2023-07-23

Content of nitrate and nitrite in commercial and self-made beetroot juices and the effect of storage temperature

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10.1002/fsn3.3575
Food Science and Nutrition
Wiley Open Access

Title: Content of nitrate and nitrite in commercial and self-made beetroot juices and the effect of storage temperature.

Running title: Nitrate and nitrite content in beetroot juice

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Author contributions: RB and MR contributed to project conception; RB, MR, TD and PC-A analysed the data. RB, MR, TD and PC-A authors contributed to data interpretation. RB and PC-A drafted the initial paper. All authors revised and approved the final manuscript.

Abstract

Popularity of beetroot juice (BJ) is growing due to its high inorganic nitrate content (NO_3^-) and its potential physiological benefits. However, the content of NO_3^- is not indicated in most commercial BJs and it can be affected by seasonal changes and storage conditions. This study analysed the content of NO_3^- and nitrite (NO_2^-) in five and two commercial and self-made BJs, respectively, that were purchased in the summer and winter period. The effect of storage temperature (20ºC, 4ºC and -20ºC) and pH was also analysed. In non-concentrated BJs, the NO_3^- content was 34 ± 20% (P = 0.075) in the winter than in the summer. NO_3^- was fully degraded in self-made BJ after 3 days at 20ºC. This effect was attenuated by 78% and 82% when it was kept at 4ºC and -20ºC, respectively. The addition of lemon juice (5%) to self-made BJ was another useful approach to avoid NO_3^- degradation for 3 days when it was kept at 20ºC. Regarding NO_2^-, self-made BJ had higher concentration (0.097 ± 0.01 mg/mL) compared to commercial BJs (< 0.1 mg/mL; P = 0.001). The pH of self-made BJ was higher (6.3 ± 0.1) compared to commercial BJs (4.5 ± 0.3; P = 0.001). These results suggest that the content of NO_3^- in non-concentrated BJs can substantially differ across the year and this is an important factor to take into account when recommending BJs to promote some of its potential physiological benefits.

Keywords: beet, nitrate, nitrite, nitric oxide, nitrogen.

Introduction

Beetroot is one of the main dietary sources of inorganic nitrate (NO_3^-) [1], a natural ion that has been traditionally considered harmful due to the risk of formation of nitrosamines that can lead to cancer [2,3]. As a consequence, the European Food Safety Authority (EFSA) established an Acceptable Daily Intake (ADI) for NO_3^- of 3.7 mg/kg body mass/day that is still valid [4]. However, this view has substantially changed over the last decade due to new evidence suggesting that consumption of vegetables rich in NO_3^-, which can exceed the ADI levels, is safe and it can enhance nitric oxide (NO) bioavailability [5-7]. Increased NO bioavailability due to NO_3^- consumption has been associated with enhanced exercise capacity especially in moderate-trained individuals and some clinical populations [7,8]. Consequently, beetroot juice is currently listed within category A (products with the most scientific evidence to enhance exercise performance) in the Sport Supplement Framework of the Australian Institute of Sport [9].

The minimum amount of NO_3^- that can elicit improvements in exercise performance is about 5 mmol (310 mg) [8], however, the content of NO_3^- in most commercial beetroot juices is not indicated in the list of ingredients as it is not required by the law. This is important since the content of NO_3^- in vegetables can substantially change depending on several factors including environmental conditions, soil clay content, organic matter content, nitrogen fertilization and type of beet [10]. Previous research in lettuce and spinach has shown large variations in the content of NO_3^- and nitrite (NO_2^-) across different seasons being higher in the winter than in the summer [11]. Similar data in beets or beetroot juice is missing, but it can be hypothesised that similar variations can also occur across the year.

Given the potential ergogenic effects of beetroot juice, the popularity of this product is increasing among professional and recreational athletes with an average increase in the sales of beetroot of 8% per year since 2016 [12]. However, some people dislike the taste of this product [13]. One approach to make beetroot juice more attractive is by mixing it with other juices such as lemon and apple juice. They can also act as natural preservatives due to their antioxidant compounds (e.g ascorbic acid). However, the effect of adding lemon juice on the NO_3^- content of beetroot juice has not been reported. This study investigated whether the addition of lemon juice into self-made beetroot juice affects the content of NO_3^- and NO_2^- and acidity (pH) levels.

