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Nutritional, Bioactive and Physicochemical Characteristics of Different Beetroot Formulations

Diego dos S. Baião, Davi V.T. da Silva,
Eduardo M. Del Aguila and
Vânia M. Flosi Paschoalin

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Abstract

Beetroot possesses high nutritional value and is considered one of the main dietary sources of nitrate. Nitrate has increasingly attracted the interest of the scientific community regarding new physiological, nutritional and therapeutic approaches with beneficial effects on the cardiovascular system. These effects can be explained by the possible effect of dietary nitrate in stimulating nitric oxide synthesis. Dietary nitrate can be reduced to nitrite in the oral cavity, which is then decomposed to nitric oxide and other bioactive nitrogen oxides in the stomach. Beetroot administration can be conducted by several types of formulations, in order to provide a convenient and alternative source of dietary beetroot, such as beetroot juice or beetroot chips and powder. The challenge in providing a product which, in addition to being rich in nitrate, is attractive and easy to administer, while also being microbiologically safe, is increased by the limited scientific information available concerning the nutritional aspects of beetroot formulations. In this chapter, a brief review on the efficiency of different beetroot formulations on health indicators is conducted, emphasizing the effects following the intake of nitrate-enriched beetroot gel. The metabolic and hemodynamic effects of beetroot formulations in healthy and non-healthy volunteers are also discussed.

Keywords: beetroot formulations, nitrate, nitric oxide, phenolic compounds

1. Introduction

Lifestyle and inadequate eating habits expose humans to a number of risk factors for the development of chronic non-communicable diseases (CNCDs). Diets rich in saturated, *trans*-fats

and simple sugars, poor in complex-carbohydrates and fibres and associated with smoking, alcoholism, stress and sedentary lifestyles have increased the number of diseases such as obesity, diabetes, hypertension, osteoporosis and cardiovascular disorders, among others [1]. Therefore, the search for a healthy diet has been significantly emphasized worldwide [2].

Vegetables are important components of a balanced diet due to their constituents, mainly bioactive compounds, fibres, vitamins and minerals. Epidemiological studies have shown that vegetables are useful protective foods against coronary heart disease and ischemia [3]. These and other findings have raised the hypothesis increasingly recognized and biologically plausible, that inorganic nitrate (NO_3^-) in certain vegetables can provide a physiological substrate for the production of nitric oxide (NO) and other products (NO_x), which in turn cause vasodilation, decrease blood pressure and support cardiovascular function [4].

Dietary NO_3^- can be reduced to nitrite (NO_2^-) in the oral cavity by commensal bacteria that express the enzyme nitrate reductase [4]. NO_2^- then reaches the stomach where, in contact with gastric acid, it is non-enzymatically decomposed into NO and other bioactive nitrogen oxides through the NO_3^- - NO_2^- /NO pathway [5]. The theory that dietary NO_3^- and NO_2^- may stimulate the endogenous synthesis of NO has increasingly been noted, and a new physiological, therapeutic and nutritional approach for these anions has arisen [6].

Dietary administration of beetroot, a vegetable NO_3^- source, can be conducted through several types of formulations, in order to provide a convenient and alternative source of beetroot, instead of consuming the whole vegetable. Some beetroot formulations must be offered in large amounts to reach effective NO_3^- concentrations, making it difficult to convince individuals to adhere to certain proposed interventions [7–9]. In this context, this paper discusses the recent advances in beetroot administration, pointing out plant species, nutritional composition and the effect of the ingestion of different beetroot formulations on NO production and the ensuing effects on hemodynamic parameters.

2. Beetroot cultivation

The Beetroot species *Beta vulgaris* L. belongs to the Quenopodiaceae family and originated in regions of Europe and North Africa, where they are cultivated in mild to cold temperatures (10–20°C). Cultivation in climatic conditions with higher relative humidity and higher temperature favours the development of pests and diseases, altering the internal colour and taste of the plant, making it less sweet, also reducing plant productivity by about 50% [10]. This plant species prefers soils rich in organic matter, with pH ranging from 5.5 to 6.2. The production cycle can range from 60 to 100 days, in summer or winter, depending on the cultivar and cultivation mode [10, 11].

The plant has a root system composed of a main root and smaller roots reaching up to 60 cm in depth, with lateral branching. It also possesses a tuberous, purplish-red, part, globular in shape, with a sweet taste, which develops almost on the surface of the soil [12]. The beetroot plant is biennial, requiring a period of intense cold to go through the reproductive stage of the cycle. The appearance of elongated leaves around the stem and the tuberous part occurs in the

vegetative phase, while floral tassel emission occurs with the production of seeds comprised of glomeruli during the reproductive stage [11].

According to some authors, *Beta vulgaris* L. beetroots can be divided into three subspecies: (a) *Beta vulgaris* ssp. *adanesis*, formed by a distinct group of semi-annual plants, with a great decline in auto-fertilization, with specific morphological characteristics; (b) *Beta vulgaris* ssp. *maritima*, formed by a large complex of morphological types that occur in a vast geographic area; (c) *Beta vulgaris* ssp. *vulgaris*, which groups all domesticated cultivars [13].

According to Lange et al. [13], subspecies *Beta vulgaris* ssp. *Vulgaris* cultivars can be subdivided into four other groups: (1) **Leaf Beet Group**, a cultivar with edible leaves and petioles and with roots with no significantly increased diameter; (2) **Sugar Beet Group**, a white coloured strain grown in the US and Europe for sugar production; (3) **Fodder Beet Group**, a cultivar intended for feeding herds and (4) **Garden Beet Group**, the only group cultivated in Brazil that has an edible tuberous part.

3. Nutritional composition of beetroot (*Beta vulgaris* L.)

The beetroot species *Beta vulgaris* L. is considered a good source of dietary fibre, minerals (potassium, sodium, iron, copper, magnesium, calcium, phosphorus and zinc), vitamins (retinol, ascorbic acid and B-complex), antioxidants, betalains and phenolic compounds, and possesses high nutritional value due to its high glucose content, in the form of sucrose [5, 14, 15]. According to data presented by the United States Department of Agriculture (USDA) for macronutrients, 100 g of raw beetroot has an energy value of 43 kcal, 9.56 g of carbohydrates, 1.61 g of proteins, 0.17 g of total lipids, 2.8 g of total dietary fibre and 6.76 g of total sugars [15].

