

## Evaluation and Characterization of Nutritional, Microbiological and Sensory Properties of Beet Greens

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### Abstract

Growing interest of consumers on incorporate healthy foods into their diets has triggered extensive research in finding and developing new food products that meet their expectations. Leaves of beet plants are an alternative source of appreciable value albeit they are usually discarded at the time of beetroot harvest. The aim of this study was to evaluate and characterize the nutritional, microbiological and sensory quality of beet greens as a prelude to the development of new products from them. Beet greens represents approximately 50% of harvested material. The proximate composition of leaves and its anti-nutritional factors (oxalate, phytates and tanins) were in the range of commonly consumed leafy vegetables, standing out for its high contents of protein, fat, fiber and iron and low content of phytates and tanins. Contents of phytochemical compounds, especially carotenoids and betalains, were higher than those reported for commonly consumed leafy vegetables, resulting in a high antioxidant potential.

Microbiological counts were in the typical range for fresh consumed leafy vegetables while, sensorial quality resulted adequate with highly appreciated characteristics in beet greens such as red veins and green blade. This work sets the basis for several recovery possibilities such as development of a minimally processed product for fresh consumption and the extraction of phytochemical compounds from this resource.

**Keywords:** Beet Leaves; Recovery; Underutilized; By-Products; Characterization; Bioactive Compounds

### Abbreviations

TW: Total Weight; GW: Green Leaves Weight; Y: Yield; TPC: Total Phenolic Content; RSC: Radical Scavenging Capacity; TLC: Thin Layer Chromatography; Bx: Betaxanthins; Bc: Betacyanins; TC: Total Chlorophyll; Chla: Chlorophyll-a; Chlb: Chlorophyll-b; C: Carotenoids; FT: Fresh Tissue; DT: Dry Tissue; LAB: Lactic Acid Bacteria.

### Introduction

The production and processing of vegetables generates a large number of by-products [1], reaching in some cases losses of up to 75% of the harvested material [2]. Contributing to this phenomenon, the leaves of plants that are grown for consumption of its roots (i.e. carrots, beets, turnips) are usually discarded at harvest as a waste. The development of sustainable solutions for food waste management represents one of the main challenges for our society. Traditionally, these by-products have been recovered as livestock feed or composting [2] and, more recently, for production of

bioethanol [3]. However, during the last decade a great number of researches have shown that vegetable by-products are promising sources of high-value compounds such as fibers, antioxidants, essential fatty acids, antimicrobials, among others [1, 4]. These features have led to an increase in the interest in recovery of underutilized vegetable by-products for its use in human feeding or as alternative sources of beneficial healthy compounds. Moreover, considering that the world population growth projections predict serious problems of food availability for future generations, the search for alternatives to achieve the comprehensive utilization of natural resources along with the development of new food sources constitute a challenge that scientists must address now to respond in advance to this concern [5].

One of the horticultural products that generate large quantities of underutilized biomass is beet (*Beta vulgaris L.*). Although beet was originally grown for the consumption of their leaves [6], nowadays the beetroot is the main product obtained. Particularly in

Argentina, beet leaves constitute a by-product that is not exploited and is discarded as a waste. The beetroot is rich in carbohydrates, fibers, proteins and minerals, such as sodium, potassium, calcium and iron [6]; and also provides important phytonutrients like polyphenols, carotenoids, betacyanin's and betaxanthins, among others [7]. Thus, it is expected that beet greens also have high levels of these nutrients.

Given this background, the aim of this study was to evaluate and characterize the nutritional and anti-nutritional profile together with the microbiological and sensory quality of beet greens in order to evaluate the potential of this underutilized resource for the development of new food products.

### Materials and Methods

#### Plant Material and Sample Preparation

Beet plants (*Beta vulgaris* L. var. Conditiva) were grown and harvested in Escobar, Argentina. Twelve lots were randomly selected in surrounding fields of the horticultural belt. Each lot represented an independent replicate, and was constituted by 6 - 8 plants. Once harvested, beet plants were immediately precooled in refrigerated containers and transported to the laboratory within the first post-harvest hour.

#### Yield

Beet plants were cut 5 cm below the base in order to separate leaves from stems and roots. The whole plant (TW) and the greens (GW) were weighed. Then, the yield (Y) was calculated as:  $Y = GW / TW \cdot 100$ .

