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Original Research Article

Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria

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ABSTRACT

Beta vulgaris L. (beetroot) contains high amounts of biologically active substances including betalains and inorganic nitrate. We determined the amounts of various compounds (minerals, betalains, oxalic acid, phenolic acids, and sugars) in juice prepared from seven different beetroot varieties cultivated in Upper Austria. Large differences were found between the varieties for some substances (such as nitrate), whereas others showed only minor variation (certain minerals and sugars). The total betalain content was found to range between 0.8 and 1.3 g/L fresh juice (about 60% betacyanins and 40% betaxanthins) that accounted for 70–100% of the total phenolics content. Other detected phenolics were hydroxycinnamic acids, which accounted for up to 2.6% of total phenolics. Nitrate content varied 10-fold between single varieties. Sugar composition was similar in all varieties with an average total content of about 7.7%, consisting of 95% sucrose. Only minor differences in the concentration of oxalic acid (0.3–0.5 g/L fresh juice) were found between the varieties. In addition, 16 commercial juices and four powders were analyzed for their nitrate contents, as its metabolic product nitric oxide has been reported to provide cardiovascular benefits. Large variations of the nitrate levels, ranging from 0.01 to 2.4 g/L, were found.

1. Introduction

Beetroot (or red beet) is a cultivated form of *Beta vulgaris* subsp. *vulgaris* (*conditiva*) and describes a number of varieties of edible taproots that are grown throughout the Americas, Europe, and Asia. In contrast to their fellow subspecies *Beta vulgaris* subsp. *vulgaris* (*altissima*), known as sugar beet, the sugar content in the *conditiva* beetroot subspecies is about 2 times lower (U.S. Department of Agriculture, 2013). Therefore, beetroot is grown for food uses (pickles, salad, juice) rather than for sugar production. In contrast to other fruits, the main sugar in beetroot is sucrose with only small amounts of glucose and fructose (Bavec et al., 2010). Because fructose reduces human exercise capacity, a low fructose and a high sucrose content is preferable, for example, in sports drinks (Murray et al., 1989).

The intense red color of beetroots derives from high concentrations of betalains, a group of phenolic secondary plant metabolites. Betalains are used as natural colorants by the food industry, but have also received increasing attention due to possible health benefits in humans, especially their antioxidant and anti-inflammatory activities (Georgiev et al., 2010; Zielinska-Przyjemska et al., 2009). Other benefits include the inhibition of lipid peroxidation (Reddy et al., 2005), increased resistance to the oxidation of low-density lipoproteins (Tesoriere et al., 2003), and chemo-preventive effects (Zhang et al., 2013). The betalains that are mainly found in beetroot are betacyanins and betaxanthins (Gandia-Herrero et al., 2010). Apart from betalains, small amounts of hydroxycinnamic acids such as gallic, syringic, and caffeic acids and flavonoids have been identified (Kazimierczak et al., 2014).

Athletes, especially those in endurance sports, are the main targets of various beetroot products currently on the market. These commercial products, both juices and powders, are advertised as performance-enhancing legal nutrition supplements. The active ingredient is the inorganic nitrate (NO_3^-), which is reduced by bacteria in the salvia into nitric oxide (NO). Clinical studies suggest

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positive effects of increased NO levels on muscle efficiency and fatigue resistance (Bailey et al., 2009; Hernandez et al., 2012; Larsen et al., 2006) and improvements in time-trial endurance tests of hobby athletes (Cermak et al., 2012; Murphy et al., 2012). Furthermore, nitrate ingestion reduced resting blood pressure (Bailey et al., 2009; Kapil et al., 2010; Larsen et al., 2006; Webb et al., 2008) suggesting it being a nutritional agent for the prevention and treatment of hypertension and cardiovascular diseases (Lundberg et al., 2011). However, there are reports that high levels of nitric oxide are correlated with depressive states, which has to be taken into consideration when consuming excessive amounts of beetroot products (Suzuki et al., 2001). Furthermore, recent evidence suggests that ingested nitrate and nitrite results in an increased endogenous nitrosation, which may lead to formation of carcinogenic metabolites (Habermeyer et al., 2015).

In addition to the health beneficial compounds, however, beetroots also contain significant quantities of oxalic acid. Oxalic acid is a strong metal ion chelator interfering with iron and calcium metabolism and can lead to the formation of nephroliths (Holmes and Assimos, 2004; Salovaara et al., 2002).

In the work described here the biochemical composition of juice prepared from seven different popular beetroot varieties grown in Upper Austria was analyzed, focusing on minerals, betalains, sugars, oxalic acid, and phenolic acids. In addition, the respective antioxidant potential of each juice was determined. Furthermore, the content of selected anions found in fresh juice was compared to the one detected in more than 20 commercial beetroot juices, concentrates, and powders. The results of this study highlight the large variation in certain biologically active substances found in popular beetroot varieties. This information will be of special interest as it is hoped to facilitate the development of functional beetroot products with a strong focus on health beneficial effects.

2. Materials and methods

2.1. Materials and reagents

(+)-Catechine, AAPH (2,2′-azobis(amidinopropane) dihydrochloride), ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)), acetonitrile, fluorescein sodium salt, Folin-Ciocalteu reagent, methanosulfonic acid, phenolphthalein, potassium persulfate, potassium phosphate, sodium bicarbonate, sodium hydroxide, trifluoroacetic acid (TFA), 2,4,6-tripyridyl-s-triazine, ascorbic acid, iron(III)chloride-hexahydrate, acetate trihydrate, glacial acetic acid, oxalic acid, and Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid] were purchased from Sigma Aldrich (Schnelldorf, Germany). Standards for Na^+, Cl^-, Al^{3+}, Cu^{2+}, Fe^{2+}, K^+, Mn^{2+}, Ni^{2+}, Zn^{2+}, Mg^{2+}, and Ca^{2+} (Certipur, cation multi-element standard III) and antimony tartrate solution were from Merck-Millipore (Vienna, Austria). Potassium dihydrogen orthophosphate, potassium permanganate, and ammonium molybdate were purchased from VWR (Vienna, Austria). Sulfuric acid was obtained from Fisher Scientific (Vienna, Austria). Gallic acid, syringic acid, caffeic acid, and chlorogenic acid were purchased from Extrasynthese (Genay, France).

2.2. Beetroot selection

A total of 7 different beetroot varieties, 16 commercial beetroot juices, and five commercial beetroot powders were analyzed for their biochemical composition. The following beetroot samples were analyzed.

