PROVITAMIN A ACTIVITY OF BRAZILIAN CARROTS: LEAVES AND ROOTS, RAW AND COOKED AND THEIR CHEMICAL COMPOSITION[1]

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SUMMARY

The purpose of this study was to determine through carotenoid analysis, the provitamin A value of two carrots (*Daucus carota* L.) cultivars (Brasilia and Beta3), leaves and roots, raw and submitted to two ways of cooking: boiling and microwave. Proximate analysis results are also presented for a better characterization of these vegetables (moisture, ash, lipids, proteins, fiber and total carbohydrates). The main carotenoids sources of provitamin A in this vegetable, both in leaves or roots was β-carotene and α-carotene. α-carotene shows half of the provitamin A value of β-carotene. Samples of the Brasilia cultivar presented the better provitamin A value both for leaves and roots. The results for raw samples of the Brasilia cultivar were 464.48 RE/100g for leaves and 606.42 RE/100g for roots. There were no significant losses of provitamin A with the boiling or microwave cooking methods used.

Key words: carotenoids, provitamin A, carrot leaves, carrots.

RESUMO

ATIVIDADE PROVITAMÍNICA A DE CENOURAS BRASILEIRAS; FOLHAS E RAIZES, CRUAS E COZIDAS E SUA COMPOSIÇÃO QUÍMICA. O objetivo do presente trabalho foi determinar, através da análise de carotenóides, o valor pró-vitamínico A de dois cultivares de cenouras (Brasilia e Beta 3) de folhas e raízes de cenoura (*Daucus carota* L.), cruas e submetidas a dois tipos de cozimentos: fervura e microondas. A composição centesimal é apresentada para uma melhor caracterização dos vegetais (umidade, cinzas, lípides, proteínas, fibras e carboidratos totais). Os principais carotenóides fontes de pró-vitamina A, tanto nas folhas quanto nas raízes, foram o β-caroteno e o α-caroteno. O cultivar Brasilia teve os melhores valores pró-vitamínicos A, tanto para as folhas quanto para as raízes. Os resultados para as amostras cruas de cenoura Brasilia foram 464,48 ER/100g para as folhas cruas e 606,42 ER/100g para as raízes. Não houve perdas significativas no valor pró-vitamínico A com os cozimentos à ebulição e por microondas.

Palavras-chave: carotenóides, pró-vitamina A, folha de cenoura, cenoura.

1 — INTRODUCTION

The purpose of this study was to determine the provitamin A value through carotene analysis of leaves and roots of two cultivars of carrots, raw and submitted to two ways of cooking. The proximate analysis is also presented for a better characterization of these vegetables.

The study of carrot roots as a conventional source of provitamin A and of carrot leaves as an alternative source, will be usefull for a future updating of a Brazilian Food Composition Table. In Brazil there are no complete and updated Tables with the nutrients composition (21).

The carrot leaves are edible (12) and may offer the consumers an option of an inexpensive and easily obtainable source of provitamin A.

Carrot roots (*Daucus carota* L) have a good economic value and nutritive value, and are a source of provitamin A. In Brazil, the main production areas are located in the Southeast and Southern regions. The main producer of carrots is the State of São Paulo, followed by Minas Gerais, Paraná, Rio Grande de Sul, Rio de Janeiro and Santa Catarina (12).

In the present study, two cultivars were used: Brasilia and Beta 3.

Brasilia, which is a cultivar grown nationwide, shows good resistance against burning of leaves caused mainly by *Alternaria dauci* e *Cercospora carotae* (12). Beta 3 cultivar is a result of genetic breeding currently under testing in Brazil by Asgrow do Brasil Sementes Ltda.

In 1991, according to the latest published Annual Bulletin of CEAGESP – Companhia de Entrepostos e Armazéns Gerais de São Paulo (6), the main produce market in São Paulo, 1,554,505 bundles of 2 kg of carrots with leaves were sold in the market.

2 — MATERIAL AND METHODS

2.1 – Material

Samples of roots and leaves of two cultivars of carrots: Brasilia (ADB) and Beta 3 (SM- III) were obtained from Asgrow do Brasil Sementes Ltda. in the raining season of the 1995.