### Nutritional Quality

#### Proximate Composition

Moisture, ash, crude protein, fat and dietary fiber were analyzed by the AOAC methods [8]. Carbohydrates and energy content (kcal kg<sup>-1</sup>) were calculated using the methodology suggested by Greenfield and Southgate [9]. For mineral metallic elements (calcium, iron and zinc), samples were pre-treated according to AOAC 968.08 method and then, the quantification by Atomic Absorption Spectrometer AA400 (Perkin Elmer, Massachusetts) was conducted.

#### Anti-Nutritional Factors

The phytate content was determined according to the methodology proposed by Amalraj and Pius [10]. Oxalates and tannins determinations were performed in external laboratories according to AOAC techniques, with spectrophotometry and capillary electrophoresis methods, respectively.

#### Antioxidant Capacity and Phytochemical Composition

Total antioxidant capacity, total phenolic compounds, chlorophylls (total, a and b), carotenoids, betaxanthins and betacyanin's were measured in beet greens samples.

For antioxidant capacity and total phenolic content, an extraction was conducted following the methodology proposed by Viacava, *et al.* [11]. Antioxidant capacity was evaluated through the DPPH radical scavenging assay and the total phenolic content (TPC) was determined using the Folin-Ciocalteu method. For

both cases, the methodology described by Viacava *et al.* [11] was followed. The total phenolic content was calculated by using Gallic acid as standard and expressed as mg of Gallic acid equivalent per kg of dry tissue (DT) weight (mg kg<sup>-1</sup>) and antioxidant capacity was expressed as radical scavenging capacity (%RSC).

For polyphenols identification, first a thin-layer chromatography was carried out. For this, 1g of sample was taken and a methanol extraction was performed. Then, 5 mL of the methanol extract were concentrated to 2 mL, and a second extraction was carried out using a mixture of water and ethyl acetate.

The organic phase was separated and concentrated to 1 mL. About 10 µL of this solution was plated on silica gel thin layer chromatography (TLC). Reference standards (Sigma Aldrich) were used. Two chromatographic systems were considered: ethyl acetate- formic acid- acetic acid- water (100:11:11:26) and methanol-chloroform (2:8) + an acetic acid drop. For detection, NH<sub>3</sub> vapor was used and the read was performed at 254 nm UV. For the phenolic compounds quantification, an HPLC Shimadzu Prominence equipment was used, with a LC-AT pump, an UV-visible detector with a diode array SPD-M 20 A, a column oven CTO-10ASVP and a Rheodyne injector. Solution LC software was used for data analysis. The chromatographic separations were performed on a HPLC Kinetex C18-2.6µ - 4.60 mm column (Phenomenex). The mobile phase flow rate was 0.8 mL/min and consisted of a gradient of 1% phosphoric acid in water (A) and 1% phosphoric acid in Acetonitrile (B). The UV-vis spectra were recorded in the 210 - 600 nm range and the chromatograms were acquired at 325 nm. The injection volume was 20 µL.

Calibration curves were done with a solution of standard quercetin, kaempferol and Rutin (Sigma Aldrich, HPLC grade). Three measurements were done for each sample and results were expressed in mg kg<sup>-1</sup> of leaf on a fresh weight basis.

Betaxanthins (Bx) and betacyanins (Bc) were determined following the methodology proposed by Moßhammer, *et al.* [12] and were reported as mg kg<sup>-1</sup>. Total chlorophyll (TC), chlorophyll-a (Chla), chlorophyll-b (Chlb) and carotenoids (C) contents were determined according to AOAC methods [8]. Results were reported as mg kg<sup>-1</sup> DW.

### Microbiological Quality

Fresh tissue samples (10g) were homogenized with 90 mL of sterile 0.1 % peptone water (Biokar Diagnostics, France) in a stomacher (Interscience Laboratories Inc. BagMixer® 400P, France) for 120 s. Decimal dilutions were prepared with sterile 0.1% peptone water and plated in duplicate. The mesophilic and psychotropic aerobic bacteria count were determined in plate count agar (Biokar Diagnostics, France) after 24 - 48h at 37°C and 5 d at 5°C, respectively; lactic acid bacteria were determined in agar Man Rogosa Sharpe (Biokar Diagnostics, France) with a double layer, after 3 - 5 d at 37°C; total coliform bacteria were determined in neutral red bile lactose crystal violet (Merck, Germany) with double layer, after 24 - 48 h at 37°C; and yeast and molds counts were determined in yeast extract glucose chloramphenicol agar (Biokar Diagnostics, France)

after 48-72 h at 28°C. The results were expressed as log CFU g<sup>-1</sup>.

