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Chemical characterization of leaves of organically grown carrot (Dacus carota L.) in various stages of development for use as food

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Original

Chemical characterization of leaves of organically grown carrot (*Dacus carota* L.) in various stages of development for use as food

Caracterização química de folhas de cenoura (Dacus carota L.) de cultivo orgânico, em diferentes estádios do desenvolvimento, visando seu aproveitamento como alimento

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Abstract

In Brazil, street markets and vegetable distributors discard vegetable leaves and stems, including those of carrot (*Dacus carota L.*). Seeking to reduce the waste of vegetable parts, this study characterized chemically the leaves of organically grown carrot in three stages of development to determine the best time for their removal and consumption as food. The leaves were dehydrated in an oven at 70 °C for 43 hours and analyzed for chemical composition, antioxidant activity, chlorophyll content, fatty acid composition, and also calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), manganese (Mn), iron (Fe), zinc (Zn), and copper (Cu) contents. The analyses indicated 100 days of development as the ideal stage for the removal and consumption of carrot leaves with good antioxidant activity requiring only 63.78 ± 0.5 mg.L^{-1} methanol leaf extract to inhibit 50% of the concentration of the free radical DPPH (2,2-diphenyl-1picrilidrazil), and total protein and alpha-linolenic acid (18:3 n-3/LNA) contents of 18.23% ± 2.8 and 876.55 ± 20.62 mg.100 g^{-1} of dry matter, respectively.

Keywords: *carrot leaves; development; antioxidant activity.*

Resumo

No Brasil, nas feiras livres, mercados e distribuidores de vegetais, são cortadas e descartadas as folhas e/ou ramas destes alimentos, incluindo as folhas de cenoura (*Dacus carota L.*). Visando diminuir este desperdício, realizou-se o presente trabalho, que teve como objetivo caracterizar quimicamente as folhas de cenoura de cultivo orgânico em três estádios de desenvolvimento e assim indicar a melhor época para a retirada e consumo na alimentação. As folhas foram desidratadas em estufa a 70 °C por 43 horas e, em seguida, fez-se as análises de composição centesimal, atividade antioxidante, clorofila total, composição em ácidos graxos e dos minerais cálcio (Ca), sódio (Na), potássio (K), magnésio (Mg), manganês (Mn), Ferro (Fe), zinco (Zn) e cobre (Cu). Baseado nas análises realizadas foi indicado o estádio de 100 dias de desenvolvimento como o ideal para a retirada e consumo das folhas de cenoura que apresentou bons resultados de atividade antioxidante, necessitando de apenas 63,78 ± 0,5 mg.L^{-1} de extrato metanólico da folha para inibir 50% da concentração do radical livre DPPH (2,2-difenil-1picrilidrazil), proteína total (18,23% ± 2,8) e de ácido graxo alfa linolênico (18:3n-3/AAL) 876,55 ± 20,62 mg.100 g^{-1} de matéria seca.

Palavras-chave: *folhas de cenoura; desenvolvimento; atividade antioxidante.*

1 Introduction

Brazil is a country with large diversity and production of fruits and vegetables; however, large amount of parts that are currently wasted can be used as an alternative, low-cost source of nutrients to increase the nutritional value of the diet of poor people. Nevertheless, due to the lack of knowledge and/or appropriate processing technology, parts of various plants, such as roots, fruit, and leaves are discarded by consumers and the food industry (SOUZA, 2006).

Carrot is a major vegetable crop in Brazil and has a high economic value for the country. The 2008/09 crop of the State of Paraná was approximately 5,000 ha, after São Paulo and Minas Gerais, which cultivated more than 12,000 and 9,000 ha, respectively. The average production of carrot is around 230,000 tonnes per year (STEINER; ECHER; LEITE, 2009).

