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Evaluation of beetroot (*Beta vulgaris L.*) leaves during its developmental stages: a chemical composition study

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Abstract

Beetroot leaves (*Beta vulgaris L.*) are commonly cut off and discarded before using its bulb due to lack of knowledge of how to use them. Aiming at using these leaves, in the present study, *in natura* and dehydrated beetroot leaves were chemically characterized in terms of fatty acid composition, proximate composition, minerals, total phenolic compounds (TPC), and antioxidant activity by DPPH• in different stages (60, 80, and 100 days) of development. The beetroot leaves showed significant levels of protein and lipids in all developmental stages, and all proximate composition nutrients decreased during these maturation stages; the highest content was observed at 60 days. The Fe content decreased during the developmental stages (from 342.75 to 246.30 mg.kg^{-1}), while the content of K increased (from 13,367.64 to 20,784.90 mg.kg^{-1}). With regard to to fatty acid composition, linolenic acid was present in the greatest quantity, and it increase up to 2.58 mg.g^{-1} (*in natura*) and 40.11 mg.g^{-1} (dehydrated) at 100 days of development. The n-6/n-3 ratios were low in all stages. The TPC and antioxidant activity by DPPH• changed during the developmental stages. The TPC was highest in the 100-day dehydrated leaves (15.27±0.12 mg GAE.g^{-1} FW), and the 50% inhibition of DPPH• (IC_{50} 89.52 μg.mL^{-1}) were better in the 60-day *in natura* leaves. This study shows that all developmental stages produced satisfactory results, and therefore, these leaves can be reused as food. The antioxidant activity and the chemical constituents, mainly the ω-3fatty acid, increased during the stages of development.

Keywords: beetroot leaves, minerals, fatty acid, alpha-linolenic acid, antioxidant activity.

1 Introduction

Epidemiological studies have demonstrated the protective effect of fruit and vegetable intake against chronic and degenerative diseases such as cancer (Padilha & Pinheiro, 2004; Kabat et al., 2010). Brazil is a major producer of fruit and vegetables, which are widely consumed, and of other less used food sources. Recent studies have investigated the composition profiles of underutilized foods and their co-products in attempt to investigate their potential as functional foods (Pereira et al., 2003; Almeida et al., 2009; Boroski et al., 2011).

Beetroot (*Beta Vulgaris L.*) belongs to the Chenopodiaceae family and is originally from temperate climate regions of Europe and North Africa. In Brazil, it is grown in the South and Southeast regions (77% of the total produce), and the annual yield is 30-40 tons per hectare, which corresponds to an average production of 280 tons. In street markets, indoor markets, and fruit and vegetable distribution centers, their leaves are cut off from the bulb to be used as organic fertilizer and animal feed or are discarded into the environment as waste (Amaral et al., 2004; Mello et al., 2008). Beetroot leaves are underused due to lack of proper knowledge, specially of their nutritive value and how to cook them and also because of dietary habits (Vilhena & Silva, 2007).

Among vegetable leaf constituents, fatty acids stand out, especially polyunsaturated fatty acids of the omega-3 series such as alpha-linolenic acid, because they play an important role as structural membrane lipids, particularly in nerve tissue and the retina, and are precursors of eicosanoids (Institute of Medicine of the National Academies, 2002). The consumption of these fatty acids can prevent diseases such as rheumatoid arthritis, coronary diseases, colon, prostate, and brain cancer, and other diseases common in western societies (Connor, 2010). Alternative and complementary sources of omega-3 are necessary to change the dietary ratio of omega-6 and omega-3 (n-6/n-3), especially in western countries, where this ratio has remained as 1:1 for many generations. However, in the last years, this ratio has changed and reached 17:1, with major consequences for public health (Simopoulos, 2002).

Antioxidants are compounds that inhibit or slow down the oxidation of lipids and other molecules through the neutralization of free radicals (Zheng & Wang, 2001). Among the antioxidants present in *in natura* vegetable leaves, phenolic compounds are found in great amounts, as well as in vegetables, fruit and medicinal plants (Abdel-Hameed, 2009). Phenolic compounds, which result from the secondary metabolism of plants and are formed in stress conditions (infections, UV rays, and others), stand out for their wide distribution in nature and

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their antioxidant activity (Arbos et al., 2010). They are important because they can retard the development of coronary and cardiovascular diseases, cancer, and intestinal inflammatory diseases (Chun et al., 2005; Pawlowska et al., 2006).