### Sensory Quality

Sensory quality of samples was determined by 4 judges, aged 28 - 60 years, with sensory evaluation experience in leafy vegetables. The coded samples were randomly presented to the judges who evaluated the sensory parameters (colour, texture, defects and overall visual quality) using a descriptive scale of 1 - 9, where 9: best, excellent; 5: acceptance limit; 1: fully objectionable.

### Statistical Analysis

All results are expressed as LSMEAN values (means estimators by the method of least squares) together with their standard deviations (n = 12). Statistical analysis was performed using SAS software version 9.0.

## Results and Discussion

### Yield

Biomass of beet leaves obtained after cutting and separating beetroots and stems represented between 25 and 75% of the total harvested material, with an average of 51.8 ± 20.1%. The proportion of leaves in beet plants is very high and implies that a half, on average, of the biomass obtained when harvesting the produce will be lost, as this portion is not exploited. This low yield of the crop not only involves the specific loss of biomass, that was quantified here, but leads, in turn, greater losses for producers when considering the resources (water, labour, soil, fertilizer, among others) invested in the production of this raw material, reducing the efficiency of the whole process. Moreover, producing food that will not be consumed leads to unnecessary CO<sub>2</sub> emissions in addition to loss of economic value for the food producer [13].

Postharvest losses of fresh vegetables as well as development of different strategies and technologies to reduce them have received especial attention in the last years [14]. According to many studies, farmers have been losing between 30 - 40% (in developed countries) and 80% (in developing countries) of the value of their fruits and vegetables before they reach the consumer [15]. These biomass losses occur at different stages from farm to table (harvest, transportation, central markets and retail markets). Thus, losses of 50% at harvest represent a real high value. The use of this resource currently underutilized for development of new food products requires the characterization of its nutritional, microbiological and sensory quality as the first step for evaluating different alternatives of use.

### Nutritional Quality

#### Proximate Composition

The proximate composition of beet greens is presented in Table 1. The nutrient content found in beet greens samples were within the range of those found in leafy vegetables such as spinach, lettuce and arugula, as it is reported in the reference database from USDA [16]. According to the results obtained in this study, the beet greens stand out for their high content of protein, fat, fiber and iron, which are on the upper bound of the range.

Component	Content
Moisture (g kg <sup>-1</sup> )	913.0 ± 8.1
Carbohydrate (g kg <sup>-1</sup> )	10.30 ± 0.9
Ash (g kg <sup>-1</sup> )	14.8 ± 0.1
Protein (g kg <sup>-1</sup> )	24.7 ± 0.3
Total fat (g kg <sup>-1</sup> )	7.9 ± 0.3
Dietary fiber (g kg <sup>-1</sup> )	29.3 ± 0.3
Iron (mg kg <sup>-1</sup> )	25.4 ± 0.1
Zinc (mg kg <sup>-1</sup> )	4.1 ± 0.2
Calcium (mg kg <sup>-1</sup> )	525.0 ± 7.3
Energy Value (Kcal kg <sup>-1</sup> )	211.4 ± 0.4

**Table 1:** Proximate Composition of Beet Greens. \*

\*Data expressed as means ± standard deviations (n = 12).

### Antinutritional Factors

One of the main problems that could be involved in the exploitation of underutilized green leafy vegetables for human consumption is the presence of antinutritional compounds [17]. In general, green leafy vegetables can accumulate high concentrations of oxalates, tannins and phytates, among others, known inhibitors of mineral absorption, especially calcium [10]. Beet leaves analysed in this work presented an oxalate content of  $8328.6 \pm 122.2$  mg kg<sup>-1</sup> of fresh tissue (FT). It is well known that beet is one of the plants with higher content of oxalates [18] and taking into account that the content of this anti-nutrient is usually higher in the leaves of plants [19], it is not surprising that the levels found in this work were slightly higher than those reported for beet roots (760 - 6750 mg kg<sup>-1</sup>, [20]). Although the oxalate content in beet leaves resulted high, it is similar to the content found in other leafy vegetables that

are usually consumed in fresh salads. Particularly, oxalate content in spinach was extensively studied presenting values in the range of 4000 - 17650 [20]. Therefore, the oxalate content of beet leaves does not constitute a constraint for the development of a product for fresh consumption. In this case, like for other leafy vegetables, the only restriction would be associated with the recommendation of American Dietetic Association that establish a limit of dietary oxalate intake of 40 to 50 mg per day [20,21] for people who have a tendency to form kidney stones.

