Comparison of Total Antioxidant Potential, and Total Phenolic, Nitrate, Sugar, and Organic Acid Contents in Beetroot Juice, Chips, Powder, and Cooked Beetroot

Julia Vasconcellos¹, Carlos Conte-Junior², Davi Silva¹, Anna Paola Pierucci³, Vania Paschoalin¹, and Thiago Silveira Alvares^{4,*}

¹Chemistry Institute, Federal University of Rio de Janeiro, 21941-909, Rio de Janeiro-RJ, Brazil 2 Department of Food Technology, Fluminense Federal University, 24230-340, Niterói-RJ, Brazil ³Nutrition Institute, Department of Basic Nutrition and Experimental, Federal University of Rio de Janeiro, 21941-090, Rio de Janeiro-RJ, Brazil
⁴Nutrition Institute, Nucleus of Pasic Nutrition and Pistotics, Esdeval U ⁴Nutrition Institute, Nucleus of Basic Nutrition and Dietetics, Federal University of Rio de Janeiro, 27979-000, Macaé, Brazil

Received May 18, 2015 Revised August 22, 2015 Accepted September 10, 2015 Published online February 29, 2016

*Corresponding Author Tel: +55-22-2759-3431 Fax: +55-21-2562-7266 E-mail: alvares@macae.ufrj.br

pISSN 1226-7708 eISSN 2092-6456

© KoSFoST and Springer 2016

Abstract Beetroot is a vegetable rich in nitrate (NO₃⁻), antioxidants and phenolic compounds that are related to improvements in cardiovascular function and exercise performance. However, it is unknown if convenient forms of beetroot administration provide different amounts of these nutrients. The total antioxidant potential (TAP), total phenolic (TPC), sugar, organic acid, and NO₃⁻ contents of beetroot juice (BJ), chips (BC), powder (BP), and cooked beetroot (CB) were compared. Significant (p<0.01) differences in chemical compositions and functional properties were found between beetroot formulations. Higher amounts of TAP and organic acids were observed in BC and BP, compared with the other formulations. BJ exhibited the highest contents of total sugars, TPC, and NO₃⁻. All beetroot formulations were suitable and advantageous based on taste preferences and convenience for consumers and for nutrient amounts required to meet dietary recommendations.

Keywords: vegetable, antioxidant, beetroot, drying method

Introduction

Consumption of vegetables has been associated with reduced risks of cancer, type 2 diabetes mellitus, hypertension, coronary heart disease, and stroke (1,2). Great interest has developed in consumption of antioxidants from foods in order to balance the level of free radical production for normal cell function. There is now considerable interest in elucidation of potential antioxidant and phenolic compounds of foods. Furthermore, consumption of nitrate-rich vegetables $(NO₃⁻)$ has been proposed for enhancement of cardiovascular function (3).

Beta vulgaris L., or beetroot, is a vegetable of the Chenopodiaceae family that is widely consumed in traditional western cooking (4). This vegetable represents a renewable and cheap source of nutrients, like carbohydrates (5). However, specific interest in beetroot has arisen due to the fact that it is a rich source of a number of polyphenolic compounds $(6,7)$ and $NO₃⁻ (3)$.

Beetroot contains pigments called betalains that are composed of vulgaxanthin I, vulgaxanthin II, indicaxanthin, betanin, prebetanin, isobetanin, and neobetanin, and a pool of phenolic compounds that includes phenolic acids, flavonoids, and organic and inorganic acids (4,8). Furthermore, beetroot also contains smaller amounts of other compounds, such as ascorbic acid, which may further increase the total antioxidant capacity. Previous studies have demonstrated the potential antioxidant effect of betalains (9) and their bioavailability in humans (10). The results of these studies have indicated important information for stimulation of people to consume foods such as beetroot that are rich in betalain as a nutritional strategy to provide protection against oxidative stress-related disorders.

Recently, consumption of $NO₃⁻$ rich beetroot juice has been proposed for enhancement of blood perfusion (10), restoration of endothelial function (11), and improvement in exercise performance (12,13). These properties may be due to the effect of NO_3^- present in beetroot juice for stimulation of endogenous synthesis of nitric oxide (NO).

Drinking beetroot juice and eating beetroot chips and/or beetroot powder can provide a convenient and alternative source in place of consumption of the whole vegetable. Limited scientific information is available concerning nutritional aspects of different forms of beetroot. This study was carried out to compare the total antioxidant potentials and total phenolic, $NO₃⁻$, sugar (glucose, fructose, and sucrose) and organic acid (citric, malic, and ascorbic acids) contents

of beetroot juice, chips, powder, and cooked beetroot.