Carrot leaves, for example, can be added to soups, broths, etc. In addition to various nutrients, they have omega-3 and omega-6 fatty acids, which are scarce in nature (ALMEIDA et al., 2009). Omega-3 (alpha-linolenic acid - LNA - 18:3n-3) and omega-6 (linoleic acid - AL - 18:2n-6) are considered essential but cannot be synthesized by mammals, and therefore must be obtained through diet. It is necessary to search for alternative and complementary sources of omega-3 polyunsaturated fatty acids to change the ratio of the sum of polyunsaturated fatty acids omega-6/omega-3 (n-6/n-3) in diet. It must be kept low to avoid the incident of malnutrition-related diseases (SIMOPOULOS, 2002b).

Antioxidants are used to preserve and extend the shelf life of food by retarding lipid oxidation reactions that directly affect the quality of food. In the body, antioxidants are responsible for inhibiting the oxidation of proteins, lipids, and other oxidizable substrates, thus slowing aging and the onset of diseases caused by free radicals formed during metabolism. Free radicals

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damage cellular structures and consequently may affect the immune functions (HALLIWELL, 1995). Cell membranes are composed of saturated and unsaturated fatty acids. The attack of the very susceptible unsaturated chains by free radicals affects the integrity of the cells.

The content of Chlorophyll, the pigment found in plants in general, can be correlated with the potential photosynthetic activity, proteins, nutrients, and micronutrients available in different stages of development of leaves (RAJCAN; DWYE; TOLLENAAR, 1999).

In view of the nutritional potential of vegetable leaves, especially of organically grown carrot, this study aims to evaluate the mineral content, antioxidant activity, chlorophyll content, and fatty acid composition, especially the omega-3 content, and investigate a possible correlation between these parameters and three different leaf development stages and thus indicate the best time for the removal of the leaves for consumption and obtain the best nutritional properties.

2 Material and methods

Samples of leaves of organically grown carrot were provided by a farm certified by the Biodynamic Institute of Paraná, Maringá, Brazil. The leaves were collected at three different stages of development, at 40, 80, and 100 days after sowing. Carrots are harvested on day 100 for commercialization.

The leaves were selected by visual appearance and integrity. Before removal from the root, the total chlorophyll content was determined with equipment Minolta SPAD-502 (Soil Plant Analysis Development) (ZOTARELLI et al., 2003).

After removal, the leaves were washed, dehydrated according to Almeida et al. (2009), and analyzed in triplicate for moisture, ash, minerals, and total protein following the AOAC (ASSOCIATION OF ANALYTICAL CHEMISTRY, 1998) methods. The extraction and determination of total lipids followed the methodology of Bligh and Dyer (1959). The fatty acid total lipids were obtained according to the method of Hartman and Lago (1973) modified by Maia and Rodrigues (1993) for subsequent chromatographic analysis. Fatty acid esters were then separated in fused silica capillary column CP 7420 100% cyanopropyl (100 m long, 0.25 mm internal diameter, 0.25 mm stationary phase) installed in a Varian gas chromatograph model CP3380 equipped with a flame ionization detector. Fatty acids in the samples were determined by comparison of peak retention times with those of Sigma (USA) standards.

Fatty acids were quantified in mg.100 g^{-1} of sample using the internal standardization method with tricosanoate methyl ester ($C_{23:0}$) (Sigma, USA) as the internal standard. The calculations were made using the method of Joseph and Ackman (1992), Equation 1:

$$M_x = A_x \times M_p \times F_{CT} / A_p \times F_{CEA} \times M_A \quad (1)$$

where: M_x = mass of fatty acid x in mg.g^{-1} total lipids; M_p = mass of internal standard in milligrams; M_A = mass of total lipids in grams; A_x = area of fatty acid x; A_p = area of the internal standard; F_{CT} = theoretical correction factor; and F_{CEA} = conversion factor for methyl ester fatty acids.

Methanolic extracts were prepared using 10 g of sample in 100 mL of methanol under magnetic stirring for 5 hours. After filtration, the extracts were concentrated under reduced pressure at 40 °C before the determination of the antioxidant activity.