4. Bioactive beetroot compounds

Plants are generally considered important sources of substances that perform bioactive functions, favouring human health, good organ function, disease control and contributing to longevity [16–19]. Insufficient intake of bioactive compounds from plant sources is considered an important risk factor for the development of chronic and non-communicable diseases.

Bioactive compounds may have different physiological targets and mechanisms of action. Many of these compounds show antioxidant action due to their potential for oxi-reduction of certain molecules, while others have the capacity to compete for active enzymatic and receptor sites in various subcellular structures or may modulate the expression of genes encoding proteins involved in intracellular mechanisms of defence against oxi-degenerative processes of molecules and cellular structures [20].

4.1. Betalains

Beetroots are a major source of betalains, classified as one of the 10 plants with the highest antioxidant activity, determined by the IC₅₀ value, i.e. the extract's ability to reduce low-density

lipoproteins (LDL) oxidation by 50%, as reported by Vinson et al. [21]. Betalains are present in the tuberous part of the plant, conferring its red-purple coloration, and act as antioxidant agents [12]. In addition, anti-inflammatory, hepatic protective and anti-cancer properties have also been attributed to this class of compounds [22, 23].

Betalains are heterocyclic compounds and water-soluble nitrogen pigments responsible for conferring various types of coloration in flowers, vegetables and fruits [24]. Betanin, a compound belonging to one of the betalain classes, has excellent stability at pH 4 and 5 and reasonable stability between pH 3 and 4 and pH 5 and 7. The stability of these molecules is affected by factors such as exposure to light, high temperature (i.e. cooking processes), high water activity and the presence of oxygen [23–27]. In addition, they can also be degraded by enzymes, such as polyphenoloxidases and peroxidases, released during food processing [28, 29]. Betanins are formed from a common precursor, betalamic acid, with more than 50 structures identified so far. Furthermore, these pigments are present in acid form due to the presence of several carboxyl groups and, are, therefore not classified as alkaloids [30].

Betalains have a general structure that contains betalamic acid, accompanied by a radical R1 or R2, where the substituents may be simple hydrogen or a more complex radical. The variability of the substituent groups comes from the different sources of these pigments and determines their hue and stability. According to their chemical structure, betalains are divided into two subclasses: betaxanthins, responsible for orange-yellow colorations, such as vulgaxanthin I and II and indicaxanthin, and betacyanins, responsible for red-violet colorations, such as betanin, prebetanin, isobetanin, neobetanin, amaranthine, gomphrenin and bouganvillein [12, 24] (**Figure 1**).

Previous studies have shown that betacyanins and betaxanthins are capable of absorbing visible light [31–33], and the structural differences between these compounds reflect on their

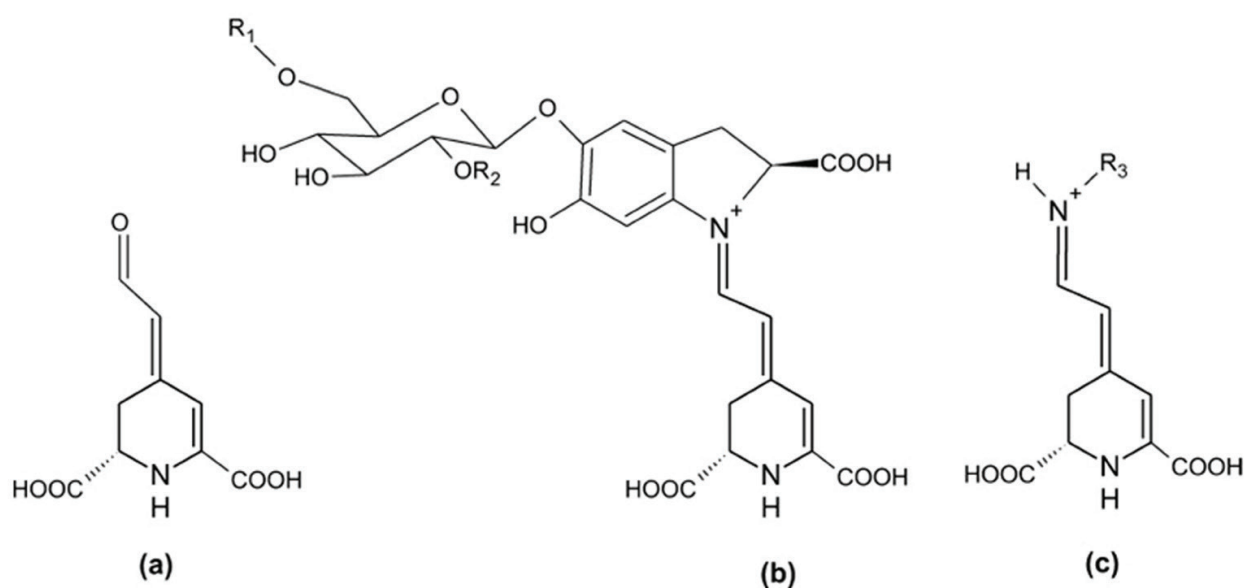


Figure 1: General structures of betalamic acid (a), betacyanins (b) and betaxanthins (c). Betanin: R₁ = R₂ = H. R₃ = amine or amino acid group. *Reproduced from Azeredo et al. [31].

light absorbing capacity. Approximately 50 types of betacyanins and 20 types of betaxanthins have been described. Beetroots contain both, approximately 75–95% betacyanin and 5–25% betaxanthine [24, 33]. The content of these pigments is present in higher concentrations on the vegetable skin, gradually decreasing towards the inner parts of the beet (pulp and crown) [25, 33, 34].

Betalains are widely used in the food industry as natural food dyes, and have shown *in vitro* and *in vivo* antioxidant capability, attributed mostly to betacyanins [33, 35, 36]. Previous studies have demonstrated that low concentrations of the betanin pigment, a beta-cyanine-like compound, present in beetroots, were able to inhibit *in vitro* oxidation of low-density lipoproteins (LDL) by H₂O₂-activated metamioglobin, as well as oxidizing agents, such as lipoxygenases and cytochrome 'c' [37]. Motivated by this evidence, several studies have evaluated the intake of foods rich in this pigment for the prevention and treatment of diseases triggered by oxidative processes in humans [31, 37, 38]. For example, Cai et al. [35] observed that betalains obtained from plants belonging to the Amaranthaceae family presented higher antioxidant activity than that of ascorbic acid, a traditional antioxidant.