Materials and Methods

Samples All beetroots used in this study were Beta vulgaris L., Family Chenopodiaceae. Conventional beetroot was acquired in the city of Rio de Janeiro in Southeastern of Brazil (22°54'S 43°10'W) between March-April of 2013. Each beetroot formulation (beetroot juice-BJ, beetroot chips-BC, Beetroot powder-BP and cooked beetroot-CB) was subjected to 3 extraction trials with subsequent analysis in triplicate.

Beetroot juice (BJ) Beetroots were thoroughly washed in tap water, sanitized using a chlorine solution (0.5%), and processed in a centrifuge blender (Model CE700; Black & Decker®, Uberaba, Brazil). The resulting juice had a 91.55±0.75% moisture content without any chemical additives.

Beetroot chips (BC) Beetroots were sliced (3-8 cm wide and 2-4 mm thick), frozen at −20°C for 48 h, and subsequently freeze-dried (Model Liotop P1040; Liobras, Sao Carlos, Brazil). The final product had an 11.4±0.68% moisture content. For BC analysis, freeze-dried samples were crushed in a portable blender (Pratic[®] Cadence, Navegantes, Brazil), weighed, and diluted.

Beetroot powder (BP) Beetroot powder was obtained from BJ as described above. BJ was dried in a Mini Spray Dryer (Model Büchi 190; Büchi Laboratoriums Technik AG, Flawil, Switzerland) at respective inlet and outlet temperatures of 180 and $65\pm3^{\circ}$ C with a 0.7 mm nozzle and a 6 mL/min feed. Powder was collected and stored in a desiccator until analysis. The final product had a 3.67±0.87% moisture content without any chemical additives. BP samples were weighed and diluted prior to analysis.

Cooked beetroot (CB) Beetroots were peeled and boiled at 100°C for 40 min in a 10 L pot in approximately 6 L of distilled and deionized water. After boiling, beetroots were sliced. Cooked beetroot had a 90.67±0.50% moisture content. Beetroots were put into a juice extractor (Model CE700; Black & Decker®) and processed into juice for further analysis.

Total antioxidant potential (TAP) analysis Beetroot samples (1 g of dry weight) from each formulation were vortexed (Phoenix®, Araraquare, Brazil) with 9 mL of deionized water and centrifuged at $4,500 \times g$ for 10 min at 4° C. Supernatants were filtered throughout a 0.45 µm cellulose membrane filter (MF Millipore®, Darmstadt, Germany) and the resulting filtered beetroot samples were transferred to amber vials and stored at –80°C prior to testing. TAP analysis was performed using HPLC following the method of Glód et al. (14). The injection volume was 20 µL and the HPLC system was equipped with a 5 µm reversed-phase C18 column (Kromasil®, Bohus, Sweden) (250 ×4.6 mm, I.D.) and a fluorescence detector (Model RF-10AXL; Shimadzu®, Kyoto, Japan) monitoring excitation and emission at respective wavelengths of 312 and 428 nm. The mobile phase at 1.0 mL /min was a 100 mM sodium phosphate buffer at pH 6.6. The TAP value was obtained based on a percentage difference as (15):

$$
\text{TAP } (\%) = [((S^0_{\text{HTPA}} - S_{\text{HTPA}})/S^0_{\text{HTPA}})] \times 100
$$

where S_{HTPA} is the surface area of the chromatogram generated via a Fenton reaction (14) with the beetroot sample, and S^0 _{HTPA} is the surface area of the chromatogram generated via a Fenton reaction without a beetroot sample. Results were expressed as TAP (%).

Total phenolic content (TPC) analysis TPC analysis was performed as described previously by Sánchez-Rangel et al. (16). Beetroot samples (1 g of dry weight for each formulation) were homogenized in 20 mL of 100% methanol (17) by vortexing (Phoenix[®]) and centrifuged at 10,000×g for 15 min at 4°C. Absorbance values at 765 nm were determined using an Asys UVM340 microplate reader spectrophotometer (Biochrom, Holliston, MA, USA). Absorbance values were compared to a gallic acid standard curve and results were expressed as gallic acid equivalents (GAE) $mg·g^{-1}$ on a dry weight basis.