- *Varieties*: Ägyptische Plattrunde, Bolivar, Forono, Mona Lisa, Moronia, Redval and Robuschka.
- *Juices*: Alnatura Rote Bete Saft, Beutelsbacher Demeter Rote Bete Saft, Bio Primo Rote Bete Saft, Demeter Voelkel Rote Bete Saft, Eden Bio Rote Rübensaft, Eden Rote Bete Most, Fit Rabbit bio sport drink, Hasenfit Bio 100% Rote Rübe, Ja Natürlich Gemüsesaft, Ja Natürlich Österreichischer Rote Rübensaft, Natürlich für uns Bio Rote Rübensaft, Rabenhorst Rote Bete Bio Direktsaft, Beetroot Vinitrox, REWE Bio Rote Bete Saft, Spar Natur pur Bio Rote Rübensaft and Zurück zum Ursprung Bio Rote Rübensaft.
- *Powders*: Beet Juice Powder (Powderpure, The Dalles, OR, USA), Bioservice Rote Rübe Pulver (Bioservice Zach, Gmünd, Austria), Greif 100% Rote Bete Pulver (Greif, Seesen, Germany), Rote Bete Farbstoffpulver (Chr. Hansen GmbH, Nienburg, Germany).

The commercial juices were acquired in local supermarkets and powders were ordered from online vendors in Europe and the USA. All selected beetroot varieties, prepared juices and powders showed an intense red coloring.

2.3. Agricultural details and sample preparation

All beets grown for this study were planted on 6 May 2013 in a single field approximately 15 m × 30 m in size, in Hargelsberg, Upper Austria (latitude: 48.15°N, longitude: 14.42°E, altitude: 324 m). An Atlantic maritime climate that is characterized by low pressure fronts, mild air from the Gulf Stream, and precipitation is most prevalent in this northern region of Austria (about 900 mm total annual precipitation; mean annual air temperature of 9 °C). Each beetroot cultivar was seeded in standard rows on a total area of 12 m^2. The soil consisted of heavy to clayey loam with a soil value of 42, a humus content of 2%, and a pH of 6.8. Immediately after seeding a nitrate-phosphate-potassium (N-P-K) fertilizer (N-P-K ratio of 14:10:20; 600 kg per hectare) was used for manuring (corresponding to 84 kg nitrogen, 26.2 kg phosphate, and 100 kg potassium per hectare). No additional fertilizer was added. Beetroots in the ripe-state were harvested by hand on 1st September 2013, immediately cleaned with water, cut with a knife (root and nodality), and stored at 2–8 °C for a maximum of two days until juice preparation. Juice from single beets of each cultivar was prepared using a conventional juice maker (Kenwood JE 850 XXL, De'Longhi-Kenwood GmbH, Wiener Neudorf, Austria) and stored at −20 °C. On average one kg of beetroots yielded about 0.5 L of juice. All analyses were performed on juices prepared from 3 individual beets of the 7 tested varieties. The mean values obtained from those 3 beets were compared to each other. In addition, the variations between the individual beets of each variety were analyzed.

2.4. Soil quality determination

The trace element content in soil samples was analyzed according to ISO11464:2006. Briefly, soil samples were mixed with hydrochloric acid and nitric acid and heated under reflux conditions for two hours. After cooling, the solution was passed through filter paper and analyzed using inductively coupled plasma optical emission spectrometry (Ultima 2; Horiba Jobin Yvon, Bensheim, Germany). Each sample was measured in triplicate.

2.5. Total phenolic content (TPC)

Total phenolic content was determined using Folin-Ciocalteu reagent as described previously (Lanzerstorfer et al., 2014). Briefly, the samples were centrifuged at 10,000 rpm for 10 min and the supernatant was used for total phenolic quantitation: 1.05 mL ddH_2O was mixed with 12.5 μL sample supernatant and 62.5 μL Folin-Ciocalteu reagent. The mixture was allowed to stand for

3–6 min at room temperature followed by addition of 125 μL sodium bicarbonate solution (200 g/L). After 60 min of incubation at room temperature in the dark, absorbance was measured at 720 nm. Total phenolic content was expressed as (+)-catechine equivalents in mg/L sample. Each juice was measured in triplicate.

2.6. Oxygen radical absorbance capacity (ORAC) measurements

The ORAC assay was performed as described previously with slight modifications (Lanzerstorfer et al., 2014). A mixture of 150 μL fluorescein (10 nM) was used as the target of free radical attack, and 25 μL of AAPH (240 mM) was used as a peroxyl radical generator at 37 °C combined with 25 μL of each sample. Trolox standards ranged from 6.25 to 100 μM. Fluorescein (4 mM) and 1 mM TROLOX stock solutions were prepared in 10 mM potassium phosphate buffer pH 7.4, and stored at 2–8 °C for up to 3 days. AAPH stock (240 mM) was prepared using potassium phosphate buffer and used within 10 h after preparation. Samples were centrifuged (5 min; 10,000 rpm) prior to measurement. The supernatant was further diluted (1:200) with potassium phosphate buffer, pH 7.4. Measurements were performed in 96-well plates. In short, 150 μL of fluorescein was pipetted into each well and 25 μL of the standard or diluted sample was added. The plate was incubated at 37 °C for 30 min in the dark followed by addition of 25 μL AAPH solution per well. The decrease in fluorescence of fluorescein was determined by collecting readings at excitation of 485 nm and emission of 520 nm every minute for 90 min on a plate reader (POLARstar Omega; BMG LABTECH, Ortenberg, Germany). The ORAC value was calculated using the ORAC plug-in of the Omega MARS plate reader software. Each juice was measured in triplicate.

2.7. Ferric reducing ability of plasma (FRAP) measurements

The FRAP assay was performed based as described by Benzie and Strain (Benzie and Strain, 1996). FRAP reagent was prepared by mixing 10 parts acetate buffer (300 mM, pH 3.6) with one part 2-4-6-tripyridyl-s-triazine (10 mM in 40 mM HCl) and one part iron(III)chloride-hexahydrate (20 mM in ddH_2O) and used immediately. Beetroot juices were diluted 1:10 in ddH_2O, and then 300 μL FRAP reagent was mixed thoroughly into 10 μL of each diluted sample. Absorbance was measured at 593 nm immediately and after 10 min incubation at 37 °C using a plate reader device. FRAP results for each sample were calculated using a dilution series of Trolox. As a positive control for each assay a 1 mM ascorbic acid standard was analyzed. Each juice was measured in triplicate.