The antioxidant activity of the leaf extracts was assessed through their free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical based on the method proposed by El-Massry, El-Ghorab and Farouk (2002). The antioxidant activity is the amount of DPPH consumed by a given amount of an antioxidant substance. The concentration of antioxidant needed to inhibit the initial DPPH concentration by 50% is called IC_{50}. The larger the consumption of DPPH by the sample, the lower its IC_{50} and the greater its antioxidant activity. The results were expressed as percent inhibition of the DPPH radical (% inhibition DPPH•), which was calculated with Equation 2.

$$\%\ \text{Inhibition DPPH}^{\bullet} = \frac{\left(\text{Abs}_{\text{DPPH}\bullet} - \text{Abs}_{\text{sample}}\right)}{\text{Abs}_{\text{DPPH}\bullet}} \times 100 \quad (2)$$

where $Abs_{DPPH•}$ is the absorbance of the DPPH• solution without extracts, and Abs_{sample} is the absorbance of the sample solution.

The tests were carried out in triplicate and the extract concentration providing 50% inhibition (IC_{50}) was obtained by plotting the concentrations of the extract solutions versus percent inhibition.

The results were submitted to variance (ANOVA) analysis and Tukey's test (5% probability) using the software Statistica 5.0 (STATSOFT, 1995).

3 Results and discussion

The chlorophyll contents of fresh carrot leaves in three stages of development (40, 80, and 100 days) were determined. The mean values are shown in Table 1.

There was no significant difference between the values for the first two stages of leaf development presented in Table 1. This fact is probably due to seasonal factors, mainly those related to sunlight intensity, since they promote photosynthesis. Therefore, the production of chlorophyll can also inhibit this process by photo-inhibition since the amount of chlorophyll is an indicator of susceptibility to seasonal factors. There was a significant increase in chlorophyll content from the second to the third stages of leaf development.

Table 1. Mean values of chlorophyll of fresh carrot leaves in three stages of development.

Development stage (n. of days)	*AU
40	$22.8^{b} \pm 6.80$
80	$23.3^{b} \pm 6.21$
100	$34.6^{a} \pm 8.02$

*Arbitrary units given by SPAD-502. Mean values ± standard deviation. Different letters in the same column indicate significant differences at 5% by Tukey's test.

The mean proximate moisture, total protein, ash, and total lipids in dehydrated leaves of carrot in the three stages of development are shown in Table 2.

Table 2 shows no significant differences in the total lipid content of the carrot leaves in the three stages of development; however, there was a slight decrease in the total protein content in the third stage. While moisture decreased in dehydrated leaves, the other compositions (ash, total proteins, and total lipids) increased resulting in a better nutritional value.

The antioxidant activity (IC_{50}) of the methanol extracts of the dehydrated carrot leaves in the three stages of development (40, 80, and 100 days) are presented in Table 3.

The values in Table 3 show no significant difference between the two last stages of development, but there was an increase in antioxidant activity towards the third stage. Although the leaves were dehydrated (heating), they presented good antioxidant activity showing that the dehydrated product is a source of natural antioxidants. Importantly, there was no loss of sensory quality due to dehydration, and the product color, odor, and flavor were kept and were very similar to those of fresh leaves.

According to Bulbovas et al. (2005), the low antioxidant activity at 40 days in compared to that at 80 and 100 days is probably due to environmental factors that promote oxidative stress, which in turn induces the production of reactive oxygen species. The same can be observed in Table 3 since the antioxidant activity of the leaves increased with the development time (100 days).

Plants subject to stressful environmental conditions may not show visible signs of injury, but their antioxidant system can be greatly altered by temperature, humidity, UV light irradiation, air pollution, and other factors that are unfavorable to plant development. The antioxidant system is also very important for the lipid oxidation inhibition in plant cells (HASLAM, 1996).

Table 2. Proximate composition of dehydrated leaves of carrot in three different stages of development.