Nitrate (NO₃⁻) analysis Beetroot samples were dissolved, diluted, centrifuged, filtered, and stocked following the above mentioned method for TAP analysis. Prior to analysis, a further dilution of 1:800 was performed. NO_3^- analysis was performed using HPLC as described previously by Alvares et al. (18) with modification of the sample preparation procedure described above for TAP analysis. NO_3^- was converted to nitrite $(NO₂⁻)$ enzymatically using nitrate reductase EC 1.6.6.2 (Roche Diagnostics, Mannheim, Germany) from Aspergillus spp. with filtered beetroot samples. After conversion, 100 µL of the filtered beetroot sample was incubated at 24° C with 10 μ L of 316 mM of 2,3-diaminonaphthalene (DAN) in 0.62 M HCl for 10 min, followed by addition of 5 µL of 2.8 M NaOH and immediately subjected to HPLC analysis. The HPLC device was equipped with a 5 µm reversed-phase C8 column (Discovery®, Bellefonte, PA, USA) (150x4.6 mm, I.D.) with a 5 µm reversed-phase C18 guard column (Ascentis®, Bellefonte, PA, USA) (50x4.6 mm, I.D.) and a fluorescence detector (Model RF-10AXL; Shimadzu®) monitoring excitation and emission wavelengths at 375 nm and 415 nm, respectively. The mobile phase at 1.3 mL/min was a 15 mM sodium phosphate buffer (pH 7.5) and methanol (50:50, v/v). Results were expressed as mg \cdot kg⁻¹ of NO₃[−] on a dry weight basis.

Sugar analysis Beetroot sample dissolution, dilution, homogenization, centrifugation, filtration, and storage were performed as described above for TAP analysis. Sucrose, fructose, and glucose analyses were performed as described previously by Hernández et al. (19), with modification of the method flow, mobile phase, and temperature as cited below. A volume of 20 µL of each filtered beetroot sample was used and analysis was performed using an HPLC system equipped with a 5 µm carbohydrate analytical column (Prevail®, Deerfield, IL, USA) (250x4.6 mm, I.D.) with a 5 µm guard column (Prevail®) (7.5x4.6 mm, ID) and an evaporative light scattering detector (Model LT II; Shimadzu $^{\circledR}$) at 40°C. The mobile phase at 1.1 mL/min was acetonitrile and water (75:25, v/v) for isocratic elution. Results were expressed as mg·g⁻¹ on a dry weight basis.

Organic acid (OA) analysis Beetroot samples were dissolved or diluted, homogenized, centrifuged, and stored as performed above for TAP analysis. Organic acid (OA) analysis was performed as previously described by Bavec et al. (20) for citric, malic, and ascorbic acids. A volume of 20 µL of each filtered beetroot sample was analyzed using an HPLC system equipped with an HPX-87H Aminex fermentation monitoring column (Bio-Rad Laboratories Inc®, Hercules, CA, USA) (150×7.8 mm, I.D.), a cation H⁺ Micro-Guard column (Bio-Rad) (30×4.6 mm, I.D.), and a photodiode array detector (Model SPD-M20A; Shimadzu®) monitoring absorbance at 210 nm. The mobile phase at 0.6 mL/min was 4 mM sulfuric acid for isocratic elution. Results were expressed as mg·g⁻¹ on a dry weight basis.

Total solids analysis Total solids analysis in juice was performed using by using an infrared moisture analyzer (MA35M; Sartorius[®], Goettingen, Germany) (21).

Data analysis A one-way analysis of variance (ANOVA) was used for identification of differences in TAP, TPC, $NO₃⁻$, and sugar and organic acid contents between BJ, BC, BP, and CB. When a significant F value was found, additional post-hoc tests with Bonferroni adjustment were performed. Statistical significance was set at the p<0.01. Values were expressed as a means±standard deviation (SD). All analyses were performed using the commercially available statistical package IBM SPSS[®] Statistics version 22 for Mac (Chicago, IL, USA).

Results and Discussion

Total antioxidant potential (TAP) TAP values of BC, BP, CB, and BJ are shown in Fig. 1. Significantly (p <0.01) higher values were found for BC (95.70±0.53%) and BP (95.31±0.68%) than for CB (85.79±0.61%) and BJ (80.48 \pm 0.25%). There was no significant (p >0.01) difference between BC and BP. Higher TAP (%) values for BP and BC were related to drying of those formulations that removed water, thereby concentrating nutrients (22). Drying probably increased antioxidant activities due to concentration of bioactive molecules in the food matrix without large loss. TAP (%) differences between different beetroot formulations may also have been related to high levels of ascorbic and citric organic acids with antioxidant activities (23,24). Some betalain antioxidants can by effectively stabilized by ascorbic

Fig. 1. Total antioxidant potential (TAP) of beetroot juice (BJ), beetroot chips (BC), beetroot powder (BP), and cooked beetroot (CB) expressed as a percentage. Values are presented as a mean±SD. Different letters indicate significance at $p<0.01$.

acid (25), and in BC and BP formulations high contents of ascorbic acid may have contributed to an increase in TAP (%) values.