2.8. Minerals, phosphate and trace elements

The minerals K^+, Na^+, Mg^{2+}, Ca^{2+}, Cl^- and the salts NO_3^- and SO_4^{2-} were quantitated by ion chromatography (Dionex ICS1000; Thermo Fisher Scientific, Vienna, Austria) as previously described (Lanzerstorfer et al., 2014). The column heater was set at 30 °C, and a Dionex DS6 heated conductivity cell was adjusted at a sensitivity of 100 and range of 2,000. For cation analysis an Ionpac CS 12A 4-mm column coupled to a CSRS 300, 4-mm suppressor was used for the separation. The mobile phase consisted of 20 mM methanosulfonic acid, the flow rate was 1 mL/min, and sample injection volume was 25 μL. For anion analysis an Ionpac AS14 4-mm column coupled to an ASRS 300, 4-mm, was used.

Phosphate concentrations were measured using a phosphomolybdate method (Shyla et al., 2011). The combined working reagent was mixed in the following order: 100 mL ammonium molybdate solution (13 g ammonium molybdate in 100 mL ddH_2O) and 300 mL sulfuric acid (9 mol/L) were mixed. This solution was diluted with 100 mL of an antimony potassium tartrate solution (0.35 g antimony potassium tartrate in 100 mL ddH_2O). As a phosphate standard, potassium dihydrogen orthophosphate was used. The samples were passed through a 0.45 μm filter and 500 μL of the sample, 2 mL ddH_2O water, 100 μL ascorbic acid solution (10 g ascorbic acid in 100 mL ddH_2O), and 200 μL ammonium molybdate solution were mixed and filled up to 5 mL with ddH_2O. Absorbance was measured at 880 nm.

The trace elements Mn^{2+}, Cu^{2+}, Fe^{2+}, Ni^{2+}, and Zn^{2+} were quantitated by ICP-OES. ICP measurements were performed using an Ultima 2 device from Horiba Jobin Yvon (Bensheim, Germany). The following emission lines were used: Mn^{2+}: 257.610; Ni^{2+}: 221.647; Zn^{2+}: 213.856; Cu^{2+}: 324.754, and Fe^{2+}: 234.349. Samples were diluted 1:10 in 1 M nitric acid and ionized using a 1000-W setting. Nebulization was done using 1 bar pressure and a constant flow of carrier gas (14 L/min) was applied. Concentrations of each ion were calculated using pure standards.

2.9. Quantitation of phenolic compounds

Beetroot phenolics were analyzed using a method previously used in our lab for the analysis of fruit juices (Lanzerstorfer et al., 2014). For reversed-phase chromatography (RPC) analysis, a Jasco LC-2000 Plus Series system comprising of a quaternary pump with built-in degasser, autosampler, temperature-controlled column compartment and diode array detector (DAD) equipped with Chrompass software (all from Jasco Corporation, Tokyo, Japan) was used. Separation was performed on a Hypersil ODS C18 column (250 mm × 4.6 mm inner diameter, 5 μm particle size; Thermo Fisher Scientific, Vienna, Austria). Column temperature was set at 40 °C and elution was carried out at 0.8 mL/min. The following conditions were used for HPLC-DAD analysis: Mobile phase A contained 0.1% trifluoroacetic acid in water. Mobile phase B contained 80% acetonitrile and 0.1% trifluoroacetic acid. The starting conditions were 97.5% A and 2.5% B. Elution was performed with a linear gradient: The proportion of B was increased to 10% at 20 min, 20% at 32 min and 50% at 45 min. Sanitation was done using 100% B for 5 min followed by re-equilibration with starting conditions for 5 min.

The injection volume for all samples was 20 μL and eluted substances were detected using multiple UV wavelengths from 200 to 600 nm. Anthocyanins were detected as un-resolved multiple peaks showing UV-absorption between wavelengths 280–550 nm, hydroxycinnamic acids were detected at 310 nm and flavonols at 360 nm. Standards for hydroxycinnamic acids and flavonols were used to generate individual calibration curves. The limit of detection (LOD) was defined as signal to noise ratio of 2:1 and limit of quantitation (LOQ) as 4:1. For hydroxycinnamic acids, LOD of 0.05 mg/L and LOQ of 0.2 mg/L were defined with a linear range of 1–1,000 mg/L. For flavonols LOD of 0.1 mg/L and LOQ of 0.3 mg/L were defined with a linear range of 0.1–100 mg/L.

Quantitation of identified phenols was done using UV absorption by reference substances of known concentrations prepared in ddH_2O. Chlorogenic acid could not be quantitated due to co-elution with the main betacyanins.

2.10. Sugar content determination

For sugar analysis a Jasco LC-2000 Plus Series system comprised of an analytical pump with external degasser, autosampler, temperature-controlled column compartment, a Jasco RI-2031 Plus detector and a UV-Vis detector equipped with Chrompass software (all from Jasco Corporation, Tokyo, Japan) was used (Lanzerstorfer et al., 2014). Analysis of sucrose, glucose, and fructose was done on the same HPLC system. Separation was performed on an Aminex HPX-87 H300 carbohydrate column

(300 mm × 7.8 mm inner diameter, 9 μm particle size, BIO-RAD, Hercules, USA). Column temperature was set to 80 °C and isocratic elution was carried out at 0.8 mL/min. As mobile phase 5 mM sulfuric acid in ddH_2O was used. Samples were predigested for 5 h at room temperature with pectinase (10 μL per 15 mL sample), centrifuged for 10 min at 15,000 rpm followed by 0.45-μm filtration to remove any remaining solids before analysis. The injection volume for all samples was 20 μL and eluted substances were detected at 210 nm and by refractive index. Limit of detection (LOD) was defined as signal-to-noise ratio of 2:1 and limit of quantitation (LOQ) as 4:1. LOD was 0.1 g/L and LOQ was 0.5 g/L for sucrose; 10 mg/L and 20 mg/L for glucose; and 1 mg/L and 5 mg/L for fructose, respectively.