Mineral elements are essential in human diet due to their various bodily functions, such as the macro minerals Ca and Mg in the formation of bones, teeth, and tissues. Fe is a component of the hemoglobin molecule, which is essential for the transport of oxygen and cell breathing. These elements are found in cereals, fruit, and in roots and leaves of vegetables (Ekholm et al., 2007; Kawashima & Soares, 2003).

Some scientific studies on foods have reported changes in chemical composition at different stages of development of fruits, vegetables, and leaves (Bulbovas et al., 2005; Telci et al., 2009; Oliveira et al., 2011; Celi et al., 2011; Leite et al., 2011; Peiretti et al., 2013). However, there are no studies that address the different stages of development of beetroot leaves; therefore, the goal of the present study was to investigate the proximate composition, the mineral and fatty acid contents, and the antioxidant activity of *in natura* and dehydrated beetroot leaves at different stages of development (60, 80, and 100 days) in order to investigate the use of their leaves as food. Today, in literature there are studies available on the reuse of cassava (Silva et al., 2012; Carvalho et al., 2002) and carrot leaves (Pereira et al., 2003; Almeida et al., 2009; Boroski et al., 2011; Leite et al., 2011), but studies on beet leaf are unprecedented.

2 Materials and methods

2.1 Samples

Leaves of beetroot organically produced in the municipality of Maringá, Paraná State (23°25' south and 51°57' west), Brazil, were harvested 60, 80, and 100 days after planting although harvesting is commonly done after 80 days.

Harvest was performed at 60, 80, and 100 days between May and August 2010 (three harvest seasons). Different stages of maturation were investigated because the beetroots can be removed at 60 or 100 days. *In natura* beetroot leaves (NBL) were kept at 4 °C and analyzed up to 36 h after harvesting. Dehydrated beetroot leaves (DBL) were obtained by drying *in natura* leaves in an air-circulation oven (Quimis Model Q-314M292) with optimized air circulation (Visentainer et al., 2009), according to Almeida et al. (2009) The beetroot leaves were dried in order to increase their useful life.

2.2 Proximate composition

Moisture, ash, and crude protein contents were determined in accordance with AOAC - Association of Official Analytical Chemists (2000). Total lipids were extracted using the Bligh and Dyer method (Bligh & Dyer, 1959). Total carbohydrates were estimated by difference, and the energetic value was calculated considering the following energy conversion factors: carbohydrate 4 Kcal.g^{-1} (17 KJ.g^{-1}), protein 4 Kcal.g^{-1} (17 KJ.g^{-1}), and lipid 9 Kcal.g^{-1} (37 KJ.g^{-1}) (Brasil, 1998).

For mineral determination, the samples were digested in a muffle at 600 °C for 6-8 h until complete organic matter decomposition and were recovered with nitric acid solution (5% v/v) (approximately 90 °C). K, Ca, Mg, Fe, Cu, Zn, Co, Mn, and Na were quantified using a flame atomic absorption spectrophotometer Analytikjena novAA 300 (equipped with software winAAS) as mg of mineral *per* kg of sample using the technical parameters of calibration according to Table 1. All samples were analyzed in three replicates.

2.3 Chromatographic analysis

The fatty acid methyl esters (FAME) were prepared by methylation of the total lipids (TL), as described by the Hartman & Lago method (Hartman & Lago, 1973). Methyl esters was separated by gas chromatography using a Shimadzu 14-A (Kyoto, Japan) gas chromatograph equipped with a flame ionization detector (FID) and a fused silica capillary column CP-Select CB-FAME (100 m x 0.25 mm id., 0.25 μm film thickness, Varian, USA). The sample splitting rate was 1:100, and the samples (2 μL) were injected in triplicate. The operation parameters were as follows: detector temperature 230 °C, injection port temperature 220 °C, and column temperature 150 °C, which was programmed to increase up to 185 °C at 2 °C min^{-1} and next up to 225 °C at 10 °C min^{-1} and held at this temperature for 20 min for a running time of around 42 min. The peak areas were determined by the Workstation 5.0 (Varian) acquisition program. For the identification of fatty acids, retention times were compared with those of standard methyl esters (Sigma, USA).