Stages of development (n. of days)	Moisture (%)	Total protein (%)	Ash (%)	Total lipids (%)
40	5.01^{a} ± 0.10	24.04^{a} ± 0.81	13.83^{b} ± 0.11	4.84^{a} ± 0.21
80	5.14^{a} ± 0.41	24.48^{a} ± 0.32	13.86^{b} ± 0.11	5.03^{a} ± 0.81
100	5.73^{a} ± 0.51	18.23^{b} ± 2.80	15.32^{a} ± 0.04	4.75^{a} ± 0.71

Results expressed as % of dry matter. Mean ± standard deviation. Different letters in the same column indicate significant differences at 5% by Tukey's test.

Table 3. Antioxidant activity of dehydrated leaves of carrots in three stages of development.

Stages of development (n. of days)	IC_{50} ($\mu g.mL^{-1}$)
40	73.35^{a} ± 2.01
80	67.78^{b} ± 2.20
100	63.78^{b} ± 0.52

Mean ± standard deviation. Different letters in the same column indicate significant differences at 5% by Tukey's test.

The composition and quantification of fatty acids in dehydrated carrot leaves are shown in Table 4.

Table 4 shows the saturated, unsaturated, and polyunsaturated fatty acids in dehydrated carrot leaves. The saturated fatty acid that had the largest concentration was palmitic acid (16:0), which ranged from 306.01 ± 5.97 to 388.98 ± 8.88 mg.100 g^{-1} leaves in different stages of development. We also found significant amounts of stearic acid (18:0), ranging from 160.04 ± 3.60 to 349.77 ± 9.07 mg.100 g^{-1}, in the different stages of development and little significant amounts of myristic (14:0), behenic (22:0), and lignoceric (24:0) acids. The major monounsaturated fatty acids (MUFA) were palmitoleic acid (16:1 n-7) and oleic acid (18:1 n-9), and the major polyunsaturated fatty acid (PUFA) was alpha-linolenic acid (LNA - 18:3 n-3) with concentrations ranging from 701.91 ± 21.90 to 1162.56 ± 33.34 mg.100 g^{-1} and the polyunsaturated fatty acid (AL - 18:2 n-6), with 339.21 ± 6.07 to 495.19 ± 7.38 mg.100 g^{-1}. The concentrations of these two PUFA were significantly higher in the first stage of development, decreased in the second stage, and increased again in the third stage at 100 days. This means that the antioxidants present in the leaves inhibited the oxidation of fatty acids preserving them and the cellular structures of the leaves over time. The same behavior was observed for the major fatty acids found.

No VLC-PUFA (polyunsaturated fatty acid of very long chain) was found in the carrot leaves; although studies have shown their presence in some species (SIMOPOULOS, 2002a).

According to the Department of Health and Social Security of the United Kingdom (1984), PUFA/SFA ratio values lower

Table 4. Fatty acid composition (mg.100 g^{-1}), sums, and ratios in dehydrated leaves of carrots in three stages of development. Mean values ± standard deviation.