Several other studies have demonstrated the role of betalains in the protection of several cellular components against oxidative stresses [38–40]. For instance, Reddy et al. [41] evaluated the efficiencies of betanin, anthocyanin, lycopene, bixin, β -carotene and chlorophyll separately and combined in inhibiting lipid peroxidation, cyclooxygenase enzymes and in the proliferation of human tumour cells. The authors observed that among the tested pigments, betanin, lycopene and β -carotene were more efficient at inhibiting lipid peroxidation.

The antioxidant potential of betalains has been attributed to the molecular structure of these compounds, which reflect their ability to donate hydrogen to reactive species. Regarding betaxanthins, an increase in the number of hydroxyl and imino residues promotes the elimination of free radicals, while glycosylation reduced activity and acylation increased antioxidant potential in betacyanins [34]. The antioxidant activity of betalains can be increased according to the number and position of the amino and hydroxyl groups in the molecule, with the C-5 position of the hydroxyl group in the aglycone responsible for increasing their antioxidant activity [31, 35].

Beetroots are the main commercial source of betalains (concentrate or powder) in the food industry, with the use of betanin restricted as a natural dye added to different foods like gelatins, desserts in general, confectionery, dry mixes, dairy products and beef and chicken-derived products [33]. Betalains are unstable and are degraded in the presence of light and oxygen and destroyed at high temperatures. The daily intake limit of betalains has not been established. In Brazil, current legislation dictates that the use of natural red dye obtained from beets is permitted in foods and beverages [33].

4.2. Phenolic compounds

Phenolic compounds present in plants are derived from the aromatic amino acid phenylalanine and are formed by two aromatic rings (A and B), linked by three carbon atoms that form an oxygenated heterocycle (ring C) [42, 43] (**Figure 2**). These compounds are secondary

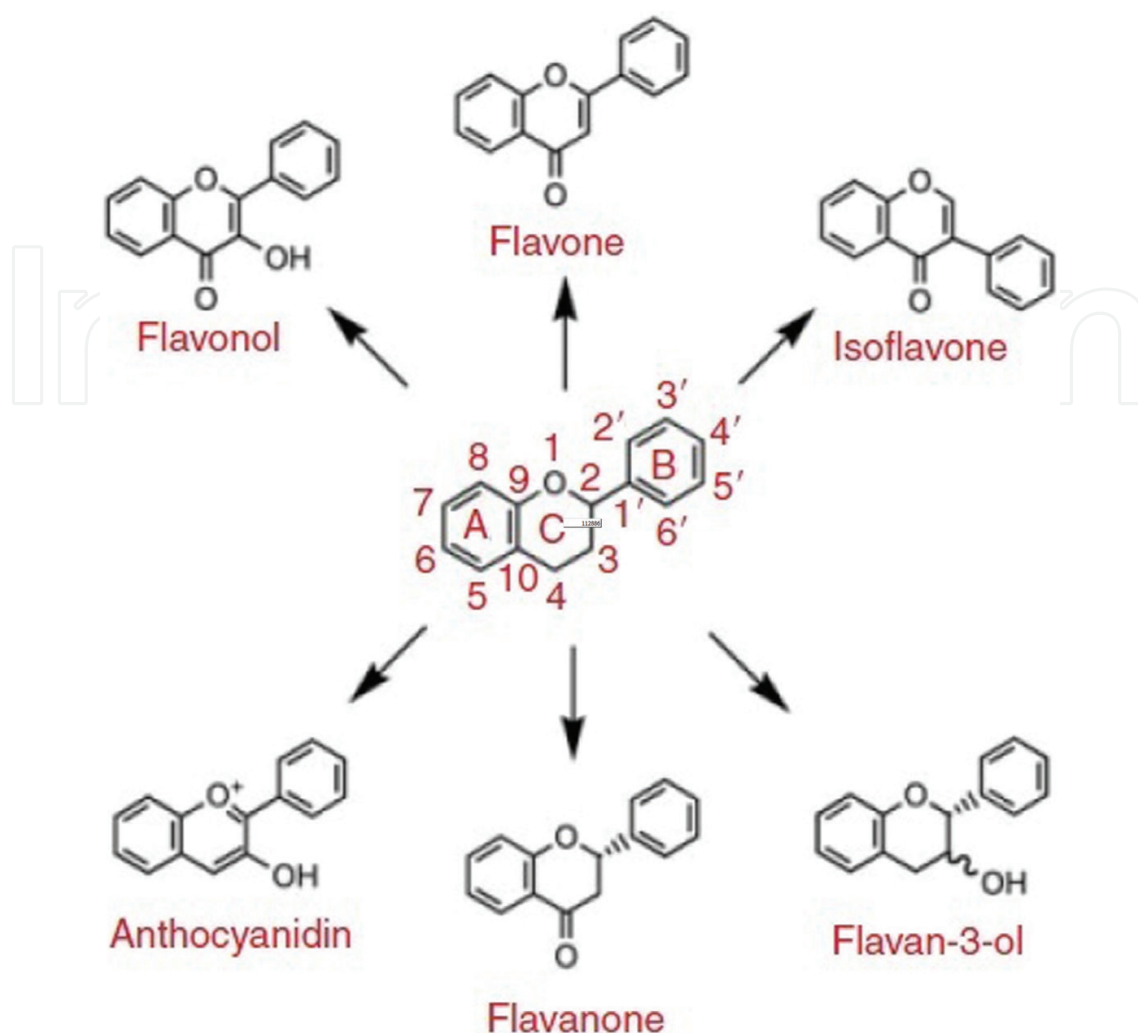


Figure 2: Chemical structure of the major flavonoids. *Reproduced from Crozier et al. [43].

metabolites in fruits and vegetables and are generally involved in the defence against pathogens and/or against ultraviolet radiation. In addition, many volatile phenolic compounds are responsible for the assignment of sensory characteristics, or flavour, in various foods commonly included in the human diet [44].