Table 1. Calibration parameters for an atomic absorption spectrophotometer.

Element	Wavelength (nm)	Spectral bandwidth (nm)	Flame type	Current lamp (mA)
Ca	239.9	0.2	acetylene/nitrous oxide	10
K	404.4	0.5	Acetylene	5
Mg	202.6	1.0	acetylene/nitrous oxide	4
Fe	248.3	0.2	Acetylene	5
Cu	324.8	0.5	acetylene/nitrous oxide	4
Zn	213.9	1.0	acetylene/nitrous oxide	5
Co	240.7	0.2	acetylene/nitrous oxide	7
Mn	279.2	0.2	acetylene/nitrous oxide	5
Na	330.3	0.2	Acetylene	5

Quantification (in mg fatty acid g^{-1} of total lipids) was performed against tricosanoic acid methyl ester as an internal standard (23:0), as described by Joseph & Ackman (1992). Theoretical FID (flame ionization detector) correction factor values were used to obtain concentration values (Visentainer, 2012). Fatty acid contents were calculated in $mg.g^{-1}$ of total lipids using Equation 1.

$$FA = A_x \times W_{is} \times CF_x / A_{is} \times CF_{AE} \times W_A \quad (1)$$

where FA is mg of fatty acids per g of total lipids, A_X is the peak area (fatty acids), A_{IS} is the peak area of the internal standard (IS) methyl ester of tricosanoic acid (23:0), W_{IS} is the IS weight (mg) added to the sample (in mg), W_X is the sample weight (in mg), CF_X is the theoretical correction factor, and CF_{AE} is the conversion factor necessary to express results as mg of fatty acids rather than as methyl esters. The results were converted from mg fatty acid *per* g of total lipid to mg fatty acid *per* g of leaves. All samples were analyzed in three replicates.

2.4 Antioxidant activity

Extract preparation: NBL and DBL were mixed with methanol in the ratio of 1:10 (w/v) under magnetic stirring for 5 h in the dark. After filtration, the methanolic extracts were concentrated under reduced pressure at 40 °C.

DPPH assay: The antioxidant capacity of the leaf extracts was studied against DPPH free radical scavenging effect, according to El Massary et al. (2002).

Different aliquots of methanolic extract solution (2.0 mg/mL) were mixed with 2.0 mL of DPPH methanolic solution (4.70×10^{-2} $mg.mL^{-1}$). The mixture was thoroughly mixed on a vortex-mixer and kept in the dark for 30 min. Next, absorbance was measured at 517 nm using a spectrophotometer (Cary Win UV 50, Varian) against a methanol blank without DPPH. The results were expressed as percent inhibition of the DPPH radical, which was calculated according to Equation 2, as follows.

$$\%\ \text{Inhibition DPPH}\bullet = (Abs_{DPPH\bullet} - Abs_{sample}) / (Abs_{DPPH\bullet}) \times 100 \quad (2)$$

where $Abs_{DPPH\bullet}$ is the absorbance of the DPPH$^{\bullet}$ solution without extracts and Abs_{sample} is the absorbance of the sample solution.

The analyses were carried out in triplicate, and the extract concentration providing 50% inhibition of DPPH (IC_{50}) was obtained by plotting the concentrations of the extract solutions versus percent inhibition. All samples were analyzed in three replicates.

Total phenolic compounds (TPC): The TPC were estimated according to the Folin-Ciocalteau method with some modifications for plant extracts (Kujala et al., 2000; Wu et al., 2005). An aliquot of 0.25-mL of the extract methanol solution (2.50 $mg.mL^{-1}$) was mixed with 0.25 mL of Folin-Ciocalteau reagent previously diluted with water 1:1 v/v, 0.50 mL of a saturated sodium carbonate solution, and 4.0 mL of water. The mixture was left to rest at room temperature for 25 min and then centrifuged at 2000 rpm for 10 min. The supernatant absorbance was measured at 725 nm using a spectrophotometer (Cary Win UV 50, Varian). The results were expressed as milligrams of gallic acid equivalents *per* g of leaves (mg $GAE.g^{-1}$). All samples were analyzed in three replicates.