A study conducted by Figiel (26) demonstrated a greater antioxidant potential of raw beetroots in comparison with freeze dried beetroots. Jiménez-Monreal et al. (27) found no significant losses in the TAP (%) value when comparing CB and raw beetroots. In this study, TAP values of CB were compared with processed beetroots and not with natural, unprocessed vegetables, as was done previously. Amongst the formulations evaluated here, CB and BP were both processed using heat treatment. BC exhibited high TAP (%) values with no difference between BP and BJ. Therefore, beetroot TAP should be further studied before and after processing.

Total phenolic content (TPC) TPC values for BC, BP, CB, and BJ are shown in Fig. 2. BJ (3.67±0.61 mg·g⁻¹) and CB (2.79±0.23 mg·g⁻¹) exhibited significantly (p <0.01) higher TPC values than BC (0.75±0.06 GAE mg·g⁻¹) and BP (0.51±0.07 GAE mg·g⁻¹).

No significant (p>0.01) difference in TPC values between BC and BP was observed (Fig. 2). The BC and BP forms of beetroot that underwent freeze drying or spray drying showed lower TPC values than BJ and CB, probably due to selective loss of compounds during the drying process as phenolics are hydrosoluble compounds that may have been lost with water. BJ was not subjected to thermal processing or drying and the TPC dry weight basis values obtained were higher than for other formulations.

Recommendations for the daily intake of phenolics and other antioxidants have not yet been established. Chun et al. (28) estimated the American average daily intake of phenolics from fruits and vegetables at 450 mg of GAE of fresh weight (FW). Asparagus had the highest TPC value of 64.15±2.46 of GAE mg⋅100 g⁻¹ of FW. In this study, levels of TPC on a FW basis were 66.29±5.46 and 26.08 ± 2.12 GAE mg $\cdot100$ g⁻¹ in BC and CB, respectively. BP exhibited 49.35 ± 6.78 and BJ exhibited 31.04 ± 5.16 mg $\cdot100$ g⁻¹.

 $NO₃⁻$ analysis The $NO₃⁻$ contents of the 4 beetroot forms analyzed in this study are shown in Fig. 3. BJ (12,252.90±1105.30 mg·kg⁻¹) showed significantly (p<0.01) higher $NO₃⁻$ concentrations than BC

Fig. 2. Total phenolic contents (TPC) of beetroot juice (BJ), beetroot chips (BC), beetroot powder (BP), and cooked beetroot (CB) expressed in Gae many CD

Fig. 2. Total phenolic contents (TPC) of beetroot juice (BJ), beetroot

chips (BC), beetroot powder (BP), and cooked beetroot (CB) expressed

in GAE mg·g⁻¹. Values are presented as means±SD. Different le indicate significance at $p<0.01$.

 $(2,031.2\pm144.00 \text{ mg} \cdot \text{kg}^{-1})$, BP $(1,683.50\pm264.48 \text{ mg} \cdot \text{kg}^{-1})$, and CB $(1,649.66\pm114.26 \text{ mg} \cdot \text{kg}^{-1})$. There were no significant $(p>0.01)$ differences in NO_3^- concentrations between BC, BP and CB. Losses of hydrophilic CB may have been due to dilution in cooking water.

A study conducted by Raczuk et al. (29) evaluated 20 CB samples and reported $\overline{\text{NO}_3}^-$ contents of 1,306±405 mg·kg⁻¹ of FW. This value was higher than the CB contents reported herein of 153.9±10.66 mg·kg[−]¹ of FW. Values may have differed due to analytical techniques used as the HPLC method is more sensitive and specific, compared with other spectrophotometric methods, such as the Griess reaction (30).

 $NO₃⁻$ present in beetroot juice promotes enhanced performance during cycling and running (12,13). Athletes and consumers seeking a healthy lifestyle are constantly looking for specific ingredients, sometimes called super foods, and dietary supplements that improve health and physical performance (31). The BP formulation can be added to drinks or water, salads, soups, and yogurts, and BC can be consumed as a snack during physical exercise. Both formulations can be practical forms of dietary nitrate ingestion, being consumed by athletes before training or competitions.

Sugar analysis Glucose, fructose, sucrose and total sugar contents for BC, BP, CB, and BJ are shown in Table 1. Formulations showed significant (p<0.01) differences in total sugar contents in a descending order of BJ (963.41±13.98 mg·g⁻¹), BC (627.96±11.39 mg·g⁻¹), BP $(444.05\pm26.08 \text{ mg} \cdot \text{g}^{-1})$, and CB $(249.51\pm0.22 \text{ mg} \cdot \text{g}^{-1})$. Sucrose was the dominant sugar in all 4 beetroot formulations while glucose and fructose were present in smaller amounts, probably because the

Fig. 3. Nitrate $(NO₃⁻)$ contents of beetroot juice (BJ), beetroot chips (BC), beetroot powder (BP), and cooked beetroot (CB) expressed in **Fig. 3. ↑**
(BC), be
mg·kg^{−1} $mg \cdot kg^{-1}$. Values are presented as means \pm SD. Different letters indicate significance at p<0.01.

root is the storage organ of plants and energy in beetroots is stored in the form of sucrose (32).