Beetroots are also an adequate source of phenolic compounds, and several studies have been carried out on the identification and evaluation of the antioxidant content and capacity of phenolic compounds present in this vegetable [45]. Previous studies have shown that beetroot tuber juice is particularly rich in phenolic compounds and that these components remain active even after *in vitro* digestion trials [45, 46]. Kujala et al. [25, 36] reported the presence of ferulic acid, flavonoids and phenolic amides, mainly in beetroot skin, which confer strong antioxidant activity to this food.

Flavonoid concentrations and species present in beetroot formulations should be better evaluated so that the intake of this vegetable in the human diet is as efficient as possible, since flavonoids can be lost in vegetable processing. In a recent study, Silva et al. [9] developed

a beetroot gel formulation, obtained from beetroot juice and powder, enriched in flavonoid content. While the beetroot juice contained 0.42 mg of quercetin equivalents per gram (QE/g), phenolic compounds in the gel were three times higher, reaching 1.37 mg QE/g. Guldiken et al. [47] determined flavonoid concentrations in beetroots processed through different forms of cooking, such as oven drying, canning, puree, processed juice, jelly and fresh beetroots, and found values ranging from 1.26 to 2.61 mg of rutin equivalents (RE)/g. Kazimierczak et al. [48] reported average flavonoids values of 0.41–1.16 mg/g in beetroots obtained by conventional and organic planting, respectively.

Phenolic compound composition and concentrations also vary according to the cultivar. Kujala et al. [36] studied four distinct beetroot cultivars by high-performance liquid chromatography (HPLC) and HPLC- electrospray ionisation-mass spectrometry (HPLC-ESI-MS) and NMR techniques and identified four flavonoids, betagarin, betavulgarin, cochliophilin A and dihydroisorhamnetin, whereas Georgiev et al. [49] identified the presence of catechin hydrate, epicatechin and rutin. The importance of flavonoids in the human diet has been ascribed to their horticultural effect, contributing to chemoprevention of DNA damage caused by several carcinogenic factors [49].

4.3. Saponins

These compounds vary extensively in structure and are widely distributed in plants. They are triterpene glycosides wherein the aglycone is covalently linked to one or two sugar chains through a glycosidic ester (C-28) or ether (to C-3) bond. Saponins exhibit several biological activities, such as anti-viral, anti-diabetic and anti-haemolytic properties, and have been, therefore, well studied [50].

Few studies are available regarding the evaluation of saponin content in beetroots. The occurrence of saponins in these species was characterized in a study conducted by Mroczek [51], where 11 saponins derived from oleanoic acids were identified using reverse phase liquid chromatography coupled with electrospray ionization mass spectrometry (LC-ESI/MS/MS). As with flavonoids, the content and species of saponins may vary according to the cultivar, with concentrations varying from 7.66 to 12.2 mg/g dry weight in three different beetroot cultivars (*Beta vulgaris* L.). Oleanoic acid is of importance among the saponins identified in the beet, since it is capable of causing a marked hypoglycemic effect [52]. Saponin content may also vary according to beetroot processing. In the study conducted by Silva et al. [9], saponin content was almost three times higher in beetroot gel compared to juice, of 22 and 8.22 mg/g, respectively.

4.4. Dietary NO_3^- and NO_2^-

NO_3^- and NO_2^- are compounds formed by a single nitrogen bonded to three or two oxygen atoms, respectively. NO_3^- is a nitric acid salt, while NO_2^- is a nitrous acid salt, and both can be obtained from endogenous and/or exogenous sources. NO_3^- are relatively inert but are transformed into NO_2^- by bacteria in the mouth of enzymatic pathways in the body. NO_2^- can be metabolically converted into NO via the L-arginine/NO pathway. This pathway was discovered

in 1916 by Mitchell et al. [53] and confirmed by Green et al. [54] and Leaf et al. [55]. In addition, increased NO_2^- -plasma concentrations have been derived from this pathway, with no contribution from dietary NO_3^- intake. Vasconcellos et al. [7] and Baião et al. [8] confirmed this assertion by supplementing healthy and physically active volunteers with juice and beetroot gel, respectively. The authors observed an increase in urinary NO_2^- concentrations after juice and beetroot gel consumption, but no change was observed after consumption of a placebo (PLA, juice and beetroot gel with reduced NO_3^- content).

After ingestion, dietary NO_3^- is well absorbed in the upper gastrointestinal tract. About 25% of NO_3^- absorbed via the dietary route is captured by the salivary glands. In the oral cavity, NO_3^- is reduced to NO_2^- by the enzyme NO_3^- -reductase. Upon being swallowed, NO_2^- formed from dietary NO_3^- is decomposed non-enzymatically into NO, which is then rapidly oxidized to NO_2^- [56]. Thus, an increase in urinary NO_2^- concentrations after administration of beetroot juice or gel but not after ingestion of a PLA is expected. For this reason, many studies have used urinary or plasma NO_2^- as the main marker for NO production.

The major dietary intake of NO_3^- and NO_2^- by the occidental population comes from vegetables (approximately 85%). The content of these anions in plants vary according to the vegetable tissues. For example, NO_3^- in plant organs can be classified from highest to lowest, as petiole > leaf > stem > root > tuber > bulb > fruit > seed [57]. In addition, environmental factors (atmospheric humidity, temperature, water content and exposure to sunlight and irradiation), agricultural factors (type of crop, fertilization, soil conditions, herbicide use, amount of available nitrogen, availability of other nutrients, plant genotype and transport and storage conditions) also influence NO_3^- levels in plants [58]. NO_2^- , on the other hand, is not found naturally in fruits and vegetables; it is unstable and rapidly oxidized to NO_3^- . However, human exposure to NO_2^- is about 70–80% derived from the additives used during food processing, such as meats, bakery products and cereals to improve taste and appearance, and to prevent the growth of foodborne pathogens and the secretion of toxins, such as the botulin toxin [59].

5. NO

NO is an endogenously produced molecule in the form of a gas with a very short half-life (5–10 s), low molecular weight (30.01 g/mol) and in standard temperature and pressure, moderate solubility in water (1.9 mM, at 25°C). It has a better solubility in polar solvents and in biological systems, and is more concentrated in lipophilic environments (cell membranes and hydrophobic domains of proteins) [60, 61].