On the other hand, tannin content resulted in  $84.6 \pm 9.4$  mg kg<sup>-1</sup> FT. This is a low value if compared with those reported by other authors for different leafy vegetables consumed in a fresh way. In this sense, Amalraj and Pius [10] informed values between 860 and 4240 mg kg<sup>-1</sup> in a variety of green leafy vegetables commonly consumed in India. Gupta, *et al.* [22] observed a wider range, between 150 and 13300 mg kg<sup>-1</sup> in the underutilized green leafy vegetables of India. Furthermore, Somsu, *et al.* [23] found contents between 30 and 13530 mg kg<sup>-1</sup> in the commonly consumed vegetables of Thailand. Thus, tanins content in the raw material under study does not constitute a restriction for the development of a product for fresh consumption.

Finally, phytate content in beet greens was  $34.3 \pm 4.0$  mg kg<sup>-1</sup> FT. This is also a low value compared with those reported for other leafy vegetables. Singh *et al.* [24] found values between 130 - 500 mg kg<sup>-1</sup> in 25 traditional vegetables of Andaman and Nicobar Islands. Gupta *et al.* [22] found contents between 4.2 - 130.3 mg kg<sup>-1</sup> in underutilized leafy vegetables of India. Ndlovu and Afolayan [25] found a phytate content of 1171, 652 and 1464 mg kg<sup>-1</sup> in coliander, cabagge and spinach, respectively. Moreover,

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the antinutritional effects of phytates are usually evaluated not only through the total content of this compound, but also through molar ratios respect mineral compounds [26]. Particularly, phytates form a complex with Zn, reducing its bioavailability when the phytate: Zn molar ratio is higher than 15 [27]. In the same way, Lazarte, et al. [28] have confirmed a possible kinetic synergism between calcium and zinc with phytate, leading to the formation of a calcium-zinc-phytate complex when the [phytate][Ca]/[Zn] molar ratio is higher than 200 [28,29]. These ratios resulted in 0.83 and 10.85 in beet leaves, respectively, values considerably lower than those recommended ones. Moreover, Phytate: Fe and Phytate: CA molar ratios were 0.11 and 0.004, being lower than the limits recommended for these indices (1 and 0.17, respectively) [28]. Therefore, if a fresh product for human consumption is developed from beet leaves, phytates level is not likely to compromise the zinc, iron or calcium absorption.

### Phytochemical Composition

Table 2 presents results obtained for antioxidant capacity, total phenolic compounds, chlorophyll (a, b and total), carotenoids and betalainic compounds.

Parameter	Result
Antioxidant capacity (% RSC)	70.91 ± 13.54
Total polyphenols (mg kg <sup>-1</sup> )	305.8 ± 79.2
Chlorophyll-a (mg kg <sup>-1</sup> )	4977.1 ± 993.8
Chlorophyll-b (mg kg <sup>-1</sup> )	1988.3 ± 509.6
Total chlorophyll (mg kg <sup>-1</sup> )	6960.9 ± 1232.7
Carotenoids (mg kg <sup>-1</sup> )	2479.1 ± 387.6
Betacyanin's (mg kg <sup>-1</sup> )	1554.8 ± 512.5
Betaxanthins (mg kg <sup>-1</sup> )	2437.0 ± 506.5

**Table 2:** Nutritional Quality of Beet Greens. \*

\*Data expressed as means ± standard deviations (n = 12).