Beetroot juice had significantly (p <0.01) higher sugar contents, compared with the other beetroot formulations since juice was not subjected to thermal processing or drying. CB exhibited the lowest level of total sugars, compared with BJ, BC, and BP, probably due to loss of sugars via solubilization in water during the cooking procedure (33). Drying processes promote water removal that can lead to loss of water-soluble components of the food matrix. On the other hand, the drying processes for both BC and BP concentrated sugars, leading to higher sugar contents than for CB and lower sugar contents than for BJ. Similar results were observed in a previous study conducted by Rodríguez-Sevilla et al. (33) who reported a significant reduction of total sugars in beetroot processed at 121° C for 15 min in an autoclave (46.9±1.06 mg·g⁻¹ of FW for total sugars and 43.4±0.76 mg⋅g⁻¹ of FW for sucrose), compared with raw beetroot (73.0±10.16 mg·g⁻¹ of FW for total sugars and 66.8±9.34 mg∙g^{−1} of FW for sucrose). Bach *et al*. (32) reported a reduction of up to 72% in the sugar contents of different types of beetroot after boiling with mean values of total sugars of 5 variations of raw and cooked beetroots of 54.57±9.00 and 39.60±3.90 mg·g⁻¹, respectively.

Lower levels of total sugars and sucrose observed in this study for CB (22.50±0.05 mg·g⁻¹ of sucrose and 23.26±0.04 mg·g⁻¹ of total sugars on a wet weight basis) than for other beetroot formulations may have been influenced by a prolonged cooking time of 40 min. The previously mentioned studies used cooking times of 15 and 8 min (32). The difference in the sugar content reported by Bach et al. (32) and this study may have been due to differences in the varieties

Fructose were present in smaller amounts, probably because the (32) and this study may have been due to differences in the varieties
Table 1. Changes in sugar contents between beetroot juice (BJ), beetroot chips (BC),

a dry weight basis					
Presentation form	Fructose	Glucose	Sucrose	Total sugars	
BJ $(mg \cdot g^{-1})$	18.05 ± 0.54 ^{a1)}	14.95 ± 0.21 ^a	930.40±13.65 ^a	963.41±13.98 ^a	
BC ($mg \cdot g^{-1}$)	13.23 ± 0.84^b	$11.46 + 0.25^{b}$	603.27 ± 10.30^b	627.96+11.39 ^b	
BP $(mg \cdot g^{-1})$	$7.69 + 0.07$ ^c	6.87 ± 0.05 ^c	429.48+25.96°	444.05±26.08 ^c	
CB $(mg \cdot g^{-1})$	3.63 ± 0.02^d	4.51 ± 0.01 ^c	241.37±0.25 ^d	$249.51 + 0.22$ ^d	

¹)Values are presented as means±SD. Different letters in the same column indicate significance at p <0.01.

Table 2. Changes in organic acid contents between beetroot juice (BJ), beetroot chips (BC), beetroot powder (BP), and cooked beetroot (CB) in **Table 2**
mg∙g^{−1}

$mg \cdot g^{-1}$ on a dry weight basis					
Presentation form	Citric acid	Ascorbic acid	Malic acid	Total organic acids	
BJ $(mg \cdot g^{-1})$	9.81 ± 0.32^{b1}	3.62 ± 0.09 ^c	$15.36 \pm 0.69^{\circ}$	28.80±0.94°	
BC $(mg \cdot g^{-1})$	13.34 ± 3.85 ^a	5.79 ± 0.78 ^b	16.39 ± 0.90^b	35.53 ± 3.97^b	
BP $(mg \cdot g^{-1})$	16.21 ± 0.68 ^a	11.57 ± 0.49 ^a	20.14 ± 0.76 ^a	$47.93 + 1.32$ ^a	
CB $(mg \cdot g^{-1})$	3.67 ± 0.09 ^c	$1.68 + 0.11$ ^d	$8.57 + 0.15$ ^c	$13.93 + 0.24$ ^d	

¹⁾Values are presented as means±SD. Different letters in the same column indicate significance at $p<0.01$.

of beetroot analyzed.