The physiological importance of NO is due to the fact that it exerts a second-messenger function, activating or inhibiting several molecules, regulating vascular tone, and acting as an effector of the immune system and neurotransmission. Unlike other intracellular messengers, NO depends on redox reactivity to associate with a receptor or enzyme, not on its molecular structure. In addition, NO is not stored *in vivo* as other neurotransmitters but rather is synthesized on demand and rapidly diffuses into the target tissue and easily and quickly penetrates into other cells due to its small size and lipophilic characteristics [4, 61].

NO has several health benefits. It participates in the regulation of vascular tonus and interacts with the iron of the prosthetic heme group of the soluble guanylate cyclase enzyme (GCs), activating the production of cyclic guanosine monophosphate (cGMP) and consequent relaxation of adjacent smooth muscle cells [62]. In the immune system, it shows cytotoxic and cytostatic effects promoting the destruction of microorganisms, parasites and tumour cells [63]. This compound can also act as a neurotransmitter in the central and peripheral nervous system, facilitating the release of other neurotransmitters and hormones [64]. Other studies have reported that NO may also act on circulating blood cells (monocytes and platelets) for the maintenance of vascular homeostasis and control of smooth muscle cell proliferation and growth, as well as activation and aggregation of platelets, leukocytes and adhesion molecules present in the inflammatory process [65].

6. Production of different beetroot formulations

In the last few years, aiming at obtaining a convenient and alternative source of dietary NO_3^- , different beetroot formulations have been tested, with different nutritional compositions, intending to promote beneficial health effects (**Figure 3**). The challenge is to provide a product that, besides being rich in NO_3^- , is attractive, easy to administer and microbiologically safe.

The first formulation described in several studies for dietary NO_3^- supplementation was beetroot juice [8, 66–73]. For example, Baião et al. [8] produced beetroot juice by thoroughly washing the vegetables in tap water, sanitizing them in a chlorine solution (0.5%) as recommended by the Brazilian Health Ministry legislation ANVISA (MS, Resolution RDC No. 216 of 15/09/2004) and preparing them a food centrifuge processor. The beetroots were processed without adding any additional water.

Other authors have used water, fruit juice or other substances with low NO_3^- content to offer the PLA solution to volunteers [66, 67, 72, 73]. To obtain free- NO_3^- beetroot juice, Baião et al. [9] took the juice prepared in food processor centrifuge and transferred it to a sterile bottle containing an NO_3^- specific anion-exchange resin. After 1 h, the juice was loaded into a sterile glass column and eluted with the aid of a vacuum pump. The free- NO_3^- beetroot juice was similar in colour, taste, appearance and texture to the original. Both enriched and free- NO_3^- beetroot juices were administered to volunteers in order to achieve the health benefits.

Kaimainen et al. [74] and Vasconcellos et al. [7] prepared beetroot powder after freeze-drying beetroot juice in a spray dried system. Vasconcellos et al. [7] also produced beetroot chips. To produce beet powder, beetroots were sanitized in a chlorine solution (0.5%) and after beetroot juice production, the juice was dried at respective inlet and outlet temperatures of 180 and $65 \pm 3^\circ\text{C}$ with a 0.7 mm nozzle and a 6 mL/min feed. Beetroot chips were obtained by cutting the beetroot vertically into slices of 3–8 cm wide and 2–4 mm thick, in order to obtain thin round slices. The slices were then frozen at -20°C for 48 h, and subsequently freeze-dried.

Recently, Silva et al. [9] developed a new formulation, a beetroot gel, mainly aimed at the administration of NO_3^- to athletes during sports competitions. The gel was prepared from