2.11. Quantitation of betalain content

Betalain content was quantitated for each sample as described previously (Stintzing et al., 2003). The concentration of betalains was calculated as the sum of the concentrations of betacyanins and betaxanthins. In short, the absorption of betacyanins at 536 nm and betaxanthins at 485 nm were measured and concentrations were calculated using the following formula:

$$\text{betacyanins (betaxanthins) content (in mg/L)} = \frac{A \times \text{DF} \times \text{MW} \times 1000}{\epsilon \times i},$$

where $A = A_{536nm} - A_{650nm}$ (betacyanins) or $A_{485nm} - A_{650nm}$ (betaxanthins); DF = dilution factor; MW (molecular weight) = 550 g/mol (for betacyanins) or 339 g/mol (for betaxanthins); ε = 60,000 (molar extinction coefficient in $L \times mol^{-1} \times cm^{-1}$ for betacyanins) or 48,000 (for betaxanthins); i = path length (cm). Samples were pre-diluted with ddH_2O water and measured in triplicate.

2.12. Oxalic acid content determination

Oxalic acid content was determined using the permanganese reduction method. Beetroot juice was diluted 1:10 with ddH_2O, and 200 μL of diluted sample was mixed with 600 μL ddH_2O and 100 μL of 1 M H_2SO_4. The sample was heated to 75–85 °C and titrated with a 0.02 M $KMnO_4$ solution until the solution remained colorless. The concentration of oxalic acid was determined using a reference curve generated by pure oxalic acid. All samples were measured in triplicate.

2.13. Statistical analysis

Statistical differences between the data sets were determined by two-way ANOVA followed by Tukey's multiple comparison test using GraphPad Prism (version 6.00 for Windows; La Jolla, California, USA).

3. Results and discussion

3.1. Soil composition and beetroot yield

Natural determinants such as soil composition, total annual precipitation, local climate, and hours of sunshine affect the accumulation of nutrients and phytochemicals in plant parts and fruits (Kishima et al., 1995; Lee and Wiley, 1981). To minimize the influence of these external factors the investigated beet varieties were grown on a single field. To demonstrate a uniform distribution of soil constituents over the entire area of the field, soil samples were taken at six different sites prior to planting of the seedlings. The trace elements Al^{3+}, Cu^{2+}, Fe^{2+}, K^{+}, Mn^{2+}, Ni^{2+}, and Zn^{2+} were quantified for each soil sample. The measured contents for each element are summarized together with the averaged contents in Table S1. Iron and aluminum were the most abundant with, on average, 22 and 19 mg per g dry weight, respectively. Potassium and manganese were medium abundant with 2.5 and 1 mg per g, whereas the contents of zinc, copper, and nickel were below 50 μg per g of soil. The concentrations of trace elements in this region of Austria were in line with information from public databases (Tulipan and Freudenschuß, 2014). For all trace elements, only minor variation was observed between the six different locations.

The 7 beetroot varieties cultivated for this study represent the majority of the varieties currently grown in Austria, both in private and conventional farming, with an annual harvest of about 6,000 tons in total (Austria, 2013). The average yield for beetroot in conventional farming in 2013 was 4.3 kg/m^2 (Austria, 2013). According to an important Austrian supplier of seeds (Saatgut, Linz, Austria), the beetroot varieties Mona Lisa, Forono, and Robuschka are of special importance for agriculture in Austria. In addition, the variety Ägyptische Plattrunde is especially popular in organic farming. The resulting yields for each variety in our study, in kg/m^2, are given in Table 1. All varieties showed comparable yields between 2.55–3.25 kg/m^2 (70% on average) with the exception of Robuschka yielding only 0.93 kg/m^2. Compared to the six other varieties investigated for this study, this variety made up only one-third of the harvest, reducing its agricultural value significantly. In addition, the weight of the individual beets that were used for subsequent analysis in this study is indicated in Table 1. In line with the lower yield, Robuschka beets were found to be the smallest ones, with a mean weight of 214 g or 58% of the averaged beet weight. In general, the observed yield performance was found to be in a similar range as reported in other studies. For example, the yield of Moronia has been shown to be about 3 kg/m^2 under completely different growth conditions, which is almost identical to the values obtained in our study (Ijoyah et al., 2008).

3.2. Quantitation of minerals and trace elements

In order to characterize potential differences in the content of selected compounds between different beetroot varieties, 3 individual beets of each variety were analyzed. As indicated in Table S2, the mean coefficient of variation (%CV) of all analyzed minerals and trace elements varied between 11% (PO_4^{3-}) and 36% (Cu^{2+}) for the 3 individual beets of each variety. Analysis of variance indicated that the variation between different varieties was significantly higher for Cu^{2+}, Fe^{2+}, Zn^{2+}, and Mn^{2+} ions, while Ni^{2+} ions showed only minor variation. The averaged values for the 7 analyzed varieties are indicated in Table 2. Since the trace element content in the soil varied only marginally, we conclude that the observed differences in the beetroot juices were variety-specific.

For minerals the variety-specific difference was higher than those of individual beets for PO_4^{3-} and especially NO_3^{-} (Table 2

Table 1
Yields of 7 beetroot varieties under study. Values were obtained from 3 beets of each variety.

Variety	Yield [kg/m^2]	Mean weight [g]	SD	%CV
Mona Lisa	3.17	344	64.5	18.8%
Moronia	3.25	293	35.2	11.9%
Redval	3.15	362	50.2	13.9%
Ägyptische Plattrunde	2.55	366	41.7	11.2%
Robuschka	0.932	214	31.1	14.7%
Forono	3.13	279	75.5	26.9%
Bolivar	2.93	329	23.4	7.07%

Table 2
Trace element and mineral contents of 7 beetroot varieties under study. ± indicates SD from 3 individual measurements.