2.5 Statistical analysis

Analysis of variance (ANOVA) was used to test the difference between means (stages of development), which were analyzed by the Tukey test at 95% ($p < 0.05$) level of significance using the STATISTIC software version 7.0 (StatSoft, 2004).

3 Results and discussion

3.1 Proximate composition

Table 2 presents the proximate composition of *in natura* (NBL) and dehydrated (DBL) leaves at different stages of maturation (60, 80, and 100 days). After dehydration, the leaf constituents were concentrated thus increasing the values of ashes, crude protein, total lipid, carbohydrate, and energy as compared to those of *in natura* leaves. According to Table 2, the moisture values of NBL and DBL were significantly different ($P < 0.05$). NBL harvested at 60, 80, and 100 days lost 93, 90, and 88% water during drying, respectively. As for the ash contents, DBL had significant values ($g.kg^{-1}$) ranging from 145.06±0.98 (100 days) to 184.33±6.81 (60 days). The crude protein contents of DBL ranged from 264.12±21.10 $g.kg^{-1}$ (60 days) to 310.25±3.63 $g.kg^{-1}$ (100 days); thus it can be considered a promising protein source for those who have limited access to animal protein. The ash and protein contents found in the DBL were higher than those found by Kinupp & Barros (2008) in 52 vegetable species and 36 hydroponics.The ash and protein contents of DBL were greater than the values

Table 2. Proximate composition of *in natura* (NBL) and dehydrated (DBL) beetroot leaves at different stages of development.

Development Stages (days)	Moisture ($g.Kg^{-1}$ leaves)	Ash ($g.Kg^{-1}$ leaves)	Crude Protein ($g.Kg^{-1}$ leaves)	Total Lipids ($g.Kg^{-1}$ leaves)	Carbohydrate† ($g.Kg^{-1}$ leaves)	Energy ($Kcal.kg^{-1}$)*
NBL 60	870.42^{a}±0.51	22.71^{a}±0.06	40.37^{a}±2.18	12.95^{a}±1.89	53.93^{a}±2.57	474.35^{a}±5.72
NBL 80	892.14^{b}±1.06	17.58^{a}±0.03	39.57^{a}±0.19	9.22^{a}±0.47	41.35^{a}±1.39	397.58^{a}±1.77
NBL 100	898.28^{c}±0.52	15.82^{a}±0.46	38.16^{a}±1.15	7.86^{a}±0.85	39.88^{a}±1.02	372.97^{a}±4.46
DBL 60	57.42^{d}±2.19	184.33^{b}±6.81	264.12^{b}±21.10	86.42^{b}±5.21	410.19^{b}±14.44	3,350.19^{b}±37.42
DBL 80	85.08^{e}±0.73	156.09^{c}±0.61	287.53^{bc}±23.01	105.63^{bc}±9.07	365.66^{c}±14.33	3,472.02^{c}±43.16
DBL 100	105.90^{f}±1.58	145.06^{d}±0.98	310.25^{c}±3.63	127.32^{c}±9.48	307.21^{d}±8.35	3,584.56^{d}±77.96

Results ± standard deviation. NBL = *in natura* beetroot leaves, DBL = Dehydrated beetroot leaves. †Values were calculated by difference. *Expressed in $Kcal.Kg^{-1}$. Means followed by the same superscript letter in the same column are not significantly different ($p < 0.05$). All chemical analyses were performed in triplicate.

reported by Pereira et al. (2003) and Leite et al. (2011) for dehydrated carrot (*Dacus carota L.*) leaves. The carbohydrate content was higher in 60-day leaves and decreased during the developmental stages (from 53.93± 2.57 to 39.88±1.02 g.kg^{-1}). The carbohydrate contents of NBL were higher than the values reported by Ohse et al. (2012).