Another important issue that has not been analysed especially in commercial beetroot juices is the stability of NO_3^-. While small shots (70 ml) are easily consumed at once, large bottles (0.5 – 1 L) can last longer and the storage conditions can affect the NO_3^- content. Fresh beetroot juice (from natural beets) may contain bacteria that can reduce NO_3^- into NO_2^- increasing the concentration of the second [14]. This is relevant because while inorganic NO_3^- is safe even at high doses, NO_2^- can cause serious harm at considerably lower levels. Thus, it is important to consider the content of NO_2^- in beetroot juice as well. In this study, we investigated the effect of 3 different storage temperatures (room: 20ºC; fridge: 4ºC; freezer: -20ºC) on the content of NO_3^- and NO_2^- in commercial and self-made beetroot juice.

In summary, the main goals of this study were to: 1) analyse the content of NO_3^- and NO_2^- of commercial and self-made beetroot juices at different periods of the year; 2) analyse the effect of the storage temperature on the content of NO_3^- and NO_2^- in commercial and self-made beetroot juice; 3) investigate the effect of adding lemon into self-made beetroot juice on the NO_3^- and NO_2^- content and acidity (pH). According to this, the main hypotheses of this study were that: 1) NO_3^- content of commercial and self-made beetroot juice will differ across different periods of the year; 2) NO_3^- in commercial and self-made beetroot juice will be degraded more quickly at higher temperatures; 3) the addition of lemon juice into self-made beetroot juice will reduce NO_3^- degradation in self-made beetroot juice.

Methods

We analysed the NO_3^- and NO_2^- content in five commercial beetroot juices that are commonly used by professional and recreational athletes and two-self made beetroot juices (Table 1). Commercial juices and raw beets were purchased in June 2021 and February 2022 and stored for less than one week at room temperature or under refrigeration as recommended by manufacturers before they were analysed.

Preparation of products

Self-made beetroot juice (SBJ) was prepared using whole beets (*Beta Vulgaris*) from a local supermarket (Plymouth, UK). Beetroot was washed with tap water, and then with ultrapure water (Purelab OptionQ, Elga Veolia, UK). The outer skin and inedible parts were removed before being chopped into small pieces and weighed using an electronic scale (Precisa XB 3200C, Switzerland). Then, beetroot was juiced using an electric juicer machine (Waring 11JE65, USA). Lemon was bought in a local supermarket and juiced using a fruit juicer. Then, 2.5 mL (5%) of lemon juice was mixed with 47.5 mL (95%) of fresh beetroot juice (SBJ), which is similar to the volume of lemon juice added into some commercial beetroot juices analysed in this study (Table 1). Commercial beetroot juices were opened on the first day of analyses. All beetroot juices were filtered using a Whatman® filter paper number 1 and centrifuged at 3,500 rpm for 10 min to remove solid parts.

Analysis of nitrate (NO_3)⁻ and nitrite (NO_2^-)

All beetroot samples were centrifuged at 13,000 rpm at 4ºC for 10 min before analysis. The content of NO_3^- and NO_2^- of each product was analysed using a dedicated high-performance liquid chromatography (HPLC) analyser (ENO-30; Eicom USA) as previously described [15]. Briefly, NO_3^- and NO_2^- were separated on a reverse-phase separation column packed with polysterene polymer (NO-PAK 4.6 x 50 mm, EICOM, Amuza Inc, US), and NO_3^- was reduced to NO_2^- in a reduction column packed with copper-platted cadmium filins (NO-RED EICOM, Amuza Inc, US). NO_2^- was mixed with a Griess reagent to form a purple azo dye in a reaction coil. The separation and reaction columns and the reaction coil were placed in a column oven set at 35ºC. The absorbance of the color of the product dye at 540 nm was measured with a flow-through spectrophotometer (NOD-30, Eicom). The mobile phase (10% methanol, 0.15M NaCl/NH_4Cl and 0.5 g/L 4Na-EDTA) and reactor phase (10% methanol, 1.25% HCl containing 5 g/L of sulphanilamide with 0.25 g/L of N-naphthylethylenediamine) were delivered at a flow rate of 0.33 mL/min and 0.10 mL/min, respectively. A standard curve was produced by injecting 10 µL of water with sodium NO_3^- ($NaNO_3^-$ / 7631-99-4, Sigma Aldrich, UK) and sodium NO_2^- ($NaNO_2^-$ / 7632-00-0, Sigma Aldrich, UK) at different concentrations (7.8 µM, 15.6 µM, 31.2 µM, 62.5 µM, 125 µM and 250 µM). Beetroot samples were diluted 1:200 using a carrier solution containing 10% methanol, 0.15M NaCl/NH_4Cl and 0.5 g/L 4Na-EDTA. Samples were analysed (10 µL) in duplicate on the first day and single on third and seventh day given the small coefficient of variation of NO_3^- (2.1 ± 1.9%) and NO_2^- (4.8 ± 3.0%) analyses.