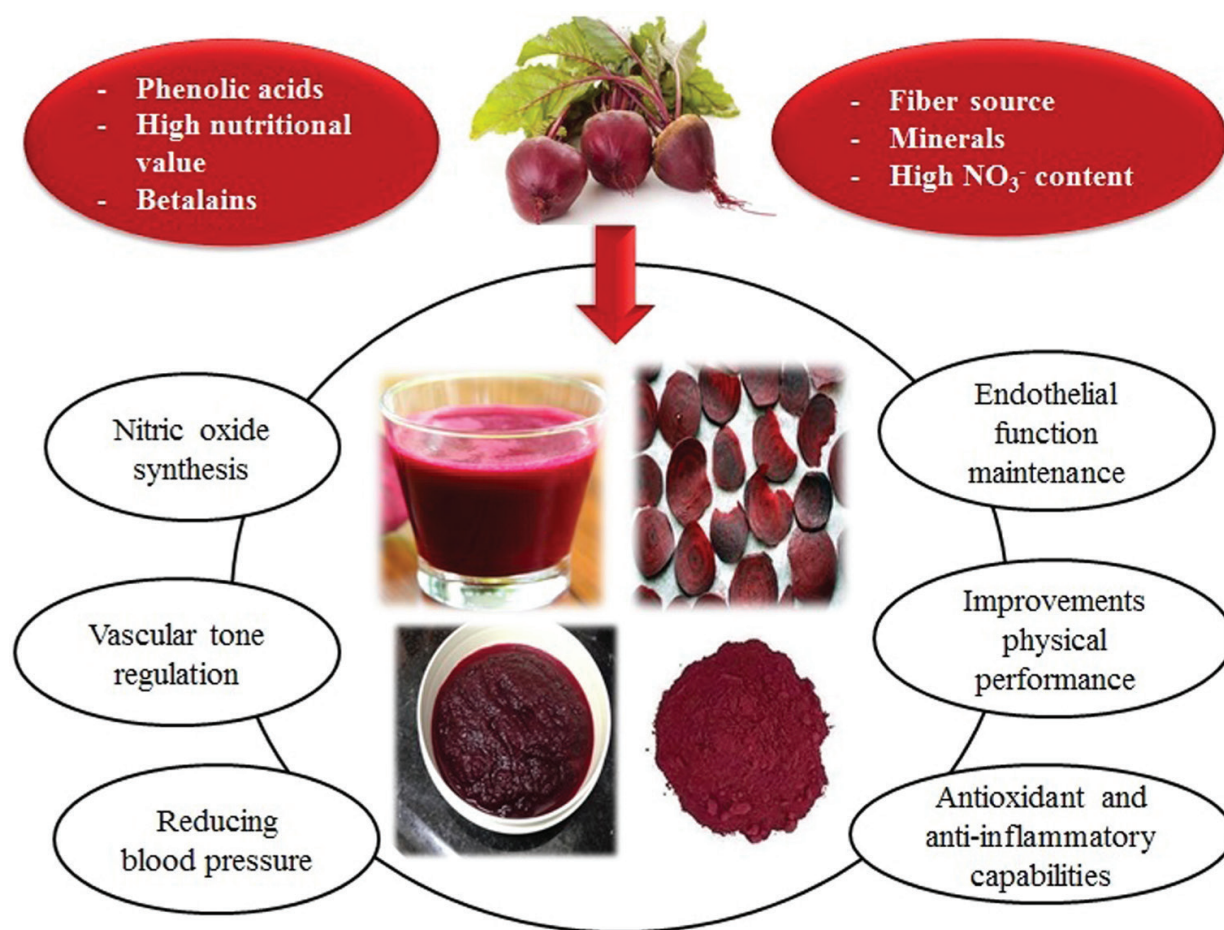


Figure 3: Different beetroot formulations and nutritional benefits.

sanitized vegetables washed in a chlorinated solution (0.5%), half of which was used for juice production, as described previously, and the other half frozen at -20°C for 48 h and then crushed in a portable blender to produce beetroot powder. The beetroot gel was formulated with a mixture of beetroot juice, beetroot powder and carboxymethylcellulose at a 90:17:3 ratio. This new beetroot-based gel presented pseudoplastic fluid characteristics, with decreased apparent viscosity as a function of increasing shear rate (deformation). This pseudoplastic behaviour can be a great advantage for the production and marketing of beetroot gel with respect to handling, packaging and yield, since the product flows smoothly, resulting in a suitable flow from the sachet into the mouth during gel ingestion. According to Rao [75], food rheology has an influence on taste and flavour perception, because of an effect related to the physical properties of the food (i.e. texture, viscosity), which affects the rate and extent to which the stimuli reach the taste buds. Colour is one of the most important food attributes, and is considered a quality indicator. The beetroot gel showed a low lightness, according to the CIE system scale ranging from 0 to 100. Visually, the produced gel had a low brightness (dark) red-violet colour, characteristic of raw beetroots, due to the characteristic colour of

betanin, a water-soluble compound derived from betalamic acid. About 101 untrained panelists comprising both males and females (using a 9-point hedonic scale: where one reflected extreme dislike and nine reflected the highest acceptability) evaluated the beetroot gel. The beetroot gel with orange flavouring received higher mean scores in all sensory attributes (flavour, aroma, texture, and overall impression). It is possible that orange flavouring positively influenced the appraisal of the texture and overall impression attributes by the evaluators. Thus, the NO_3^- -enriched beetroot gel presented advantageous rheological properties for oral administration and handling in the nutritional supplement industry. This encourages the testing of beetroot gel in larger groups, such as physically active subjects.

In their most recent study, Vasconcellos et al. [76] produced a beetroot gel with reduced NO_3^- content as previously described by Silva et al. [9] and supplemented 25 healthy runners, and also produced a PLA gel. The PLA was prepared by depleting the NO_3^- from the beetroot juice by using an A-520E anion-exchange resin. Fuji apple (*Malus pumila* species) puree was prepared by liquefying apples in a portable blender. NO_3^- -depleted beetroot juice, 16.8 g of apple puree (in substitution of beet chips), 2.8 g of carboxymethylcellulose and 1 mL of artificial orange flavour were then mixed to produce PLA gel.

Table 1 displays the centesimal composition and sugar content of the different beetroot-based formulations in 100 g. Beetroot gel presented the highest protein, lipid and total dietary fiber contents when compared to the other formulations. It is important to note that lipid amount was extremely low in all formulations. Beetroot chips had the highest energy content (in the form of kcal), carbohydrates and total sugars when compared to beetroot juice and gel.

Although beetroot is considered one of the main sources for the acquisition of dietary NO_3^- and of functional compounds with antioxidant activity, the contents of these substances vary significantly from one formulation to another. **Table 2** displays the antioxidant potential, total phenolic compounds content, flavonoids, saponins, NO_3^- and NO_2^- contents of different beetroot formulations. Beetroot gel presented the highest total phenolic content (TPC), flavonoids, saponins and NO_3^- content when compared to juice and chips. However, beetroot chips presented the highest values of the total antioxidant potential (TAP) when compared to the other formulations. Beetroot juice showed the lowest NO_3^- concentrations, while chips showed the lowest TPC, flavonoids and saponin levels when compared to the other formulations, indicating that beetroot processing leads to impoverishment of functional compounds. The NO_2^- content of all formulations was considered insignificant ($<1 \text{ mmol} \cdot 100 \text{ g}$).

Although beetroot juice has become the most commonly used formulation for the dietary administration of NO_3^- , beetroot gel showed significantly higher levels of NO_3^- and bioactive compounds. Thus, beetroot gel seems to be the most effective formulation, providing a ready, easy to administer, attractive, NO_3^- -rich food with the aim of promoting beneficial effects on the cardiovascular system.

Beetroot formulations	kcal	CHO (g)	PTN (g)	LIP (g)	TDF (g)	Total sugars (g)	Fructose (g)	Glucose (g)	Sucrose (g)	Moisture (%)	Ashe (%)
Raw	43	9.56	1.61	0.17	2.8	6.76	-	-	-	87.58	-
Juice	94.9 ± 1.7 ^b	22.6 ± 0.4 ^b	0.70 ± 0.07 ^b	0.16 ± 0.01 ^b	0.91 ± 0.31 ^c	12.1 ± 0.1 ^b	0.86 ± 0.01 ^b	2.5 ± 0.02 ^a	8.8 ± 0.03 ^b	76.1 ± 0.50 ^a	0.8 ± 0.06 ^b
Chips	365.0 ± 2.1 ^a	89.9 ± 0.52 ^a	0.97 ± 0.01 ^b	0.14 ± 0.01 ^b	3.2 ± 0.63 ^b	18.8 ± 0.01 ^a	1.4 ± 0.01 ^a	2.7 ± 0.01 ^a	14.6 ± 0.01 ^a	4.6 ± 0.57 ^b	1.0 ± 0.05 ^b
Gel	71.52 ± 1.9 ^c	13.6 ± 0.31 ^c	3.02 ± 0.09 ^a	0.56 ± 0.01 ^a	4.5 ± 0.28 ^a	9.7 ± 0.07 ^c	0.31 ± 0.01 ^c	1. ± 0.01 ^b	8.1 ± 0.05 ^b	76.14 ± 0.5 ^a	2.01 ± 0.13 ^a

The values are displayed as means ±SD. Different letters denote statistical significance between the samples at $P < 0.05$ Kcal, kilocalorie; CHO, carbohydrate; PTN, protein; LIP, lipid; TDF, total dietary fibre; g, gram.