NBL showed low total lipid contents; the highest value was 12.95 ±1.89 mg.kg^{-1} (60 days). However, in comparison to other vegetable leaves collected in South Africa (Odhav et al., 2007), for example, *Amaranthus, Chenopodium, and Physalis,* the chemical composition of NBL stands out for its amount of lipids. Drying increased the lipid content of the leaves by 85, 91, and 94% in NBL harvested at 60, 80, and 100 days, respectively. The highest carbohydrate contents were detected in DBL harvested at 60 days, and the energy values were higher for DBL harvested at 100 days.

Dehydrated leaves contained significant ash levels; nutritionally important macro- and micro minerals stood out. The macro- and micro mineral composition of DBL are presented in Table 3.

Table 3. Minerals in dehydrated beetroot leaves (DBL) (mg.kg^{-1} dry leaves) at three different stages of development.

	60 days	80 days	100 days
K	13,367.64^{a} ± 735.17	13,379.48^{a} ± 128.49	20,784.90^{b} ± 898.70
Ca	1,476.35^{a} ± 40.20	1,568.07^{a} ± 60.31	1,864.85^{b} ± 4.81
Mg	1.83^{a} ± 0.10	2.09^{b} ± 0.19	1.79^{c} ± 0.03
Fe	342.75^{a} ± 48.56	187.30^{b} ± 33.92	256.30^{ab} ± 29.49
Cu	12.76^{a} ±0.73	12.23^{a} ± 0.20	13.42^{a} ± 4.64
Zn	11.63^{a} ± 0.44	11.52^{a} ± 0.36	13.31^{a} ± 1.99
Co	1.29^{a} ± 0.27	0.54^{b} ± 0.07	0.10^{a} ± 0.01
Mn	17.59^{a} ± 1.47	15.49^{a} ±8.97	8.83^{b} ± 3.66
Na	7,907.01^{a} ± 245.41	5,534.37^{b} ± 627.70	4,724.63^{b} ± 417.32

Results ± standard deviation. DBL = Dehydrated beetroot leaves. Means followed by the same superscript letter in the same column are not significantly different ($p < 0.05$). All chemical analyses were performed in triplicate.

According to the Brazilian sanitary surveillance agency (Agência Nacional de Vigilância Sanitária, 2004), the recommended daily intake (RDI) of Fe and Cu for adults is 14 and 0.9 mg/d, respectively and the consumption of 70 g (about 2 cups) of DBL provides the recommended amounts of these micro minerals. The Fe content of DBL varied from 187.30 ± 33.92 to 342.75 ± 48.56 mg.kg^{-1} of leaves, and the largest amount was observed at 60 days. The best sources of Fe are red meat, vegetables, and grains (Pereira et al., 2003).

In the present study, as previously mentioned, good levels of Fe were found in the beetroot leaves. The beetroot leaves have more Fe than other vegetable leaves such as *Senna occidentalis* (110 mg.kg^{-1}), *Chenopodium album* (130 mg.kg^{-1}), and *Justicia fla*va (160 mg.kg^{-1}) (Odhav et al., 2007).

Beetroot leaves contain high amount of Cu, similar to the levels reported for watercress and rocket (Ekholm et al., 2007). High K contents were determined, as expected for vegetables and fruits since K^{+} is necessary for the metabolism of carbohydrates and proteins[3] and the RDI for potassium varied from 1,950 to 5,900 mg/d (Brasil, 1998).The consumption of 145 g (about 4 cups) of DBL provides the recommended amounts of potassium.

3.2 Fatty acid composition (Fatty acid content)

The presence of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids in the leaves of beetroot was investigated (Table 4). Among the SFA, pentadecilic (15:0), palmitic (16:0), and stearic (18:0) acids were identified. The MUFA identified were pentadecenoic (15:1n-9), palmitoleic (16:1n-7), oleic (18:1 n-9), and vaccenic acids (18:1n-7). The commonly PUFA present in the lipid fraction of leaves are linoleic (LA, 18:2n-6) and alpha-linolenic (LNA, 18:3n-3) acids, which belong to the ω-6 and ω-3 families, respectively.

Table 4. Concentration of fatty acids (mg.g^{-1} leaves) in *in natura* (NBL) and dehydrated (DBL) beetroot leaves at the different stages of development.