pH measurements

Measurements of pH were performed using a single electrode digital pH meter (Lutron Electronic Enterprise Co Ltd., Model PH-208, Taiwan) that was calibrated following the manufacturer's instructions prior each use.

Storage temperature

The effect of different storage temperatures on the NO_3^- and NO_2^- content was only analysed in the first batch (June 2021). Eppendorf (1.5 mL) and Falcon tubes (3 mL) were filled with each product and kept at three different temperatures (20ºC; 4ºC; -20ºC) to analyse NO_3^-, NO_2^- and pH on the first (baseline), third and seventh day using the same methods described above. All the tubes were wrapped with aluminium foil to preserve the samples from light oxidation. Samples at -20ºC were thawed the same day of the analysis. Then, all samples were centrifuged at 13,000 rpm at 4ºC for 10 min before analysis was undertaken.

Statistical analyses

Data are presented as mean ± standard deviation (SD). Differences in NO_3^- and NO_2^- content and pH between different beetroot juices were compared using a a one-way of analysis of variance (ANOVA). *Post hoc* analyses were performed using Tukey HSD. Data were analysed using the statistical software SPSS (version 28). The level of significance was set at $P < 0.05$.

Results

Juices

Raw beetroot (SBJ) in the summer (102 g) and winter (178 g) yielded 53 (52%) and 107 (60%) mL of juice, respectively.

NO_3^- and NO_2^- content in beetroot juices in the summer and winter

As expected, concentrated beetroot juice (JW2) had the highest content of NO_3^- (6.3 ± 0.2 mg/mL; P = 0.001) compared to non-concentrated commercial (JW1: 1.1 ± 0.2 mg/mL; BN: 1.1 ± 0.1 mg/mL; BT: 1.6 ± 0.2 mg/mL; CW: 0.8 ± 0.1 mg/mL) and self-made juices (SBJ: 1.4 ± 0.2 mg/mL; SBJL: 1.3 ± 0.2 mg/mL) (Figure 1A). The content of NO_3^- of concentrated beetroot juice (JW2) was similar in the summer (6.3 ± 0.2 mg/mL) and winter (6.4 ± 0.2 mg/mL; P > 0.05). Non-concentrated beetroot juices (JW1, BN, BT, CW, SBJ), had in average 34 ± 20% more NO_3^- in the summer (1.2 ± 0.3 mg/mL) than in the winter (0.8 ± 0.3 mg/mL; P = 0.075) (Figure 1A). These differences were more pronounced in JW1, BN and BT juices (from 0.7 ± 0.1, 0.5 ± 0.1 mg/mL and 0.8 ± 0.1 mg/mL in the winter to 1.1 ± 0.1, 1.1 ± 0.1

and 1.6 mg/m in the summer; P < 0.001) than in CW and SBJ (from 0.6 ± 0.1 and 1.3 ± 0.2 mg/mL in the winter to 0.8 ± 0.1 and 1.4 ± 0.2 mg/mL in the summer; P > 0.05) (Figure 1A).

Using the NO_3^- results from each product, we calculated the amount of beetroot juice that was needed to achieve the minimum dose of NO_3^- to enhance exercise performance (5 mmol of NO_3^- = 310 mg) (Figure 2). With the exception of concentrated (JW2) and self-made juice (SBJ), an average of 258 ± 162 mL more beetroot juice from the winter batches of commercial beetroot juices was needed to achieve such amount compared to the summer batches.