In a study of raw beetroot, Bavec et al. (20) reported 21.03±4.66 mg·g^{−1} of FW for total sugars, 18.88±4.85 mg·g^{−1} of FW for sucrose, 0.65±0.20 $\text{mg}\cdot \text{g}^{-1}$ of FW for glucose, and 1.49±0.19 $\text{mg}\cdot \text{g}^{-1}$ of FW for fructose. The total sugar content of BJ was greater than for other beetroot forms in this study. However, considering different formulation portions on a wet weight basis, the BC and BP formulations had higher sugar contents per g (556.13±10.09 and 427.75±25.13 mg \cdot g $^{-1}$, respectively). Thus, technological processes can be advantageous for preservation and for increasing the nutritional value of foods due to concentration of compounds. Consumption of BC can be recommended for endurance athletes before, during, and after exercise due to a high carbohydrate content. The American College of Sports Medicine (ACSM) (34) recommendation for carbohydrate intake during exercise ranges from 30 to 60 g $\cdot h^{-1}$. A BC serving portion of 17 chips (54 g) would achieve the ACSM recommendation of 30 g of carbohydrates, and the BC formulation is more convenient than other beetroot formulations, such as 8 cups of CB (1.3 kg), 3 cups of BJ (370 mL), or 11 tablespoons of BP (30 g) (34).

Organic acid (OA) analysis Organic acid contents of BC, BP, BJ, and CB are shown in Table 2. BP (47.93±1.32 mg·g⁻¹) and BC (35.53±3.97 mg·g⁻¹) showed significantly (p<0.01) higher values of total organic acids than both BJ (28.80±0.94 mg·g⁻¹) and CB (13.93±0.24 mg·g⁻¹). For all formulations, the dominant organic acids were malic acid, followed by citric, and ascorbic acids. The malic acid content did not differ significantly (p>0.01) between BJ and BC while significantly (p<0.0) higher citric acid levels were found in BC and BP than in other formulations. However, no significant (p > 0.01) difference in the malic acid content was observed between BC and BP. BP had 2x the amount of ascorbic acid found in BC and 10x more than CB.

Spray drying and freeze drying may have positively contributed to preservation of organic acids in beetroot. The BC and BP formulations, both subjected to drying, exhibited the highest levels of organic acids. During the drying process, ascorbic acid degradation was reported to be moisture and temperature-dependent (35). Concentration of nutrients as a result of drying processes were probably responsible for higher levels of organic compounds in BC and BP formulations than in BJ and CB. Higher amounts of ascorbic acid observed in BP than in BC may have also been the result of spray-drying concentration (36).

Bavec et al. (20) observed levels of malic and ascorbic acids in

beetroots varying between 1.63±0.07 and 0.30±0.06 mg·g⁻¹ of FW. respectively. Jiratanan and Liu (37) observed ascorbic acid levels of 0.15±0.00 mg·g^{−1} for raw beetroot and 0.12±0.00 mg·g^{−1} for beetroot cooked for 45 min at 115°C. Reported values of citric, malic, and ascorbic acids were similar to BJ and CB values reported herein, although previously reported values were lower than values for BP and BC reported herein. BP and BC formulations were concentrated due to drying, whereas previously reported values for BJ and CB were stated on a FW basis that included water content.

Drying techniques applied in this study resulted in higher concentrations of ascorbic acid in smaller beetroot amounts. Spray drying effectively encapsulates ascorbic acid. Pierucci et al. (36) used pea proteins to encapsulate ascorbic acid and obtained good results for vitamin release kinetics.

The Institute of Medicine (IOM) (38) has recommended levels of ascorbic acid for adult men and women of 90 and 75 mg·day⁻¹. respectively. To achieve vitamin C recommendations using beetroot formulations, 1/2 cup or 5 chips (16 g) of BC, or 1 full tablespoon (8 g) of BP should be consumed, whereas it would be necessary to consume 4 cups (530 g), and more than 1 cup (267 mL) of CB and BJ, respectively. The BC and BP formulations are, therefore, more advantageous forms of beetroot since a smaller amount is sufficient to reach the daily ascorbic acid recommendation for adults.

In conclusion, consideration of all formulations on a dry weight basis indicates that the BC and BP formulations have higher organic acid contents and total antioxidant potentials than the BJ and CB formulations. However, BJ can also be advantageous if the purpose is to achieve optimum ingestion of sugars, nitrates, and phenolic compounds.

Comparisons on a FW basis of 4 beetroot formulations were made in order to inform the scientific community and consumers about nutrient contents and functional properties of beetroots, and for comparison of serving portions of beetroot formulations. Different beetroot formulations can be advantageous based on convenience and delivery of recommended dietary nutrient intake amounts, and to meet consumer preferences.

Distinct formulations used for the administration of beetroot can increase consumption of beetroot for different nutritional needs associated with improvements in cardiovascular function and exercise performance. However, future studies for investigation of the effects of different beetroot formulations on prevention of disease associated with oxidative stress, including cardiovascular disease, and for improving exercise performance, are needed.