Fatty Acids	NBL			DBL		
	60 d	80 d	100 d	60 d	80 d	100 d
15:0	0.08^{a}±0.00	0.06^{a}±0.00	0.04^{a}±0.01	0.35^{b}±0.02	0.46^{c}±0.07	0.49^{c}±0.03
15:1n-9	0.25^{a}±0.03	0.21^{a}±0.00	0.15^{a}±0.00	1.21^{b}±0.07	1.52^{c}±0.23	1.64^{c}±0.14
16:0	0.80^{a}±0.10	0.72^{a}±0.01	0.64^{a}±0.00	7.75^{b}±0.62	9.53^{c}±1.54	11.26^{d}±0.76
16:1n-7	0.06^{a}±0.01	0.06^{a}±0.00	0.06^{a}±0.00	0.54^{b}±0.15	0.71^{bc}±0.13	0.82^{c}±0.13
18:0	0.08^{a}±0.01	0.05^{a}±0.01	0.12^{a}±0.01	0.46^{b}±0.04	1.12^{c}±0.18	2.15^{d}±0.16
18:1n-9	0.09^{a}±0.01	0.04^{a}±0.01	0.03^{a}±0.01	0.44^{b}±0.02	0.41^{b}±0.07	0.29^{b}±0.19
18:1n-7	2.04^{a}±0.12	0.18^{b}±0.01	0.14^{b}±0.01	3.04^{c}±0.11	1.88^{a}±0.31	1.98^{a}±0.24
18:2n-6	0.68^{a}±0.08	0.57^{a}±0.01	0.57^{a}±0.03	4.36^{b}±0.16	4.82^{b}±0.78	8.32^{c}±0.77
18:3n-3	1.78^{a}±0.23	1.76^{a}±0.06	2.58^{a}±0.15	18.80^{b}±0.67	27.94^{c}±3.71	40.11^{d}±4.29
Sums and ratios						
SFA*	0.96^{a}±0.10	0.83^{b}±0.01	0.80^{b}±0.01	8.56^{c}±0.62	11.11^{c}±1.91	13.90^{c}±0.78
MUFA†	2.44^{a}±0.12	0.49^{b}±0.01	0.38^{c}±0.01	5.23^{d}±0.20	4.52^{e}±0.41	4.73^{de}±0.36
ΣPUFA§	2.46^{a}±1.78	2.33^{a}±0.06	3.14^{a}±0.15	23.16^{b}±0.69	32.76^{c}±3.79	48.43^{d}±4.36
n-6/n-3	0.38^{a}	0.32^{b}	0.22^{c}	0.23^{d}	0.17^{e}	0.21^{d}
PUFA/SFA	2.56^{a}	2.81^{b}	3.92^{c}	2.71^{b}	2.95^{b}	3.48^{d}

Values are mean ± standard deviation of triplicate analyses. NBL = *in natura* beetroot leaves; DBL = dehydrated beetroot leaves. *SFA: Saturated fatty acid; †MUFA: Monounsaturated fatty acid; §PUFA: Polyunsaturated fatty acid. Different letters in the same line indicate significant differences ($P < 0.05$) by the Tukey test.

Identifying the fatty acids present in beetroot leaves, especially those of ω-6 and ω-3 families, is very important because these acids occur in larger amounts in dark green leaves than in the leaves of other vegetables due to the lipids contained in the chloroplasts (Martin et al., 2006).

LNA was the fatty acid found in the highest amount in the beetroot leaves at all the stages of development studied. This fatty acid is an important component of chloroplast membrane lipids (Simopoulos, 2002). During the vegetal development, the highest concentration of LNA was found in the 100-day leaves, with 2.58±0.15 mg.g^{-1} in NBL and 40.11±4.29 mg.g^{-1} in DBL.

The n-6/n-3 ratio has changed in the last years. The current estimates for western societies suggest a n-6/n-3 ratio of 10-20:1 instead of 1:1 (Simopoulos, 2011); thus, since it is necessary to reduce this ratio, beetroot leaves rich in n-3 can be included in the western diets. The n-6/n-3 ratios found in the present study varied from 0.17 to 0.38; therefore beetroot leaves can help maintain a healthy diet. Although the total lipid contents are low, the consumption of these leaves increase the intake of essential fatty acids such as LNA (ω-3).