* Reproduced from da Silva et al.[9]; USDA. [15].

Table 1. Proximate composition of different beetroot formulations (100 g).

Beetroot formulations	TAP	TPC	Flavonoids	Saponins	NO ₃ ⁻	NO ₂ ⁻		
	%	GAE mg	mg	mg	mmol	mg	mmol	mg
Juice	79.13 ± 0.63 ^c	1.01 ± 0.03 ^b	0.42 ± 0.01 ^b	8.22 ± 0.12 ^b	1.6 ± 0.01 ^c	217 ^c	0.10 ± 0.02 ^c	4.6 ^c
Chips	95.70 ± 0.53 ^a	0.75 ± 0.06 ^b	0.31 ± 0.02 ^b	6.37 ± 1.26 ^c	4.5 ± 0.02 ^b	279 ^b	0.13 ± 0.02 ^b	5.98 ^b
Gel	87 ± 0.1 ^b	1.98 ± 0.03 ^a	1.37 ± 0.03 ^a	22 ± 0.54 ^a	6.3 ± 0.41 ^a	390 ^a	0.15 ± 0.0 ^a	6.9 ^a

The values are displayed as means ±SD. Different letters denote statistical significance between the samples at $P < 0.05$. TAP, total antioxidant potential; TPC, total phenolic content; GAE, gallic acid equivalents; NO₃⁻, nitrate content; NO₂⁻, nitrite content.

*Reproduced from da Silva et al.[9]; Vasconcellos et al. [7].

Table 2. Antioxidant potential, total phenolic compounds, bioactive compounds, NO₃⁻ and NO₂⁻ contents of different beetroots formulation (100 g).

7. Effect of the ingestion of beetroot formulations on nitric oxide production and consequent health benefits

Due to its high NO₃⁻ content, beetroots have been used as a dietary source of this anion for the production of NO, aiming at blood pressure lowering effects [77]. The first study conducted with beetroot juice (500 mL) was performed by Webb et al. [66]. The authors offered 500 mL of beetroot juice to 14 healthy volunteers (containing ≈ 22.5 mmol of NO₃⁻). Following ingestion, significant increases in NO synthesis (plasma NO₃⁻ and NO₂⁻), stabilization of the endothelial function evaluated by the dilation of the brachial artery-mediated flow (DILA) and a significant decrease of the systolic blood pressure (SBP) by up to 10 mmHg and diastolic blood pressure (DBP) by up to 8 mmHg were observed. In addition, the decreases in SBP and DBP were correlated with increased NO synthesis. Subsequently, the juice was used in other studies that, in addition to healthy subjects, involved individuals with hypertension and associated morbidities [67, 71, 78]. Kapil et al. [78] supplemented 68 hypertensive subjects with 250 mL of beetroot juice (containing ≈ 6.4 mmol of NO₃⁻) for 4 weeks and evaluated endothelial function through DILA, plasma NO₃⁻, NO₂⁻, cGMP (other marker of NO synthesis) and monitored BP before and after the interventions. The authors observed an improvement in endothelial function through a significant increase in the mediated flow of the brachial artery and the concentration of NO₃⁻ and NO₂⁻ plasma after 4 weeks of beetroot juice ingestion. Significant SBP decreases by 7.7 mmHg and DBP by 2.5 mmHg were also observed after dietary NO₃⁻ intake.

Baião et al. [8] acutely supplemented 40 healthy volunteers (20 men and women) with 1.6 mmol of NO₃⁻ with 100 mL beetroot juice. Significant increases in urinary NO₃⁻, NO₂⁻ and NOx (NO₃⁻ + NO₂⁻) concentrations after the ingestion of this small amount of beetroot juice were found, but no differences in the NO metabolites excretion responses between men and women. Regardless of gender and body mass, urinary excretion of NO metabolites after consumption of a dietary source of NO₃⁻ increased in both men and women.

Table 3 shows studies that evaluated the effect of NO₃⁻ supplementation through different beetroot formulations regarding their efficiency on NO production and the effect

Study	Beetroot formulations	NO ₃ ⁻ concentration	Experimental population	Duration of administration (days)	Effect
Webb et al. [66]	Juice (500 mL)	22.5 mmol	14 healthy males	Acute	Increases NO ₃ ⁻ (≈16-fold) and NO ₂ ⁻ (≈2-fold) in plasma. Decreases of 10.4 ± 3 mmHg in SBP and after 24 h (-6 mmHg), decrease of 8.1 ± 2.1 mmHg in DBP.
Kapil et al. [67]	Juice (250 mL)	5.5 mmol	9 healthy subjects	Acute	Increases NO ₃ ⁻ (≈2-fold) and NO ₂ ⁻ (≈1.6-fold) in plasma. Decreases of 5.4 ± 1.5 mmHg in SBP. No effect on DBP.
Kenjale et al. [79]	Juice (500 mL)	9.0 mmol	8 elderly subjects	Acute	Increases plasma NO metabolites (NO ₃ ⁻ and NO ₂ ⁻). There was an increase in 32 s in exercise time after beetroot juice consumption before subjects reported pain due to claudication. There was a reduction 48% after beetroot juice consumption (indicating that oxygen extraction was reduced) compared to PLA after exercise.
Coles and Clifton [72]	Juice + Apple (500 g)	15.0 mmol/L	30 healthy subjects	Acute	There was a non-significant reduction in SBP and DBP in men and women after beetroot juice consumption when compared to PLA.
Hobbs et al. [69]	Juice (100 mL) (250 mL) (500 mL)	2.3 mmol 5.7 mmol 11.4 mmol	18 healthy males	Acute	Increase in urinary NO. Dose-dependent reduction with peaks of 13.1, 20.5 and 22.2 mmHg in SBP and 16.6, 14.6 and 18.3 mmHg in DBP at doses of 100, 250 and 500 mL, respectively.
Hobbs et al. [69]	Red and white beetroot bread (100 g)	1.6 mmol and 1.8 mmol, respectively.	14 healthy males	Acute	Peak differences in SBP and DBP in the order of 19.3 and 23.6 mmHg and 16.5 and 23.2 mmHg for breads enriched with white and red beetroot, respectively, in relation to the control.
Hobbs et al. (2013) [80]	Red beetroot bread (100 g)	1.1 mmol	24 healthy males	Acute	Increases NO ₃ ⁻ (≈3-fold) and NO ₂ ⁻ (≈1-fold) in plasma. Decreases peaks of 7 mmHg in SBP. No effect on DBP.
Gilchrist et al. [71]	Juice (250 mL)	7.5 mmol	27 hypertensive and diabetic type 2	15	Increases NO ₃ ⁻ (≈5-fold) and NO ₂ ⁻ (≈1.7-fold) in plasma. No effect on SBP and DBP
Bond Jr et al. [81]	Juice (500 mL)	12.1 mmol	12 healthy women	Acute	Increase plasma NO metabolite (NO ₂ ⁻). Maximum decrease of 5.0, 8.1, 6.5, 11.2 mmHg and 3.6, 2.1, 0.4, 3.1 mmHg on SBP and DBP at rest and 40, 60 and 80% VO _{2peak} in submaximal exercise, respectively.