Acknowledgments Ricky Toledano aided preparation of the English version of this manuscript. The Research Foundation of the State of Rio de Janeiro-FAPERJ (Process # E-26/111.297/2013 and E-26/ 110.309/2014) and the National Counsel of Technological and Scientific Development-CNPq (Process # 442977/2014-0) provided financial support. LioFoods® of Sao Paulo, Brazil provided support for preparation of beetroot chips.

Disclosure The authors declare no conflict of interest.

References

- 1. Liu RH. Health-promoting components of fruits and vegetables in the diet. Adv. Nutr. 4: 384-392 (2013)
- 2. Boeing H, Bechthold A, Bub A, Ellinger S, Haller D, Kroke A, Leschik-Bonnet E, Müller MJ, Oberritter H, Schulze M, Stehle P, Watzl B. Critical review: Vegetables and fruit in the prevention of chronic diseases. Eur. J. Nutr. 51: 637- 663 (2012)
- 3. Hord NG, Tang Y, Bryan NS. Food sources of nitrates and nitrites: The physiologic context for potential health benefits. Am. J. Clin. Nutr. 90: 1-10 (2009)
- 4. Lange W, Brandenburg WA, De bock TS. Taxonomy and cultonomy of beet (Beta vulgaris L.). Bot. J. Linn. Soc. 130: 81-96 (1999)
- 5. United State Department of Agriculture (USDA). National Nutrient Database for Standard Reference. Available from: http://ndb.nal.usda.gov/ndb/foods/ list. Acessed Aug. 10, 2013.
- 6. Koubaier HBH, Snoussi A, Essaidi I, Chaabouni MM, Thonart P, Bouzouita N. Betalain and phenolic compositions, antioxidant activity of tunisian red beet (Beta vulgaris L. conditiva) roots and stems extracts. Int. J. Food Prop. 17: 1934-1945 (2014)
- 7. Wootton-Beard PC, Ryan L. Combined use of multiple methodologies for the measurement of total antioxidant capacity in UK commercially available vegetable juices. Plant Food. Hum. Nutr. 67: 142-147 (2012)
- 8. Kujala TS, Loponen JM, Klika KD, Pihlaja K. Phenolics and betacyanins in red beetroot (Beta vulgaris) root: Distribution and effect of cold storage on the content of total phenolics and three individual compounds. J. Agr. Food Chem. 48: 5338-5342 (2000)
- 9. Georgiev VG, Weber J, Kneschke EM, Denev PN, Bley T, Pavlov AI. Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot Beta vulgaris cv. detroit dark red. Plant Food. Hum. Nutr. 65: 105-111 (2010)
- 10. Kapil V, Webb AJ, Ahluwalia A. Inorganic nitrate and the cardiovascular system. Heart 96: 1703-1709 (2010)
- 11. Stokes KY, Dugas TR, Tang Y, Garg H, Guidry E, Bryan NS. Dietary nitrite prevents hypercholesterolemic microvascular inflammation and reverses endothelial dysfunction. Endocrinol. Metab. 296: 1281-1288 (2009)
- 12. Murph M, Eliot K, Heuertz RM, Weiss E. Whole beetroot consumption acutely improves running performance. J. Acad. Nutr. Diet. 112: 548-552 (2012)
- 13. Vanhatalo A, Fulford J, Bailey SJ, Blackwell JR, Winyard PG, Jones AM. Dietary nitrate reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia. J. Physiol.-London 15: 5517-5528 (2011)
- 14. Glód BK, Piszcz P, Czajka K, Zarzycki PK. A New Total antioxidant potential measurements using rp-hplc assay with fluorescence detection. J. Chromatogr. Sci. 49: 401-404 (2011)
- 15. Wantusiak PM, G*ł*ód BK. Application of UV detection in HPLC in the total antioxidant potential assay. Cent. Eur. J. Chem. 10: 1786-1790 (2012)
- 16. Sánchez-Rangel JC, Benavides J, Heredia JB, Cisneros-Zevallos L, Jacobo-Velázquez DA. The Folin-Ciocalteu assay revisited improvement of its specificity for total phenolic content determination. Anal. Methods 5: 5990-

5999 (2013)