The fatty acid founds in the beetroot leaves can be compared to those of carrot leaves (Leite et al., 2011) since both have large amounts of LNA; however, beetroot leaves have a 4.7 times higher content of these acids; 100-day dehydrated carrot leaves had 856.55 mg.100g^{-1} leaves of and dehydrated beetroot leaves had 4,011.02 mg.100g^{-1}. Beetroot leaves showed low n-6/n-3 ratio, on average of 0.48 of carrot leaves (Leite et al., 2011) and 0.20 of beetroot leaves. Analyzing the PUFA/SFA ratio, it was verified that the values obtained for all samples were higher than the minimum value recommended, 0.45 (Her Majesty's Stationery Office, 1994).

In previous studies, dehydrated carrot leaves that had significant amounts of essential fatty acids such as ω-3 were used in the formulation of pasta (Boroski et al., 2011), and therefore, this enriched formulation had high levels of LNA and low n6/n-3 ratio.

Therefore, beetroot leaves can also be used in food formulations to improve its LNA levels.

3.3 Antioxidant activity by DPPH radical

Antioxidants have been largely studied in the food and agriculture fields, and the DPPH assay has been widely used since it is a simple and highly sensitive method (Moon & Shibamoto, 2009). Beetroots have been studied for their content of betalains, compounds that have antioxidant properties (Wootton-Beard & Ryan, 2011); however, beetroot leaves can also have antioxidant properties.

NBL had better antioxidant activity in the DPPH assay than DBL (Figure 1). A smaller amount of extract was necessary to inhibit 50% of the free radical (IC_{50}), 89.52 and 125.24 μg.mL^{-1} in NBL at 60 and 80 days, respectively, since the presence of water increases permeability of cell tissue, and thus it enables better mass transfer by molecular diffusion and the recovery of water soluble bioactive compounds (Jayaprakasha et al., 2001; Cheng et al., 2012). The DPPH results obtained are similar to those of organic and conventional vegetables (Arbos et al., 2010).

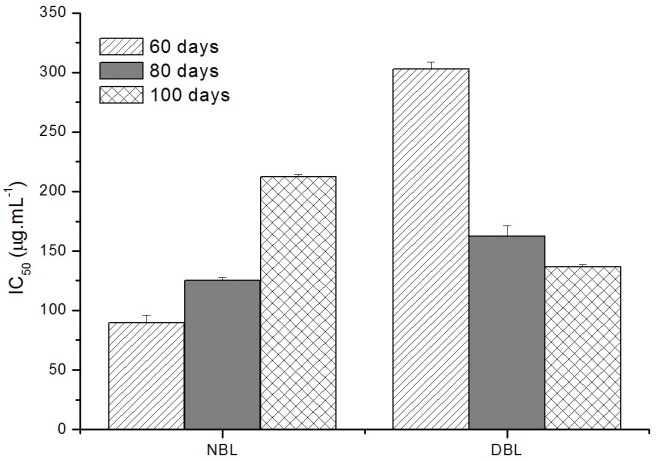

Figure 1. IC_{50} values (concentration of methanolic extract (μg.mL^{-1})) necessary to inhibit 50% of the DPPH radical for NBL and DBL harvested at different stages of development.

Assays of antioxidant activity by DPPH in leaves (IC_{50}, μg.ml^{-1}) are available in the literature, but there is no standardization in terms of the extraction of compounds responsible for such antioxidant activity; nonetheless, there are studies on leaves and plants using different extraction solvents such as water (Tung et al., 2009), ethanol (Argoti et al., 2011) and acetone:water (Liu et al., 2007).

3.4 Total Phenolic Compounds (TPC)

Different amounts of phenolic compounds and levels of antioxidant activity were observed for the different stages of development. According to Bulbovas et al. (2005), these variations are due to seasonal factors. Extreme environmental factors led to an increase in the concentration of reactive oxygen species, and consequently, in the antioxidant activity.

In plants, compounds or classes of phenolic compounds such as phenolic acids are responsible for antioxidant activity (Angelo & Jorge, 2007; Lako, 2007) and are present in vegetable leaves (Moon & Shibamoto, 2009; Fernandes et al., 2007). According to Maisuthisakula (2008), in some species of plants, the antioxidant activity is correlated with their phenolic compounds, while in others, it is not.