Variety	Trace elements [µg/L]					Minerals [mg/L]							
	Cu^{2+}	Fe^{2+}	Mn^{2+}	Ni^{2+}	Zn^{2+}	Na^{+}	K^{+}	Mg^{2+}	Ca^{2+}	Cl^{-}	NO^{3-}	SO_4^{2-}	PO_4^{3-}
Mona Lisa	173 ± 70.6	2067 ± 469	981 ± 225	43.3 ± 6.72	2195 ± 809	363 ± 61.7	2817 ± 382	251 ± 36.6	32.3 ± 7.35	247 ± 32.2	4626 ± 658	199 ± 46.4	740 ± 85.3
Moronia	22.0 ± 15.4	1551 ± 479	496 ± 98.6	38.1 ± 4.74	831 ± 183	490 ± 149	2628 ± 295	290 ± 21.7	24.6 ± 10.2	262 ± 59.4	1358 ± 742	155 ± 34.7	554 ± 109
Redval	146 ± 49.1	2052 ± 895	718 ± 183	28.6 ± 7.46	1258 ± 534	464 ± 90.3	3067 ± 345	337 ± 39.0	103 ± 13.7	259 ± 38.7	2963 ± 279	199 ± 33.6	651 ± 65.7
Ägyptische Plattrunde	261 ± 109	4034 ± 1730	1104 ± 352	19.5 ± 1.61	885 ± 195	422 ± 55.8	3324 ± 306	265 ± 63.6	116 ± 6.56	311 ± 25.3	1637 ± 565	286 ± 54.9	718 ± 87.4
Robuschka	377 ± 74.9	2421 ± 362	987 ± 431	20.4 ± 4.84	970 ± 40.9	380 ± 61.4	2900 ± 474	230 ± 19.3	30.9 ± 4.58	132 ± 32.8	564 ± 129	278 ± 51.0	1622 ± 200
Forono	98.6 ± 24.0	321 ± 92.8	352 ± 109	19.0 ± 3.06	105 ± 59.4	494 ± 168	2701 ± 519	225 ± 70.0	78.2 ± 5.31	186 ± 78.3	1730 ± 526	184 ± 58.5	721 ± 131
Bolivar	392 ± 86.3	2995 ± 1321	1196 ± 276	23.1 ± 4.82	1677 ± 531	460 ± 203	2336 ± 637	218 ± 33.4	99.3 ± 22.4	137 ± 72.6	914 ± 422	157 ± 28.1	1062 ± 254
Mean	210	2206	833	27.4	1132	439	2825	259	69.3	219	1970	208	867
SD	139	1157	318	9.74	667	51.9	317	42.5	39.1	68.3	1395	53.5	368
%CV	66.4%	52.5%	38.2%	35.5%	59.0%	11.8%	11.2%	16.3%	56.5%	31.1%	70.8%	25.6%	42.4%

and Table S2). The mean nitrate content varied between 565 mg/L (Robuschka) and 4,626 mg/L (Mona Lisa). This result is of commercial significance as the presence of high nitrate concentrations is the main reason for beetroot juice being promoted in sports as a performance-enhancing drink and for the proposed decrease in blood pressure (Webb et al., 2008). Thus, in view of the three-fold higher yield of Mona Lisa in comparison to Robuschka, a significant net productivity gain for nitrate (28 times more) would be possible. Analysis of variance showed that other minerals such as Mg^{2+} and Na^{+} with the exception of K^{+} varied to a far lesser extent. Despite the lowest nitrate levels determined in Robuschka beetroots, PO_4^{3-} was found to be most abundant in this variety (1,622 mg/L). No correlation of the quantitated trace elements and minerals could be detected when linear regression and correlation analysis was performed (data not shown). So far, data on the concentration of trace elements and minerals in juice prepared from different beetroot varieties are limited. However, the determined concentration, for example, for PO_4^{3-} in this study (about 900 mg/L, corresponding to about 450 mg/kg fresh weight), is in good agreement with published data on the phosphate content in beetroot, irrespective of the investigated variety (Petek et al., 2008; Pries, 2010). For nitrate, the determined mean concentration (1,970 mg/L, corresponding to about 985 mg/kg fresh weight) was found to be in a similar range as reported for other varieties, such as Bikor (Petek et al., 2012). In conclusion, great variations in the concentration of some minerals and trace elements, especially nitrate, were clearly observed among the analyzed beetroot varieties.

3.3. Quantitation of betalains and phenolic compounds

Beetroots contain large quantities of betalains (betacyanins and betaxanthins) that serve as color pigments (Delgado-Vargas et al., 2000). At the same time, these substances possess high antioxidant potential *in-vitro* and are thus promising candidates for health-promoting effects in humans (Jiratanan and Liu, 2004). However, detailed information about the content and composition of betalains in different beetroot varieties is limited (Kazimierczak et al., 2014; Kujala et al., 2000; Lee et al., 2014). For this study, the betalain and total phenolic contents of juices prepared from 7 beetroot varieties was determined. As shown in Table 3, the total betalain content varied between 0.8 g/L (Bolivar) and 1.3 g/L (Mona Lisa), with the mean value being 1.1 g/L. The ratio between betacyanins and betaxanthins was around 1.75 to 1 for all varieties, indicating that the betalain composition for individual varieties was similar, and only the total contents varied. A similar ratio of betacyanins and betaxanthins has been previously reported for other beetroot varieties (Kujala et al., 2002). As shown in Table S3 the differences between individual beets of the same variety were lower (%CV = 16.5) than those of the different varieties (%CV = 23.0), respectively. Thus, the betalain content appeared to be variety-specific, consistent with results reported previously (Lee et al., 2014). This finding was also confirmed by analysis of variance using 2-way ANOVA comparison.

In a next set of analyses the total phenolic content (TPC) was measured using a Folin-Ciocalteu reagent-based assay. The mean TPC varied from 0.9 to 1.3 g/L (Table 3). The TPC among the analyzed varieties was highest for Mona Lisa and Moronia (1.28 and 1.29 g/L, respectively). However, analysis of variance did not show a significant variety-specific difference of the TPC content (Table S3).

Consistently, reversed-phase chromatography was used to determine additional phenolic compounds described recently (Kazimierczak et al., 2014), such as gallic acid, chlorogenic acid, and quercetin derivatives. In contrast to this study on extracts prepared from beets, we analyzed the untreated juice without

Table 3
Total phenolic content (TPC), antioxidant capacity (ORAC and FRAP), pigments, and phenolic acids concentration of 7 beetroot varieties under study. ± indicates SD from 3 individual measurements.