The content of NO_2^- is shown in Figure 1B. SBJ (0.097 ± 0.01 mg/mL) and SBJL (0.090 ± 0.01 mg/mL) had the highest content of NO_2^- compared to the commercial beetroot juices (JW1: 0.030 ± 0.01 mg/mL; JW2: 0.035 ± 0.01 mg/mL; BN: < 0.01 ± 0.01 mg/mL; BT: 0.032 ± 0.01 mg/mL; CW: 0.023 ± 0.01 mg/mL; P = 0.001) (Figure 1B). The content of NO_2^- was slightly lower in JW1 and BT juices in the winter (JW1: 0.011 ± 0.01 mg/mL; BT: 0.017 ± 0.01 mg/mL) than in the summer (JW1: 0.030 ± 0.01 mg/mL; BT: 0.032 ± 0.01 mg/mL; P = 0.110), while JW2 had slightly higher content of NO_2^- in the winter (0.110 ± 0.01 mg/mL) than in the summer (0.097 ± 0.01 mg/mL; P = 0.101).

pH of beetroot juice

Results of pH are shown in Figure 1C. SBJ had the highest pH (6.3 ± 0.1) compared to commercial juices (mean pH from all the commercial beetroot juices = 4.5 ± 0.3; P = 0.001) and SBJL (3.6 ± 0.1; P = 0.001) (Figure 1C). Overall, the average pH of commercial juices (JW1, JW2, BN, BT, CW) was slightly lower in the winter (4.2 ± 0.2) than in the summer (4.5 ± 0.3; P = 0.239).

Effect of storage temperature on NO3-, nitrite and pH

The content of NO_3^- in juices stored at 20ºC, 4ºC and -20ºC for 1, 3 and 7 days during the summer is shown in Figure 3.

A reduction of 24% (from 6.3 ± 0.2 mg/mL to 4.8 ± 0.2 mg/mL; $P < 0.001$) and 46% (from 6.3 ± 0.2 mg/mL to 3.4 ± 0.2 mg/mL, $P < 0.001$) in NO_3^- was observed when concentrated beetroot juice (JW2) was kept at 20ºC for 3 and 7 days, respectively (Figure 3A). A similar effect was observed when JW2 was kept for 3 days at 4ºC (from 6.3 ± 0.2 mg/mL to 4.7 ± 0.2 mg/mL; $P < 0.001$) and -20ºC (from 6.3 ± 0.2 mg/mL to 3.4 ± 0.2 mg/mL; $P < 0.001$) (Figure 3B).

NO_3^- was degraded in SBJ after 3 days at 20ºC (from 1.4 ± 0.1 mg/mL to 0.04 ± 0.01 mg/mL; $P < 0.001$) (Figure 3A). This reduction was attenuated by 78% (from 1.4 ± 0.1 mg/mL to 1.1± 0.1 mg/mL) and 82% (from from 1.4 ± 0.1 mg/mL to 1.2 ± 0.1 mg/mL) when it was kept at 4ºC and -20ºC for 3 days, respectively (Figure 3B and 3C).

The addition of 5% lemon juice was also effective to fully attenuate the reduction of NO_3^- in SBJ for 3 days (from 1.3 ± 0.1 mg/mL to 1.3 ± 0.1 mg/mL) at 20ºC (Figure 3A). Furthermore, the addition of lemon juice was useful to preserve 62% of NO_3^- in SBJ (from 1.3 ± 0.1 mg/mL to 0.8 ± 0.1 mg/mL) when it was kept at 20ºC for 7 days (Figure 3A).

Regarding the NO_2^- content, an abrupt increase was observed in SBJ on day 3 at 20ºC (from (0.097 ± 0.01 mg/mL to 1.5 ± 0.2 mg/mL; $P < 0.001$) (Figure 3D). This effect was inhibited when SBJ was stored at 4ºC for 3 (from 0.097 ± 0.01 mg/mL to 0.01 ± 0.001 mg/mL) and 7 days (from 0.097 ± 0.01 mg/mL to 0.01 ± 0.001 mg/mL) and when it was stored at -20ºC for the same duration (3 days: from 0.01 ± 0.001 mg/mL to 0.01 ± 0.001 mg/mL; 7 days: from 0.01 ± 0.001 mg/mL to 0.01 ± 0.001 mg/mL) (Figures 3E and 3F). Furthermore, the addition of lemon juice to self-made juice (SBJL) was effective to inhibit the increase in NO_2^- when it was kept at 20ºC for 3 (from 0.097 ± 0.01 mg/mL to 0.087 ± 0.01 mg/mL) and 7 days (from 0.090 ± 0.01 mg/mL to 0.11 ± 0.01 mg/mL), respectively (Figure 3D).