The antioxidant capacity of beet greens was very high, placing them among the vegetables with the highest antioxidant capacity among those reported in the literature. In this way, Roy, et al. [30] tested the antiradical activity of spinach, white and Chinese cabbage, and found values of 71 ± 9.5, 32 ± 2.7, and 8 ± 1%, respectively. Liu, et al. [31] reported levels of RSC from 51.7 to 70.0 % for different lettuce varieties. In addition to this, Turkmen, et al. [32] found values of 12.2 and 67.4 % for the antioxidant activity of leek and spinach, respectively.

Among phytochemical components, phenolic compounds constitute an extended group with health beneficial action mainly related to its antioxidant activity. Epidemiological studies correlate its ingestion with a lower incidence of chronic diseases such as cardiovascular disease, diabetes and cancer [33]. The total phenolic content (TPC) in beet greens (Table 2) resulted higher than that found for other leafy vegetables. Llorach, et al. [34] reported TPC values of 18.2, 63.5, 125.5, 322.1 and 259.0 mg kg<sup>-1</sup> for iceberg, roman, continental and Red oak leaf and escarole, respectively. Turkmen, et al.

[32] found TPC values of 30.0 and 127.4 mg kg<sup>-1</sup> in leek and spinach. Also, Zhou and Yu [35] found high TPC values, in the range of 163 - 188 mg kg<sup>-1</sup>, in kale samples followed by 132 and 93 - 130 mg kg<sup>-1</sup> in rhubarb and spinach, respectively, when study the TPC of commonly consumed vegetables grown in Colorado.

The identification of polyphenols by TLC reveals the presence of quercetin, kaempferol and Rutin in the enriched methanolic extract of beet leaves. The subsequent HPLC analysis revealed that the main polyphenol on beet leaf (61 % of total polyphenols) is Rutin, with a concentration of 9.7 mg kg<sup>-1</sup>, while the other two were found in low concentrations (0.012 and 0.001 mg kg<sup>-1</sup> for quercetin and kaempferol, respectively). Rutin (quercetin-3-O-rutinoside) is a flavonoid ubiquitously found in plants [36]. It is noteworthy that rutin has shown to have beneficial health effects, including anti-inflammatory, antioxidant, anti-carcinogenic, anti-allergic, and anti-viral effects. Its potent capacity for scavenging superoxide radicals has also been demonstrated. Moreover, recent studies show that rutin supplementation from natural food sources, might improve memory impairment and decrease hippocampal pyramidal neuronal death, such as seen in Alzheimer's disease. Additionally, the ability of rutin to suppress microglial activation and proinflammatory cytokines has also been demonstrated [37].

Among the phytonutrients, plant pigments such as chlorophylls, carotenoids and betalains, which were traditionally valued for their technological applications due to their properties as colorants, are being studied for their outstanding nutritional properties. Chlorophylls and their derivatives have shown important health-promoting functions, showing anti-mutagenic, anticancer and anti-inflammatory activity [38]. Carotenoids are extremely important for their biological function as pro-vitamin A. It has also been reported the significant antioxidant activity of carotenoids that has been associated with reduced risk of developing degenerative diseases such as cancer, cardiovascular diseases, cataracts and macular degeneration [38]. Betalains are water-soluble nitrogen-containing pigments with two subclasses: betacyanins (red-violet pigments) and betaxanthins (yellow-orange pigments) [7]. These pigments have shown antimicrobial and antiviral effects and ability to inhibit cell proliferation of human tumor cells [7].

Contents of TC, Chla and Chlb in beet greens resulted significantly lower than those reported for other leafy vegetables. For example, Žnidarčič, et al. [39] studied the chlorophyll of commonly consumed leafy vegetables in Mediterranean countries, and found an average of 23162.5, 17198.0 and 5964.1 mg kg<sup>-1</sup> of dry tissue (DT) for TC, Chla and Chlb, respectively. In spite of this, the chlorophyll a/b ratio in the present work (2.5) resulted similar to that reported in other studies. Also, Sánchez-Vega, et al. [40] reported values of 12553.8 mg kg<sup>-1</sup> DT of total chlorophyll with Chla/Chlb ratio of 2.70 for spinach.

On the other hand, carotenoids content of beet greens resulted considerable higher than those reported for other leafy vegetables. For instance, Žnidarčič, et al. [39] found a C content of

615 mg kg<sup>-1</sup> DT, in average, for Mediterranean commonly consumed leafy vegetables, while Sánchez-Vega, *et al.* [40] found values of C content of 490.1 mg kg<sup>-1</sup> DT for spinach. Thus, beet leaves provide 4 times more carotenoids than another common LV.