Variety	TPC [g/L]	ORAC [mM TE]	FRAP [mM TE]	Pigments [mg/L]			Phenolic acids [mg/L]			
				Betalain total	Betacyanins	Betaxanthins	Gallic acid	Syringic acid	Caffeic acid	Ferulic acid
Mona Lisa	1.28 ± 0.223	37.9 ± 2.82	37.1 ± 6.21	1309 ± 140	807 ± 99.3	501 ± 46.7	27.7	2.02	10.3	1.24
Moronia	1.29 ± 0.175	24.0 ± 1.71	23.3 ± 2.88	1135 ± 127	633 ± 75.6	501 ± 53.9	17.8	0.865	3.14	0.335
Redval	0.85 ± 0.146	19.7 ± 6.56	17.8 ± 3.16	853 ± 80.1	466 ± 41.7	387 ± 38.6	14.4	1.34	3.03	0.751
Ägyptische Plattrunde	1.01 ± 0.271	25.7 ± 0.78	22.6 ± 4.05	933 ± 147	576 ± 87.9	357 ± 61.9	30.2	0.674	5.78	0.399
Robuschka	0.885 ± 0.136	28.5 ± 2.93	23.2 ± 2.94	767 ± 101	465 ± 69.5	301 ± 32.6	10.8	2.91	4.46	0.764
Forono	0.984 ± 0.214	23.7 ± 0.73	17.4 ± 4.03	826 ± 197	515 ± 135	311 ± 62.8	21.3	1.63	3.74	0.246
Bolivar	1.10 ± 0.257	28.0 ± 3.86	19.4 ± 4.77	789 ± 177	487 ± 86.3	301 ± 93.3	30.4	3.54	3.32	0.854
Mean	1.06	26.8	23	1103	705	397	21.8	1.85	4.82	0.651
SD	0.209	5.75	6.73	253	156	100	7.95	1.15	2.62	0.335
%CV	17.4%	21.4%	17.4%	23.0%	22.2%	25.2%	36.1%	58.1%	54.6%	58.0%

previous extraction of phenolic compounds. As expected, the vast majority of phenolic compounds found in beetroot juice belonged to the betalain family (multiple betacyanins and betaxanthins, which could not be quantitated due to unavailable standards), with the remaining compounds representing only a very small fraction. From these substances hydroxycinnamic acids represented the most abundant group with detectable concentrations of gallic, syringic, caffeic, and ferulic acids (see Table 3). Chlorogenic acid could be detected using DAD, but quantitation was not possible as it co-eluted within the main peak of betacyanins (a representative HPLC-DAD diagram, indicating retention times and maximal wavelengths of assignable compounds, is provided in the Supplementary material, Figure S1). In contrast to other published data (Kazimierczak et al., 2014), we did not detect any flavonols, most probably because of their low abundance in juice compared to extracts prepared from dry matter samples. The most abundant trace polyphenol was gallic acid with levels of 11–30 μg/L followed by caffeic acid, syringic acid, and ferulic acid. On average, these four substances accounted for only about 3% of total phenolic compounds analyzed. In summary, the calculated coefficients of variation as well as results from ANOVA-based analysis of variance illustrate that the differences between individual beets of the same variety were lower than those of the different varieties (Table 3 and Table S3), suggesting a variety-specific content of the analyzed polyphenolic acids.

Our results indicate the general dependence of the TPC on the betalain concentration, as can be seen by linear regression and correlation analysis (Fig. 1A). Considering the low concentration of other polyphenolic compounds in comparison to the one of betalains this dependence appears reasonable.

3.4. Quantitation of total antioxidant capacity

It has been shown that beetroots possess strong antioxidant capacity (Ravichandran et al., 2013; Wootton-Beard and Ryan, 2011). Here two different assays (ORAC and FRAP) were used to

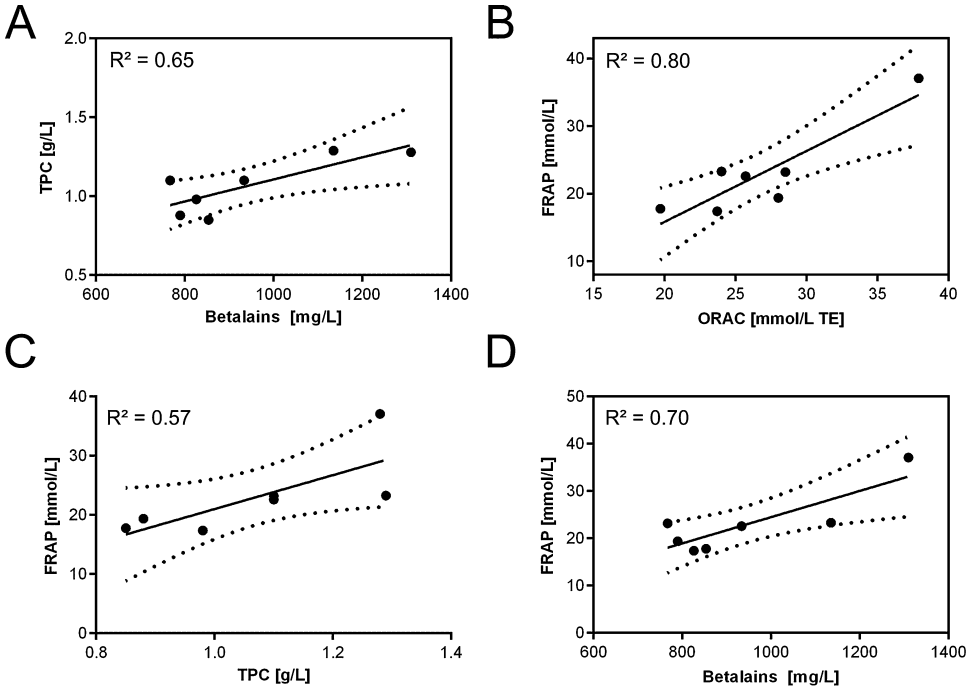

Fig. 1. Linear correlation regression analysis. Correlations between (A) betalains and TPC; (B) ORAC and FRAP; (C) TPC and FRAP; (D) betalains and FRAP; ORAC = oxygen radical absorbance capacity. FRAP = ferric reducing ability of plasma. TPC = total phenolic content. TE = Trolox equivalent.

measure the antioxidant capacity of beetroot juices prepared from 7 different varieties. The full list of ORAC and FRAP values, including those of single beets of the same variety, can be found in Table 3 and Table S3. The mean antioxidant capacity obtained from ORAC measurements ranged from 23.9 (Redval) to 37.9 mmol/L (Mona Lisa), the mean value was calculated to be 27.4 mmol/L. Those from FRAP measurements were determined to range from 17.4 (Forono) to 37.1 (Mona Lisa), with an average value of 23 mmol/L. As shown in Fig. 1B, linear regression and correlation analysis showed a significant correlation of FRAP and ORAC values, confirming the suitability of both assays. In addition, the obtained antioxidant capacity was clearly dependent on the TPC/betalain content (Fig. 1C and D). In conclusion, the antioxidant capacity of beetroot juice differs among the analyzed varieties and is dependent on the concentration of phenolic compounds. Mona Lisa was found to have the highest antioxidant capacity in both assays, consistent with the highest TPC/betalain content in this variety.