- 17. Mohdaly AAA, Hassanien MFR, Mahmoud A, Sarhan MA, Smetanska I. Phenolics extracted from potato, sugar beet, and sesame processing byproducts. Int. J. Food Prop. 16: 1148-1168 (2013)
- 18. Alvares TS, Conte CA, Paschoalin VM, Silva JT, Meirelles CM, Bhambhani YN, Gomes PS. Acute L-arginine supplementation increases muscle blood volume but not strength performance. Appl. Physiol. Nutr. Me. 37: 115-126 (2012)
- 19. Hernández JL, González-Castro MJ, Alba NI, García CC. High-performance liquid chromatographic determination of mono- and oligosaccharides in vegetables with evaporative light-scattering detection and refractive index detection. J. Chromatogr. Sci. 36: 293-298 (1998)
- 20. Bavec M, Turinek M, Grobelnik-MLakar S, Slatnar A, Bavec F. Influence of industrial and alternative farming systems on contents of sugars, organic acids, total phenolic content, and the antioxidant activity of red beet (Beta vulgaris L. ssp. vulgaris Rote Kugel). J. Agr. Food Chem. 58: 11825-11831 (2010)
- 21. Sun Q, Han Z, Wang L, Xiong L. Physicochemical differences between sorghum starch and sorghum flour modified by heat-moisture treatment. Food Chem. 145: 756-764 (2014)
- 22. Ratti C. Hot air and freeze-drying of high-value foods: A review. J. Food Eng. 49: 311-319 (2001)
- 23. Ravichandran K, Saw NMMT, Mohdaly AAA, Gabr MMA, Kastell A, Riedel H, Cai Z, Knorr D, SmetanskaI. Impact of processing of red beet on betalain content and antioxidant activity. Food Res. Int. 50: 670-675 (2013)
- 24. Attoe EL, Von Elbe JH. Degradation kinetics of betanine in solutions as influenced by oxygen. J. Agr. Food Chem. 30: 708-712 (1982)
- 25. Herbach KM, Stintzing FC, Carle R. Betalain stability and degradation-Structural and chromatic aspects. J. Sci. Food Agr. 71: R41-R50 (2006)
- 26. Figiel A. Drying kinetics and quality of beetroots dehydrated by combination of convective and vacuum-microwave methods. J. Food Eng. 98: 461-470 (2010)
- 27. Jiménez-Monreal AM, García-Diz L, Martínez-Tomé M, Mariscal M, Murcia MA. Influence of cooking methods on antioxidant activity of vegetables. J. Food Sci. 74: H97-H103 (2009)
- 28. Chun OK, Kim D-O, Smith N, Schroeder D, Han JT, Lee CY. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. J. Sci. Food Agr. 85: 1715-1724 (2005)
- 29. Raczuk J, Wadas W, Glozak K. Nitrates and nitrites in selected vegetables purchased at supermarkets in Siedlce, Poland. Rocz. Panstw. Zakl. Hig. 65: 15- 20 (2014)
- 30. Tsikas D, Fuchs I, Gutzki FM, Frölich JC. Measurement of nitrite and nitrate in plasma, serum and urine of humans by high-performance liquid chromatography, the Griess assay, chemiluminescence and gas chromatography-mass spectrometry: Interferences by biogenic amines and N(G)-nitro-L-arginine analogs. J. Chromatogr. B 715: 441-444 (1998)
- 31. Clements WT, Lee SR, Bloomer RJ. Nitrate ingestion: A review of the health and physical performance effects. Nutrients 6: 5224-5264 (2014)
- 32. Bach V, Mikkelsen L, Kidmose U, Edelenbos M. Culinary preparation of beetroot (Beta vulgaris L.): The impact on sensory quality and appropriateness. J. Sci. Food Agr. 95: 1852-1859 (2015)
- 33. Rodríguez-Sevilla MD, Villanueva-Suárez MJ, Redondo-Cuenca A. Effects of processing conditions on soluble sugars content of carrot, beetroot and turnip. Food Chem. 66: 81-85 (1999)
- 34. American Dietetic Association; Dietitians of Canada; American College of Sports Medicine, Rodriguez NR, Di Marco NM, Langley S. American College of Sports Medicine position stand. Nutrition and athletic performance. Med. Sci. Sport. Exer. 41: 709-731 (2009)
- 35. Santos PHS, Silva MA. Retention of vitamin c in drying processes of fruits and vegetables-A review. Dry Technol. 26: 1421–1437 (2008)
- 36. Pierucci AP, Andrade LR, Baptista EB, Volpato NM, Rocha-Leão MH. New microencapsulation system for ascorbic acid using pea protein concentrate as coat protector. J. Microencapsul. 23: 654-662 (2006)
- 37. Jiratanan T, Liu RH. Antioxidant activity of processed table beets (Beta vulgaris var, conditiva) and green beans (Phaseolus vulgaris L.). J. Agr. Food Chem. 52: 2659-2670 (2004)
- 38. Institute of Medicine (US) Subcommittee on Interpretation and Uses of Dietary Reference Intakes, Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. DRI Dietary Reference Intakes: Applications in Dietary Assessment. Washington (DC): National Academies Press (US) (2000)