Fatty acid	40 Days	80 Days	100 Days
14:0	36.76^{a} ± 1.13	16.92^{c} ± 1.22	31.54^{b} ± 1.40
15:0	39.38^{a} ± 1.21	19.33^{c} ± 1.05	34.92^{b} ± 1,09
15:1	91.52^{a} ± 1.30	42.93^{c} ± 2.97	77.78^{b} ± 2.76
16:0	388.98^{a} ± 8.88	306.01^{b} ± 5.97	388.93^{a} ± 8.60
16:1n-7	43.95^{a} ± 2.53	30.22^{c} ± 2.13	36.00^{b} ± 1.61
18:0	349.77^{a} ± 9.07	160.04^{c} ± 3.60	221.37^{b} ± 3.31
18:1n-9	35.86^{ab} ± 2.36	32.00^{b} ± 3.78	41.67^{a} ± 1.38
18:1n-7	4.44 ± 0.31	4.62 ± 0.74	4.56 ± 0.55
18:2n-6	483.48^{a} ± 6.66	339.21^{b} ± 6.07	495.19^{a} ± 7.38
18:3n-3	1162.56^{a} ± 33.34	701.91^{c} ± 21.90	876.55^{b} ± 20.62
22:0	12.05^{a} ± 1.32	5.84^{c} ± 0.80	8.66^{b} ± 0.36
24:0	19.95^{a} ± 1.83	8.07^{b} ± 0.71	17.99^{a} ± 0.64
	Sums and ratios		
PUFA	1646.04^{a} ± 40.00	1041.12^{b} ± 27.98	921.74^{c} ± 28.00
SFA	846.89^{a} ± 23.44	517.13^{a} ± 13.35	693.41^{b} ± 15.40
MUFA	175.77^{a} ± 6.5	109.77^{c} ± 9.62	160.01^{b} ± 6.29
∑n-3	1162.56^{a}± 33.34	701.91^{c} ± 21.90	876.35^{b} ± 20.62
∑n-6	483.48^{a} ± 6.66	339.21^{b} ± 6.07	495.19^{a} ± 7.38
n-6/n-3	0.42^{c} ± 0.004	0.48^{b} ± 0.001	0.57^{a} ± 0.005
PUFA/SFA	1.94^{b} ± 0.02	2.01^{a} ± 0.01	1.33^{c} ± 0.01

Means ± standard deviation. Different letters in the same row are significantly different at 5% according to the Tukey's test. Abbreviations: PUFA: polyunsaturated fatty acids, MUFA: monounsaturated fatty acids, SFA: Saturated fatty acids, n-6: omega-6 fatty acids, and n-3: omega 3 fatty acids.

Table 5. Minerals in dehydrated carrot leaves at 100 days of development.

Minerals (mg.100 g^{-1} dry matter)							
Ca	Na	K	Mg	Mn	Fe	Zn	Cu
0.05 ± 0.01	72.04 ± 1.70	467.55 ± 9.30	0.28 ± 0.05	0.10 ± 0.01	ND	ND	ND

Mean ± standard deviation. ND = not detected.

than 0.45 indicate unhealthy food, especially concerning cardiovascular disease. The ratios found for carrot leaves ranged from 1.33 ± 0.01 to 2.01 ± 0.01 at different stages of development.

As shown in Table 4, the PUFA-to-SFA ratio of the carrot leaves is low, ranging from 0.42 ± 0.004 to 0.57 ± 0.005 in the three stages of development studied. Vardavas et al. (2006) studied the n-6/n-3 ratio in 54 different species and only seven had ratios above 1.00. The present study shows that carrot leaves can be used as a dietary source of essential fatty acids. Balanced LA and LNA contents in diet can reduce the risk of coronary heart disease and cancer, while high intake of LA relative to LNA may cause the opposite effect. The 100-day leaves had a higher ash content (inorganic matter), as shown in Table 2. The mineral contents in these leaves in mg.100 g^{-1} of dehydrated carrot leaves are given in Table 5.

According to Pereira et al. (2003), among other minerals, carrot leaves present iron, zinc, and copper; however, these minerals were not detected in this study, as shown in Table 5. Minerals in leaves depend on soil conditions, fertilization, and plant factors.

Based on these results, 100 days was chosen as the best stage of development to remove carrot leaves for human consumption.

4 Conclusions

Carrot leaves are a choice of food with high concentration of essential fatty acids (LA and LNA) and PUFA/SFA and n-6/n-3 ratios appropriate for human consumption.

Based on the results of proximate composition, antioxidant activity, fatty acids, and minerals in dehydrated carrot leaves, it can be said that they can be used for human consumption.

Studies have shown that dehydrated carrot leaves can be added to different dishes, juices, and processed products as an alternative source of antioxidants and nutrients in food.

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