.7%.

Principal Component Analysis (PCA)

A PCA was used to estimate the relationships between the different sample types and variables shown in Figure 2. According to the PCA results, the first two axes explained 73.5% of the total variance: the first principal component (PC1) explained 42.8% and the second principal component (PC2) accounted for 30.7% of the variation (Figure 2). The eigenvalues of PC1 and PC2 were greater than one (8.14 and 5.84, respectively). The brix, N, Fe, DM%, P, protein, anthocyanin and vitamin C were highly positively associated with PC1, whereas ash content, pH, fibre and B were negatively associated with PC1; Mn, Zn, Ca, total phenolics, K and total sugar were negatively associated with the second factor (PC2). As shown in Figure 2, all four varieties (viz. Chioggia, Detroit Dark Red, Early Wonder Tall Top and Red Ace) separated concerning their biochemical and mineral constituents.

CONCLUSION

The proximate and mineral compositions, bioactive compounds (total phenolics, total anthocyanins) in the flesh tissues of different varieties of beetroot grown in Gozaria, Munshiganj were compared. The findings of this investigation allow for the conclusion that beetroot is a valuable source of macro- and micronutrients. The amounts of these nutrients in beetroot far exceed their quantity in the flesh. Therefore, special attention should be paid to the potential use of skin as an additive for improving the quality of food products, for the development of new functional products, or for use in the pharmaceutical industry. Among all investigated beetroot samples, the skins of 'Detroit Dark Red' and 'Chioggia' were the most valuable. The highest amounts of protein, fiber, ash, N, Zn, Fe, Mn, dry matter, and total sugars were found in 'Detroit Dark Red', while the 'Chioggia' contained the greatest amounts of K, and Ca. The 'Early Wonder Tall Top' contains highest amount of P, and total anthocyanins. The flesh of 'Chioggia' had a maximum total phenolic content.

COMPETING INTEREST

The authors declare that they have no competing interests.

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