The pH of all juices, except in SBJL, remained relatively stable on day 3 and 7 at 20ºC, 4ºC and -20ºC (Figures 3G, 3H and 3I). The pH of SBJL increased from day 1 to day 3 when it was kept at 20ºC (from 3.6 ± 0.1 to 4.7 ± 0.1; $P < 0.001$).

Discussion

The main finding of this study was that the content of NO_3^- in non-concentrated commercial beetroot juices was on average 34 ± 20% lower in the winter than in the summer . Differences in the content of NO_3^- in concentrated commercial (JW2) (1.9 ± 0.7%) and self-made beetroot juices (SBJ, SBJL) (5.7 ± 2.1%) were smaller.

NO_3^- is the main form of nitrogen used by crop to synthesise amino acids. They absorb NO_3^- from the soil via transporter proteins in the root cell membrane [16]. Thus, the amount of NO_3^- in vegetables depends on the level of this ion in the soil, which can substantially differ across the year. For example, in the UK it has been reported that the soil is poorer in NO_3^- in the winter because wet conditions (rainfalls) can wash out NO_3^- into the groundwater, a phenomenon known as NO_3^- leaching [17]. For this reason, it is feasible to use additional fertilizer (nitrogen) in autumn and winter in some vulnerable areas to improve the crops yield [17]. Four of the commercial beetroot juices (JW1, JW2, BN, BT) analysed in this study indicated that beetroot used was organic so nitrogen fertilizers were not supposed to be used during the growth of the crop. Interestingly, all of them, except the concentrated juice (JW2), had lower content of NO_3^- when they were bougth and analysed in the winter, which may suggest that beetroot were grown over the summer. However, this information was not provided by the commercial companies. Light conditions, use of organic matter (animal manure), and storage conditions are also important factors to take into account as they can affect the content of NO_3^- in vegetables [18-20]. Commercial companies can obtain beetroot from different locations and areas given the large amount of product needed to constantly supply the market, which can modify the content of NO_3^- in the final product. Furthermore, there is no regulation about labelling the content of NO_3^- in commercial beetroot juice, its origin or when crop were harvested. This is relevant given the potential physiological implications of NO_3^- and the variations in the content of this ion observed in this study in some commercial products. Although individuals can always choose to consume larger-than-recommended amounts, potential disadvantages to doing so include increased cost, greater volume to ingest, higher intake of oxalate and potential side effects.

Only two of the commercial juices analysed in this study (JW1 and JW2) reported an estimated value of NO_3^- in the serving size (Table 1). The first juice (JW1) claimed that the NO_3^- content was on average 800 mg per litre (0.8 mg/mL). Compared to this, we found that the NO_3^- content of this product was 38% higher in the summer batch (June 2020) (1.1 mg/mL) and 14% lower in the winter batch (February 2021) (0.69 mg/mL). On the other hand, the NO_3^- content from a concentrated product from the same commercial brand (JW2) was 47% and 50% higher in summer and winter batch compared to the claimed NO_3^- content. Our results are in agreement to a previous study indicating that the NO_3^- content of the same beetroot juice was 23% higher than the claimed NO_3^- content [21]. However, they did not compare the content of NO_3^- of the same product across different periods of the year. Furthermore, both studies, showed that commercial concentrated beetroot shots (JW2) had nearly 5 times more NO_3^- than commercial non-concentrated and fresh beetroot juice. Concentrated beetroot shots appeared in the market a decade ago to provide the minimal dose of inorganic NO_3^- (5 mmol = 310 mg/serving) that has been suggested to enhance exercise capacity in an small volume [22]. Although the method to concentrate beetroot juice is not reported in the label, this process is usually performed by removing part of the water of the juice [23].