The total phenolic contents in dried beetroot leaves (DBL) was higher than those found in *in natura* beetroot leaves (NBL), when the results were expressed as fresh weight (FW) (Figure 2a) due to the moisture content in this leaves (Table 2); the 100- day DBL showed larger amounts of phenolic compounds. However, when calculating the total phenolic content in dry weight (DW), it was observed that the values were higher in NBL than in DBL (Figure 2b). The phenolic compounds may be responsible for the antioxidant activity; however, in the present study, this was not observed. In other studies on food matrices the relationship between phenolic compounds and antioxidant activity has been observed, as reported by Michiels et al. (2012).

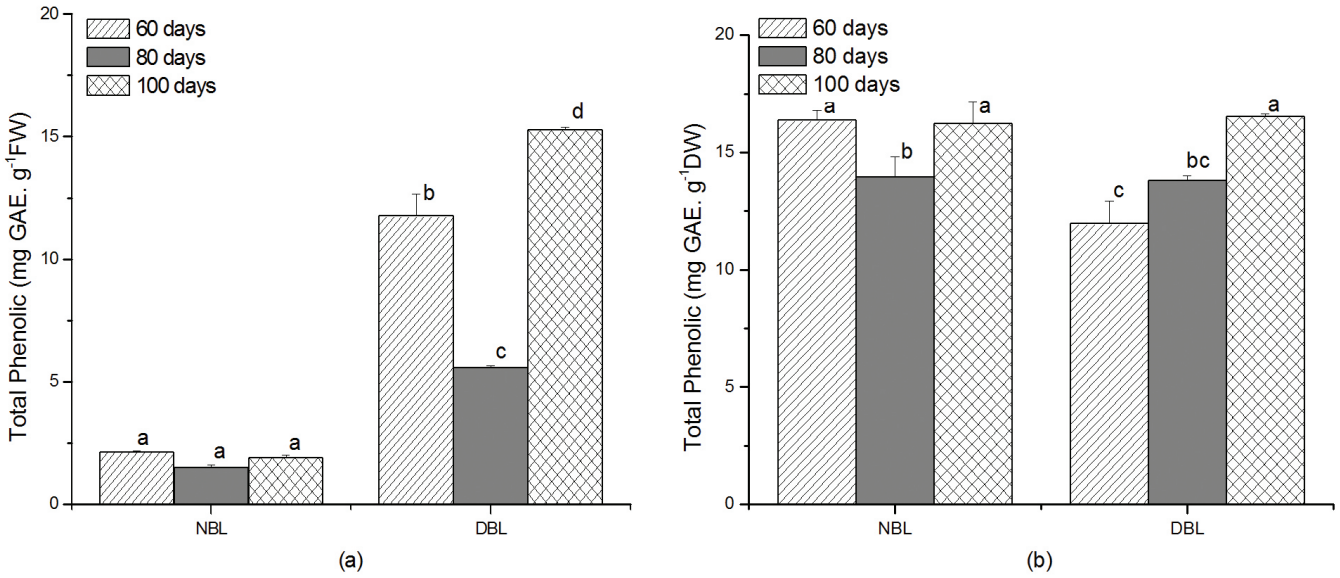

Figure 2. Phenolic compounds expressed as quantity of gallic acid equivalent (mg GAE.g^{-1}) of the methanolic extracts of NBL and DBL as (a) fresh weight (FW) and (b) dry weight (DW). Same superscript letters in the same rectangular bars in the bar chart indicate no significant difference ($p < 0.05$).

4 Conclusion

The present study showed that beetroot leaves are an excellent source of omega-3, in addition to having significant antioxidant activity and amounts of total phenolic compounds, macro- and micro minerals. The chemical constituents in the leaves changed during the development stages, and the greatest amount of omega-3 and 6 and the amount of total phenolic compounds and some minerals were found in the 100-day leaves. Protein and lipids contents were the highest in the 60-day leaves; thus, the leaves can be consumed in more than one stage of development. Therefore, it can be said that *in natura* and dehydrated beetroot leaves can be used in the preparation of broths, meals and/or added to other foods, and that the dehydrated leaves have the greatest nutritional value.

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