3.5. Quantitation of sugars and oxalic acid

Being an important nutritional factor, the total sugar content was quantitated together with the one of sucrose, glucose, and fructose. As indicated in Table 4, the average total sugar content was found to be 77.5 g/L (7.8%), with the vast majority being sucrose (94.8%) followed by glucose (3.3%) and fructose (1.9%). The quantitated concentrations were in good agreement with measurements performed on beetroot in comparison with carrot and turnip (Rodriguez-Sevilla et al., 1999). The differences between individual beets of the same variety (Table S4) were found to be in a similar range as those of the different varieties (%CV = 13). This finding was also confirmed by ANOVA-based analysis of variance. Thus, our data suggest only minor variety-specific differences in the concentration of sugar, with Ägyptische Plattrunde having the lowest and Forono the highest sugar content. In contrast to an observed correlation of Na^+ and sucrose content in sugar beets, our data exclude this dependence in the analyzed beetroot varieties (Martin et al., 2001).

Finally, the concentration of oxalic acid, a dicarboxylic acid that is known for chelating metal cations (such as calcium and magnesium) and a putative subsequent formation of kidney stones and gallstones (Holmes and Assimos, 2004; Weaver et al., 2006), was determined. In comparison to other vegetables and fruits, beetroots have been reported to contain rather large quantities (400–600 mg/100 g fresh weight) of oxalic acid (Duke, 2000). Thus, excessive consummation of beetroots may lead to negative health effects. Therefore the concentration of oxalic acid in juices prepared of each variety was determined. As indicated in Table 4, the average oxalic acid content was 412 mg/L with a %CV of 21. The highest content was found in "Mona Lisa" with 525 mg/L and the lowest content in "Forono" with 300 mg/L. Differences between individual beets of the same variety (Table S4) were found to be in a similar range as those of the different varieties (%CV = 25), suggesting only minor variety-specific differences in the concentration of oxalic acid. This finding was confirmed by ANOVA-based analysis of variance. There are no general recommendations, but a daily dose of 500 mg oxalic acid is considered to pose no health risk (Noonan and Savage, 1999). Therefore, for healthy humans the observed oxalic acid levels can be considered harmless when drinking normal amounts of juice (less than 1 L per day).

3.6. Biochemical analysis of commercial beetroot juices and powders

Currently numerous beetroot products are available on the market, both juices and powders. We analyzed the contents of chloride, nitrate, and sulfate for a total of 16 beetroot juices currently being sold in Austria: Two samples were juice concentrates, especially marketed for their performance-enhancing activity, and 14 were pure beetroot juices (conventional and organic production). The main selling point for those products stems from the assumed positive effect of nitrate. If such an effect actually exists, the concentration of nitrate in the final product is of uppermost importance for this desired effect. In Table 5 the results for Cl^-, NO_3^-, and SO_4^{2-} ions in the 16 juices are summarized. The average content for the juices were 477 mg/L for Cl^-, 909 mg/L for NO_3^-, and 182 mg/L for SO_4^{2-} ions, respectively. While the variation for SO_4^{2-} was rather low (%CV = 17.6), the one for NO_3^- was significant (%CV = 64.8), it varied between virtually no (EDEN Bio Rote Bete Most) and 2,400 mg/L (Hasenfit Bio 100% Rote Rübe). In addition to the found differences of the NO^{3-} content, ANOVA-based analysis of variance also indicated significant differences in Cl^- concentrations. No label stated the beetroot variety used as raw material, but one can assume that different varieties of beetroot were used for the different products. Furthermore, additional post-harvest processing steps (such as lactic fermentation) were not specified. While minor differences in the composition of beetroots grown under conventional or organic farming conditions have been reported (Bavec et al., 2010; Kazimierczak et al., 2014; Szopinska and Gaweda, 2013), the observed variations of the commercial juices analyzed in this study are likely to depend more on the variety of the used beetroot raw material.

Compared to juice prepared from the 7 varieties described in this study, the NO_3^- concentration in commercial beetroot juices was significantly lower. Only one analyzed variety (Robuschka) was found to have lower NO_3^- levels. In contrast, SO_4^{2-} concentrations in the commercial juices were similar to those in the 7 analyzed varieties.

Finally, four commercially available beetroot powders were analyzed. For analysis, solutions containing 50 g/L were prepared.

Table 4
Sugar and oxalic acid contents of 7 beetroot varieties under study. ± indicates SD from 3 individual measurements.

Variety	Sugars [g/L]				Oxalic acid [mg/L]
	Sucrose	Glucose	Fructose	Total	
Mona Lisa	69.0 ± 12.1	3.51 ± 0.525	1.12 ± 0.212	73.6	525 ± 65.4
Moronia	78.9 ± 6.27	2.79 ± 0.541	1.03 ± 0.286	82.6	375 ± 65.8
Redval	82.7 ± 5.53	1.26 ± 0.364	0.575 ± 0.156	84.3	375 ± 65.2
Ägyptische Plattrunde	56.3 ± 5.09	4.04 ± 0.928	1.76 ± 0.137	62.0	488 ± 129
Robuschka	65.5 ± 9.01	1.57 ± 0.373	1.42 ± 0.480	68.4	488 ± 65.4
Forono	86.9 ± 22.5	2.23 ± 1.82	2.96 ± 3.04	92.0	300 ± 172
Bolivar	75.2 ± 8.05	2.95 ± 1.18	1.71 ± 0.482	79.8	338 ± 112
Mean	73.5	2.62	1.51	77.5	412
SD	10.6	1.06	0.851	10.2	86.4
%CV	14.5%	43.3%	52.6%	13.4%	21.4%

Table 5
Chloride, nitrate, and sulfate concentration of 16 commercial beetroot juices under study. ± indicates SD from 3 individual measurements.