Regarding the effect of temperature storage on the content of NO_3^- and NO_2^-, rapid degradation of NO_3^- occurred in self-made beetroot juice (SBJ) when it was kept at 20ºC for 3 days, but this reaction was attenuated by storing it at low temperatures and by adding lemon juice (SBJL), a natural source of ascorbic acid. This is in agreement to our hypothesis suggesting that low temperatures and the addition of lemon juice can help to attenuate NO_3^- degradation in beetroot juice. Ascorbic acid is widely used in the food industry for its antioxidant and stabilising properties [24]. Two of the commercial juices analysed in this study (JW2 and BN) contained lemon juice, two more apple juice (JW1 and CW) and another one (CW) was fortified with ascorbic acid. Despite the addition of lemon juice into concentrated beetroot juice (JW2), we found a rapid reduction in the content of NO_3^- occurred over day 3 and 7 that was not attenuated at low temperatures. According to this, rapid consumption of concentrated beetroot juice is recommended to enhance NO_3^- intake. This is in agreement to the recommendations from the commercial companies indicating to keep the juice refrigerated and consume it within 3 – 7 days once opened. The addition of lemon and apple juices can also help to enhance the organoleptic characteristics of beetroot juice for some people who dislike the taste of beetroot [13].

The content of NO_2^- was very low (< 0.1 mg/mL) in all the juices at baseline, however, a rapid increase was observed in self-made beetroot juice (SBJ) on day 3 at 20ºC. This could happen due to the activity of NO_3^- reductase enzymes or microorganisms present in beetroot as the decrease of NO_3^- was accompanied by the increase of NO_2^-. From a safety point of view, the levels of NO_2^- achieved on day 3 were quite low to cause harm in healthy individuals as doses above 100 mg/kg of body mass are required to produce serious side effects in humans [25]. According to our results, the consumption of over 4 litres of beetroot juice rich in NO_2^- over a relatively short period of time may be needed to reach this quantity of NO_2^-. However, a word of caution is needed about beetroot juice overload among athletes thinking ‘the more the better’.

The pH of commercial beetroot juices was more acidic than self-made juice (SBJ), which can be related to lacto-fermentation and addition of ascorbic acid in commercial juices. Two commercial juices of this study were lacto-fermented (BN and BT), which consists of the addition of lactic acid bacteria consuming sugars to produce acid compounds and carbon dioxide by fermentation [26]. Three commercial juices also contained lemon juice (JW2 and BN) or vitamin C as an additive (CW). Addition of lemon juice into self-made beetroot juice (SBJL) is a useful approach to maintain the content of NO_3^- as we demonstrated in this study. On the other hand, further research is needed to investigate whether lacto-fermentation and/or addition of other juices can modify NO_3^- bioavailability.

This study had some limitations that are worth discussing. First, it was based on a Masters thesis that was performed during Covid-19 pandemic when students had to deal with laboratory restrictions. Bootles of five of the most consumed brands of beetroot juice in the UK were analysed in the summer (June) and winter (February) season. The batch code of each product was not recorded, but we believe that juices belonged from different batches given the time gap (8 months) between the purchase and analyses of them. Despite this limitation, our results are still interesting indicating that the content of NO_3^- can substantially differ especially in commercial non-concentrated beetroot juices. We also had limitations to increase the sample size of different beetroot juices given the duration of each chromatogram (10 min) to analyse NO_3^- and NO_2^-. We could analyse a maximum of 42 samples in a day. In the winter batches (February 2021), only baseline analyses of NO_3^-, NO_2^- and pH were performed due to time constrains. All the analyses (NO_3^-, $NO_2^-$and pH) were performed in duplicate during the first day (7 samples x 3 different temperatures) to ensure the reproducibility of the results. Regarding the effect of storage temperature, only 1.5 and 3 mL of each beetroot juice were taken and stored at the respective temperature prior to testing, which it may not represent of what would happen in larger volumes (e.g 500 mL) of juice.

In summary, this study showed that the NO_3^- content of commercial beetroot juices can substantially differ across different batches. Reduction of the content of NO_3^- in concentrated commercial beetroot juice and fresh beetroot juice occurs quickly at room temperature (20ºC). Furthermore, it is possible to obtain similar quantities of NO_3^- from self-made beetroot juice compared to non-concentrated commercial beetroot juices, but it must be kept at low temperatures (4ºC and -20ºC) and/or mixed with lemon juice to avoid NO_3^- degradation. These findings are relevant to individuals (e.g nutritionists, athletes, coaches, etc.) and researchers interested in the physiological effects of beetroot juice supplementation. Indeed, given the possible variation in the NO_3^- content of beetroot juice, scientists looking at the physiological effect of dietary NO_3^- in beetroot juice should measure the content of NO_3^-in the supplement.