Study	Beetroot formulations	NO ₃ ⁻ concentration	Experimental population	Duration of administration (days)	Effect
Kim et al. [82]	Juice (140 mL)	12.9 mmol	12 healthy men	Acute	Increases plasma NO metabolites (NO ₃ ⁻ and NO ₂ ⁻). There was no decrease in SBP and DBP after beetroot juice ingestion. There was a significant reduction in PWV 3 h after beetroot juice consumption. I was observed an increase of diameter of the brachial artery (mm) and FMD (%) through exercise intensity after beetroot and PLA juice consumption (there was no difference between the interventions).
Jajja et al. [83]	Juice (70 mL)	6.45 mmol	24 overweight subjects	21	Increases NO ₃ ⁻ in plasma and urine. There was no difference after the interventions beetroot and PLA juice in SBP and DBP.
Bondonno et al. [84]	Juice (140 mL)	7 mmol	27 hypertensive treated patients	7	Increases NO ₃ ⁻ (≈3-fold) and NO ₂ ⁻ (≈3-fold) in plasma. No effect on SBP and DBP
Kapil et al. [78]	Juice (250 mL)	6.4 mmol	34 hypertensive treated patients and 34 with hypertension	28	Increases NO ₃ ⁻ (≈5.5-fold) and NO ₂ ⁻ (≈2.7-fold) in plasma. Maximum decrease of 8.1 mmHg and 3.8 mmHg on SBP e DBP, respectively.
da Silva et al. [9]	Gel (100 g)	6.3 mmol	five healthy subjects	Acute	Increases NO ₂ ⁻ in plasma. Decreases in SBP of 6.2 mmHg and in DBP 5.2 mmHg.
Vasconcellos et al. [76]	Gel (100 g)	10 mmol	25 healthy runners	Acute	Increase NO ₂ ⁻ in urine. There was no improvement in VO _{2peak} and time to fatigue. SBP and DBP did not differ significantly at any of the investigated time points.

NO₃⁻, nitrate; NO₂⁻, nitrite; NO, nitric oxide; DBP, diastolic blood pressure; SBP, systolic blood pressure; PLA, placebo; VO_{2peak}, maxim oxygen volume; PWV, pulse wave velocity; FMD, mediated flow dilatation.
 Beetroot results from the following references [7, 9, 66, 67, 69, 71, 72, 78–84].

Table 3. Studies evaluating the effect of the ingestion of different forms of beetroot on NO production and blood pressure in healthy and hypertensive individuals.

on blood pressure of healthy and hypertensive subjects. In the study conducted by Silva et al. [9], the NO₃⁻ and antioxidant-enriched beetroot gel was administered acutely to five healthy subjects. About 60 min after a single-dose intake of 100 g of the beetroot gel containing ≈ 6.3 mmol of NO₃⁻, a 3-fold increase in plasma NO₂⁻ was observed, followed by decreases in SBP and DBP of 6.2 mmHg in 60 min and 5.2 mmHg in 120 min, respectively. In the recent study by Vasconcellos et al. [76], supplementation with NO₃⁻-enriched

beetroot gel (100 g containing ≈ 10 mmol of NO_3^-) in 25 healthy and physically active individuals resulted in a significant increase in urinary NO_3^- and NO_2^- levels after 90 min. The study also demonstrated a decrease in hypoglycaemia, sustained even in the post-recovery period, indicating that other significant metabolic changes appear to occur after NO_3^- intake. In addition, the authors also indicated that physically trained individuals would not benefit from increased levels of NO, since no improvement in physical performance was observed during aerobic submaximal exercise assessed by an aerobic exercise protocol on a treadmill (3 min warm-up of 40% peak oxygen consumption, 4 min to 90% of gas exchange threshold I and 70% (Δ) maximal end speed until volitional fatigue). However, no significant changes were observed in systolic and diastolic pressures or cortisol and lactate levels after the ingestion of the beetroot gel.

8. Conclusions

The effects of beetroot intake on cardiovascular health with regard to NO production and blood pressure are well documented in the literature. The bioactive compounds and *in vitro* antioxidant capacity of the formulations point to the importance of the inclusion of this plant as a dietary component aimed at the prevention of cardiovascular diseases, which may also aid in the cellular response to oxidative stress. The present review highlighted the existence of different beetroot formulations, among which are gels enriched in NO_3^- and bioactive/functional compounds formulated by our research group. Studies involving the effects of different forms of beetroot on a significant number of healthy individuals with cardiovascular problems, as well as *in vivo* effect in animal models, could boost the food industry in the elaboration of beetroot formulations as a food support for the population.

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Author details

Diego dos S. Baião, Davi V.T. da Silva, Eduardo M. Del Aguila and Vânia M. Flosi Paschoalin*

*Address all correspondence to: paschv@iq.ufrj.br

Federal University of Rio de Janeiro, Chemistry Institute, Rio de Janeiro (RJ), Brazil

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