Comparison of Bc and Bx contents found in beet leaves (Table 2) with values reported for other leafy vegetables is difficult especially taking into account that leafy vegetables are not a source of these nutrients, so there is scarce information about this issue. In fact, it is known that betalains are not wide-spread pigment in nature such as carotenoids and anthocyanins [41]. Betalains accumulate in flowers, fruits and occasionally in vegetative tissues of plants belonging to most families of the Caryophyllales [7].

The most important source of betanin as colouring agent is the red beetroot (*Beta vulgaris* subsp. *vulgaris*) [41], but lately, in the search for alternative betalain sources, edible fruits *Opuntia* (subfamily *Opun-tioideae*), *Hylocereus* (subfamily *Cactoideae*) and some *Mamillaria* showed very promising results [41].

Accordingly, Herrera-Hernández [42] found values of 570 and 0.41 mg kg<sup>-1</sup> DT (if an 87.5% of fruit humidity is assumed) [16] for Bc and Bx contents in ripe berry cactus fruits, which are considerable lower than values found in the present research. Thus, beet leaves could be considered as an alternative rich source of these pigments.

Summarizing, beet greens constitute a resource of high nutritional value comparable to commonly consumed leafy vegetables in its proximate composition as well as the level of anti-nutritional factors, which are not a limitation for a development of a product for fresh consumption. Additionally, the content of phytochemical compounds, especially polyphenols, carotenoids and betalains, reinforce the potential of this raw material for the development of a product of high nutritional value. Also, the high concentration of these compounds would justify the development of extraction processes of them from this underutilized resource.

### Microbiological Quality

Microbiological counts obtained for beet leaves are shown in Table 3. Mesophilic microorganisms give an estimate of total viable populations and are indicative of the endogenous microflora and the contamination undergone by the material [43]. Lots of studies have quantified this population on vegetables and a high variability related with vegetable under study, preharvest and culture conditions, among others, is found. Mesophilic counts of beet leaves are in the range of those previous studies. For example, Maffei, *et al.* [44] found that mesophilic aerobic bacteria counts were from 6 to 7 log CFU g<sup>-1</sup> for organic and conventional vegetable varieties sold in Brazil. Additionally, Seow, *et al.* [45] found counts around 5.8 - 7.3 log CFU g<sup>-1</sup> for several vegetable samples including carrots, lettuce, tomato, bean sprouts.

Microbial group	Counts (log CFU g <sup>-1</sup> )
Mesophilic aerobic bacteria	5.17 ± 0.59
Psychrotrophic bacteria	5.63 ± 0.79
Lactic bacteria	2.86 ± 0.69
Total coliform	3.74 ± 0.81
Yeasts and molds	4.63 ± 0.33

**Table 3:** Microbiological Quality of Beet Greens. \*

\*Data expressed as means ± standard deviations (n = 12).

Psychotropic microorganisms represent an important group of microorganisms in fresh vegetables, because they can multiply during storage and retail (usually carried out at 1 - 5°C). In the present research, counts of these microorganisms were similar to mesophilic ones and this pattern was also found for other vegetables. For example, Abadias, *et al.* [46] reported that psychrotrophic microorganisms' counts were highly comparable to those of mesophilic microorganisms in several varieties of lettuce (iceberg, oakleaf, trocadero and romaine) and endive from retail establishments.

Regarding to Lactic Acid bacteria (LAB), their role on keeping quality of vegetables is not clear [43]. Breidt and Fleming [47] have proposed LAB as biocontrol agents in minimally processed refrigerated foods. Their antimicrobial effects may be associated with reduced pH, the generation of hydrogen peroxide, competition for nutrients, or the production of antimicrobial compounds such as bacteriocins [48]. LAB found in beet leaves were 2 log cycles lower than that found by Ponce, *et al.* [43] in lettuce samples (4.98 - 5.64 log CFU g<sup>-1</sup>). It would be interesting to evaluate the performance of this population during refrigerated storage of samples and determine whether they are capable to control the development of other microbial population or even a pathogen microorganism.