Acknowledgement: We thank the technical and funding support of the University of Plymouth.

Declaration of interest: This study was not funded by any commercial company. We also declare to not have any conflict of interest.

Data availability: Data will be available on request from the authors.

Word count: 3904

References

1. Bailey SJ, Winyard P, Vanhatalo A, et al. Dietary nitrate supplementation reduces the O2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J Appl Physiol*. 2009;107(4):1144-55.

2. Zhang F-X, Miao Y, Ruan J-G, et al. Association between nitrite and nitrate intake and risk of gastric cancer: a systematic review and meta-analysis. *Med Sci Monit: Int Med J Experim Clin Res*. 2019;25:1788.

3. Bryan NS, Alexander DD, Coughlin JR, Milkowski AL, Boffetta P. Ingested nitrate and nitrite and stomach cancer risk: An updated review. *Food Chem Toxicol*. 2012;50(10):3646-3665.

4. EFSA, Mortensen A, Aguilar F, et al. Re-evaluation of sodium nitrate (E 251) and potassium nitrate (E 252) as food additives. *Efsa journal*. 2017;15(6):e04787.

5. Lundberg JO, Carlström M, Weitzberg E. Metabolic Effects of Dietary Nitrate in Health and Disease. *Cell Metab*. 2018;28(1):9-22.

6. Hultström M, Amorim de Paula C, Porcelli S, et al. Commentaries on Viewpoint: Can elite athletes benefit from dietary nitrate supplementation?Commentaries on Viewpoint: Can elite athletes benefit from dietary nitrate supplementation?Commentaries on Viewpoint: Can elite athletes benefit from dietary nitrate. *J Appl Physiol*. 2015;119(6):762-769.

7. Senefeld JW, Wiggins CC, Regimbal RJ, Dominelli PB, Baker SE, Joyner MJ. Ergogenic Effect of Nitrate Supplementation: A Systematic Review and Meta-analysis. *Med Sci Sports Exerc*. 2020; 52(10):2250-2261.

8. Shannon OM, Allen JD, Bescos R, et al. Dietary Inorganic Nitrate as an Ergogenic Aid: An Expert Consensus Derived via the Modified Delphi Technique. *Sports Med*. 2022; 52(10):2537-2558.

9. Australian Institute of Sport A. Supplements and sports food in high performance sport. Accessed 22/03, 2023. https://www.ais.gov.au/__data/assets/pdf_file/0014/1000841/Position-Statement-Supplements-and-Sports-Foods.pdf

10. Gallardo EJ, Coggan AR. What Is in Your Beet Juice? Nitrate and Nitrite Content of Beet Juice Products Marketed to Athletes. *Int J Sports Nutr Exerc Metab*. 2019;29(4):345.

11. Ashworth A, Bescos R. Dietary nitrate and blood pressure: evolution of a new nutrient? *Nutr Res Rev*. 2017:1-12.

12. Ysart G, Clifford R, Harrison N. Monitoring for nitrate in UK-grown lettuce and spinach. *Food Addit Contam*. Jul 1999;16(7):301-6.

13. Grand View Research. Sugar Beet Juice Extract Market Size. Accessed 26/09/2022, https://www.grandviewresearch.com/industry-analysis/sugar-beet-juice-extract-market

14. Babateen AM, Shannon OM, O'Brien GM, et al. Acceptability and Feasibility of a 13-Week Pilot Randomised Controlled Trial Testing the Effects of Incremental Doses of Beetroot Juice in Overweight and Obese Older Adults. *Nutrients*. 2021;13(3).

15. Corleto KA, Singh J, Jayaprakasha GK, Patil BS. Storage Stability of Dietary Nitrate and Phenolic Compounds in Beetroot (Beta vulgaris) and Arugula (Eruca sativa) Juices. *J Food Sci*. 2018;83(5):1237-1248.