Commercial product	Cl^- [mg/L]	NO_3^- [mg/L]	SO_4^{2-} [mg/L]
"Hasenfit" Bio 100% Rote Rübe	420 ± 8.95	2357 ± 50.1	229 ± 8.82
"Natürlich für uns" Bio Rote Rüben Saft	266 ± 1.96	1628 ± 9.74	214 ± 5.96
"Demeter Voelkel" Rote Bete Saft	357 ± 3.11	1417 ± 8.26	223 ± 1.17
"EDEN" Bio Rote Bete Saft	590 ± 3.78	1183 ± 2.57	177 ± 6.83
"Spar Natur pur" Bio Rote Rübensaft	394 ± 12.8	1112 ± 35.4	166 ± 5.94
"REWE" Bio Rote Bete Saft	766 ± 3.26	941 ± 4.23	167 ± 0.691
"Zurück zum Ursprung" Bio Rote Rübensaft	495 ± 0.134	896 ± 21.5	237 ± 15.3
"Rabenhorst" Rote Bete Bio Direktsaft	650 ± 17.11	602 ± 14.9	187 ± 3.96
"Bio Primo" Rote Bete Saft	666 ± 2.98	598 ± 22.0	161 ± 4.73
"Alnatura" Rote Bete Saft	421 ± 2.84	530 ± 4.38	173 ± 1.01
"Ja natürlich" Gemüsesaft	435 ± 7.74	513 ± 12.2	159 ± 3.67
"Beutelsbacher" Demeter Rote Bete Saft	376 ± 7.22	398 ± 7.22	155 ± 3.08
"Ja natürlich" Österreichischer Rote Rüben Saft	315 ± 15.3	388 ± 19.9	141 ± 9.61
"EDEN" Bio Rote Bete Most	548 ± 3.17	14.8 ± 0.473	135 ± 3.32
Red Beet Vinitrox Shot	4996 ± 322	3968 ± 252	1049 ± 81.4
Fit Rabbit bio Sport Drink	1727 ± 99.7	3858 ± 37.9	539 ± 33.7
Mean	816	1275	257
SD	1093	1175	231
%CV	134%	92.1%	89.9%

The results for Cl^-, NO_3^-, and SO_4^{2-} ions are shown in Table 6. According to the manufacturers, 1 kg of red beet yields about 80 g of dried powder. In our experience, 1 kg of fresh beetroots yielded about 0.5 L juice. Using these two factors, it is possible to compare juices with powders. Unexpectedly, the four powders divided into two groups when comparing the nitrate contents. Two powders contained less NO_3^- than juice prepared from a beetroot variety with the lowest NO_3^- level, and about the content of the lowest commercial juice. The remaining two powders, however, contained about 35% more NO^{3-} than the average NO_3^- level of the 7 analyzed beetroot juices and nearly 3 times more than the one of the commercial juices. Consequently, the %CV for nitrate content over all five samples was 93%, much larger than those seen in beetroot varieties or commercial juices. ANOVA-based analysis of variance also indicated a significant variety-specific difference in the NO^{3-}, SO_4^{2-} and Cl^- content.

There is good agreement that nitrate supplementation by beetroot juice improves exercise tolerance through vascular control and elevated O_2 delivery to skeletal muscles (Ferguson et al., 2013; Pinna et al., 2014; Wylie et al., 2013). Furthermore, beetroot juice was found to exhibit blood pressure lowering and vasoprotective effects (Kapil et al., 2010). In general, dietary uptake of nitrate and/or nitrite has been linked to beneficial NO-mediated physiological effects in humans, such as regulation of blood pressure, vascular control and enhanced vasodilation (Dejam et al., 2007; Lundberg and Weitzberg, 2005; Maher et al., 2008). In addition, protective effects against ischemia have been reported (Jung et al., 2006). Moreover, dietary nitrate has been shown to abate symptoms of the metabolic syndrome (Carlstrom et al., 2010).

Table 6
Chloride, nitrate, and sulfate concentration of four commercial beetroot powders under study. Powders were analyzed as 50 mg/L solutions. ± indicates SD from 3 individual measurements.

Commercial product	Cl^- [mg/L]	NO_3^- [mg/L]	SO_4^{2-} [mg/L]
"Rote Bete Farbstoffpulver", Chr. Hansen	916 ± 8.94	393 ± 2.23	289 ± 2.28
"Beet Juice Powder", Powderpure	876 ± 2.22	524 ± 2.29	444 ± 1.13
"Rote Bete Farbstoffpulver", Greif	849 ± 36.0	2517 ± 12.1	341 ± 7.94
"100% Rote Bete Pulver", Bioservice	958 ± 21.6	2721 ± 54.4	378 ± 101
Mean	900	1539	363
SD	47.8	1251	65.5
%CV	5.35%	81.3%	18.0%

However, it is worth mentioning that high amounts of nitrates may also have negative effects on human health. For example nitrous acids are formed by Gram-positive bacteria with different systemic effects on human body (Bruning-Fann and Kaneene, 1993). Furthermore, nitrite can react with hemoglobin to form methemoglobin, which cannot bind oxygen (Camp, 2007). In addition, nitrite can react with ammine derived from meat digestion generating nitrosamines (*N*-nitroso compounds, NOCs), known as mutagenic compounds with carcinogenic effects in different animal species (Gangolli et al., 1994). The association of endogenous NOC formation and human carcinogenesis still remains a matter of debate. NOCs are shown to induce tumors in the gastrointestinal tract in experimental models, but there is little evidence that these compounds are directly involved in the induction of such tumors in humans. To facilitate a holistic estimation of the consequences of a nitrate/nitrite rich diet for human health further comprehensive studies, preferentially in humans, have to be conducted (Habermeyer et al., 2015). However, there is a broad consensus that the advantages of dietary vegetables rich in nitrate outweigh potential disadvantages.

The concentration of nitrate in the analyzed juice concentrates, as long as consumed in the recommended quantity (for example 60 mL of the Red Beet Vinitrox Shot, which corresponds to 240 mg nitrate per serving), and juices (500 mL serving) is lower or only slightly higher than the acceptable daily intake (ADI) of nitrate as defined by the WHO (3.7 mg/kg body weight per day) (Speijers and Brandt, 2003).

An important point which has to be taken into consideration is the high sugar content of the prepared beetroot juices (62.0–92.0 g/L). The WHO recommendation regarding sugar consumption is about 25–50 g of sugar per day for an adult with normal Body Mass Index (BMI). According to these recommendations 300–600 mL of the prepared juice suffice to achieve the maximum daily consumption of sugars. Thus, to tap the full health potential of beetroot products without raising a problem concerning overconsumption of sugars, it will be of great importance to develop products with reduced sugar levels.

4. Conclusion

Taken together, we found significant differences in the concentration of selected compounds in juice prepared from different beetroot varieties, including especially NO_3^-. This finding is of great importance for the development and production of beetroot juice supplements with high efficacy. The variety Mona Lisa described in this study may serve as an optimal source of beetroot raw material.

Author contributions

J.Wr., O.H., and J.W. conceived and designed the experiments. J.Wr., G.W., S.H., U.M., and P.U. performed the experiments. J.Wr., G.W., and P.L. analyzed the data. J.Wr. and J.W. wrote the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfca.2015.03.005.

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