Total coliforms are widely distributed in nature and commonly found in raw vegetables. Soil, irrigation water or improper handling may explain the contamination of fresh vegetables by coliforms [43]. Total coliforms found in beet greens were within the range of those observed by other researchers in commonly consumed vegetables. Among them, Maffei, *et al.* [44] found that total coliforms count ranged from 4 to 5 log CFU g<sup>-1</sup> in organic and conventional vegetables sold in Brazil, and Seow, *et al.* [45] values of 2.1 to 5.7 log CFU g<sup>-1</sup> in fresh vegetables sold in Singapore.

Yeast and molds are usually associated with food spoilage and high counts may be a health hazard because of the mycotoxins produced by molds. The diseases caused by mycotoxins vary greatly, including carcinogen and immunosuppressive effects, among others [49]. Yeast and molds counts in beet leaves were also in the range of values reported for other commonly consumed vegetables. In this way, Mafei, *et al.* [44] found yeasts and molds counts ranged from 5 to 6 log CFU g<sup>-1</sup> in organic and conventional vegetable varieties of Brazil. Similarly, Seow, *et al.* [45] found counts of 3.2-5.2 log CFU g<sup>-1</sup> for lettuce samples.

Summarizing, microbiological quality of beet greens is in the order of that found for commonly consumed leafy vegetables. However, it is important to note that during the production of this raw material, no special care is taken in the microbiological quality of the leaves as producers are interested in roots. Even in this situation, beet greens resulted with proper microbiological quality at harvest, and this quality could be even improved with both good agricultural practices and postharvest disinfection procedures, if a minimally processed product was developed.

### Sensory Quality

Table 4 presents results obtained for sensory quality evaluation.

Parameter	Result
Colour	7.73 ± 0.63
Texture	7.77 ± 0.81
Defects	7.45 ± 0.64
Overall Visual Quality	7.64 ± 0.58

**Table 4:** Sensorial Quality of Beet Greens. \*

\*Data expressed as means ± standard deviations (n = 12).

Beet greens are characterized by having two clearly differentiated colouring areas, one related to the red midrib and vein and the other related to the green blade. These attributes were considered positive by panellists. In general, beet greens present the desirable characteristics typically required for a leafy vegetable (i.e. freshness like turgidity, bright leaves) [50]. However, all sensory parameters resulted a slightly lower than the maximum value of the scale because some of the samples presented certain discoloration with yellowish areas in the blade, broken leaves, especially those composed of larger leaves, considered as defects by panellist, and/or burns in their edges. In spite of this, in all cases scores were higher than the acceptance limit. Hence, these results indicate that this product could be easily accepted by consumers if presented in appropriate conditions. Being a completely new product, in more advanced stages it will be necessary to study acceptability and disposition to purchase with a consumer panel.

### Conclusion

Underutilization of beet leaves implies a loss of more than 50% of the harvested beet plant. It has been shown that this by-product is a source of valuable nutrients. In fact, the proximate composition of beet greens as well as its phytochemical composition revealed them to be good sources of many nutrients like iron, polyphenols and betalains that could help in overcoming micronutrient malnutrition at a negligible cost. Beet greens also present high fiber content, hence, would also serve as a natural source of it.

The anti-nutritients content in beet leaves was in the same level or even lower than other leafy vegetables that are eaten raw, like spinach. So fresh consumption is adequate in relation to this aspect, but recommendations of reduced intake must be made for people who suffer oxalosis.

The microbiological quality of this material immediately after harvest is in the same level of that observed in fresh commonly consumed vegetables. In addition, from a sensory aspect, beet greens present the desirable characteristics typically required for a leafy vegetable.

This work was the first step to address the characteristics of this plant material, which today is underutilized, and opens several possibilities to develop exploitation alternatives to manage it as a by-product with high profit from an economic and environmental perspective. In this way, extraction of some bioactives such as fiber, polyphenols, betalains, or, even the integral utilization of leaves as a fresh vegetable for human consumption could be some interesting options for the recovery of this underutilized biological source. In summary, this study has the value of giving the necessary bases for the development of different alternatives for the recovery of beet greens, underutilized material with low cost, high nutritional value and high potential for exploitation with the added benefit of reducing the environmental impact generated when discarded. So, its utilization, if managed in the right way, could generate economic, social, and environmental benefits.

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### Conflict of Interest

Any financial interest or any conflict of interest exists.

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