16. Lundberg JO, Larsen FJ, Weitzberg E. Supplementation with nitrate and nitrite salts in exercise: a word of caution. *J Appl Physiol*. 2011;111(2):616-7.

17. Thomas B, Smallwood S, Cutler C, Bescos R. The oral nitrate-reducing capacity correlates with peak power output and peak oxygen uptake in healthy humans. *Nitric Oxide*. 2019;87:43-51.

18. Dechorgnat J, Nguyen CT, Armengaud P, et al. From the soil to the seeds: the long journey of nitrate in plants. *J Experim Bot*. 2010;62(4):1349-1359.

19. Gov.UK. Using nitrogen fertilisers in nitrate vulnerable zones. 26/09/2022, https://www.gov.uk/guidance/using-nitrogen-fertilisers-in-nitrate-vulnerable-zones

20. Santamaria P. Nitrate in vegetables: toxicity, content, intake and EC regulation. *J Sci Food Agric*. 2006;86(1):10-17.

21. Weightman RM, Huckle AJ, Roques SE, Ginsburg D, Dyer CJ. Factors influencing tissue nitrate concentration in field-grown wild rocket (Diplotaxis tenuifolia) in southern England. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 2012;29(9):1425-35.

22. Santamaria P, Elia A, Serio F, Todaro E. A survey of nitrate and oxalate content in fresh vegetables. *J Sci Food Agric*. 1999; 79(13):1882-1888.

23. Jones AM, Thompson C, Wylie LJ, Vanhatalo A. Dietary Nitrate and Physical Performance. *Ann Rev Nutr*. 2018;38(1):303-328.

24. Bazaria B, Kumar P. Compositional Changes in Functional Attributes of Vacuum Concentrated Beetroot Juice. *J Food Process Preserv*. 2016;40(6):1215-1222.

25. Liao M-L, Seib PA. Chemistry of L-ascorbic acid related to foods. *Food Chem*. 1988;30(4):289-312.

26. Klewicka E, Zduńczyk Z, Juśkiewicz J, Klewicki R. Effects of Lactofermented Beetroot Juice Alone or with N-nitroso-N-methylurea on Selected Metabolic Parameters, Composition of the Microbiota Adhering to the Gut Epithelium and Antioxidant Status of Rats. *Nutrients*. 2015;7(7):5905-5915.

Table 1: Beetroot juices analysed in this study.

Brand	Code	Product	Serving size (mL)	Claimed nitrate content (mg/serving)	Characteristics
Juices					
James White	JW1	Beet it organic juice	1,000	800	90% organic beetroot juice + 10% organic apple juice
James White (300 mg)	JW2	Beet it sport	70	300	98% organic concentrated beetroot juice + 2% lemon juice
Biona	BN	Beetroot pressed juice	500	-	Beetroot juice partially lacto fermented + lemon juice
Biotta	BT	Beetroot juice	500	-	100% organic pressed beetroot juice lacto-fermented.
Cawston	CW	Brilliant beetroot juice	1,000	-	90% pressed beetroot juice + 10% pressed apple juice + vitamin C
Fresh beetroot juice	SBJ	Fresh beetroot juice 1	50	-	100% pressed beetroot juice
Fresh beetroot juice + lemon juice	SBJL	Fresh beetroot juice	50	-	95% pressed beetroot juice + 5% pressed lemon juice

Figure legends

Figure 1: Content of nitrate (NO_3^-) (A), nitrite (NO_2^-) (B), and pH (C) in commercial and self-made beetroot juices in two different periods of the year. (*a* represents statistical differences between beetroot juices; *b* represents statistical differences between beetroot juice batches in the summer and winter.

Figure 2: Estimated amount of beetroot juice required to achieve 5 mmol of nitrate (NO_3^-) from commercial and and self-made beetroot juices in the summer and winter.

Figure 3: Effect of storage temperature on the content of nitrate (NO_3^-) (A-C), nitrite (NO_2^-) (D-F) and pH (G-I) of beetroot juice at baseline (day 1) and 3 and 7 days after opening the package (commercial beetroot juice) or after preparing self-made beetroot juice. (*a* represents statistical differences between day 1 and day 3; *b* represents statistical differences between day 1 and day 7; *c* represents statistical differences between day 3 and day 7).