Each lot of approximately 500g of the roots was peeled, cut in small pieces (smaller than 1cm), and mixed. The leaves were also cut into small pieces and mixed. Samples of 5g of roots and leaves were used raw for analysis and from the same batch 5g were separated and submitted to ten minutes boiling and other 5g to microwave cooking (7 minutes at medium potency in a Panasonic Jr. model microwave oven). Fifty milliliters of distilled water were used for both cooking methods.

Analytical grade solvents and reagents were used. Sigma's β-carotene and carrot roots α-carotene were used as standards.

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2.2 – Methods

2.2.1 - Open Column Chromatography

The method used was similar to the one employed by Almeida *et al.* (1,2,3) which was based on the procedure of Rodriguez *et al.* (27) without the saponification step. The method consisted of:

A) Extraction and Isolation of Carotenes

- Extraction with cooled acetone in a Waring Blender followed by extract filtration under suction through fritted glass plate filter.
- Transfer of the extract to light petroleum in a separation funnel and washing by repeated additions of distilled water. The extract was dried over sodium sulfate and concentrated in a rotary evaporator.
- Column chromatography in a 2 x 20 cm glass column using MgO : Hyflosupercel (1 : 2) to a height of about 35cm, packed by pressing down with a rubber stopper, containing approximately 0.3cm of anhydrous Na_2SO_4 on the top. The mobile phase was light petroleum with increasing acetone concentrations. The bands were cut and eluted with acetone and then transferred to light petroleum

B) Quantitation

- Absorption spectra were recorded in a Beckman DU-70 spectrophotometer with an Epson printer.
- Quantification of each carotene was done as recommended by Davies (9) using the maximum absorbances and applying the Beer's law.

C) Confirmation of identity

- thin-layer chromatography was developed using 20 x 20cm plates with 0.25 mm thickness of silicagel 60 G, using a solution of 3% methanol in benzene as mobile phase.
- chemical reactions (9):

1. epoxide tests:
   – the presence of 5,6-epoxide group is detected by the decrease of 20 nm in the absorption spectrum, after adding drops of 0.1 NHCl to an alcoholic solution of the pigment.
   – the chromatography TLC plate, with the pigments adsorbed, was exposed to HCl vapor. The reaction is positive when the yellow spots become blue or green
2. cis-trans isomers were detected by adding an iodine solution in light petroleum to the cuvette immediately after recording the spectrum. After 5 min. of exposure to light, the spectrum was taken again. In the case of all-trans isomers there is an hypsochromic shift (to shorter wavelengths) as well as an increase in the cis peak. In the case of cis isomers, there is normally a bathochromic shift (to higher wavelengths) or no shift.

2.2.2 - High Performance Liquid Chromatography

The High Performance Liquid Chromatography was used to certify the authenticity of the carotenes identified by Open Colmn Chromatography.

The initial steps up to partition to light petroleum were identical to those described earlier for the Open Column Method. The method was similar to one employed by Granado *et al.* (15). An aliquot of the carotenoid solution was filtered in a 0.45(m membrane (Millex HV filter unit from Millipore) and injected into the liquid chromatography. Synthetic trans-β-carotene (Sigma - C-9750) and α-carotene purified from carrot roots were used as external standard. The high performance liquid cromatographic system consisted of a Shimadzu LC-9A pumping system, auto injector, and a photodiode array UV-VIS detector with computer software SPD-M6A and recorder. Chromatography was carried out, at ambient temperature with a C18 reversed phase column (Shimadzu CLC-ODS: 5(m, 4.6mm ID x 25 cm). The detector system was programed to monitor 454 nm for 18 min. The mobile phase used was an isocratic system consisting of a mixture of acetonitrile / dichloromethane / methanol (70 : 20 : 10) with a flow rate of 1.5 mL/min. All solvents used in mobile phase were from Merck HPLC grade and were filtered through a 0.45 (m PVDF membrane (Milllipore) and degassed with Helium Degassing Unit (Shimadzu) for ten minutes. The mobile phase was prepared daily to avoid changing in the retention times. (α- and β-carotene was identified by comparison of its retention time with those of authentic standards, and was calculated by peak area. All standards were measured daily by absorbance spectroscopy. After the injections, the column was washed with the mobile phase.

2.2.3 - Vitamin A evaluation

The quantification of vitamin A value was done based on the provitamin A activity of each carotenoid. As recommended by National Academy of Sciences and National Research Council (25), it was assumed that 6µg of β-carotene, which has 100% of provitamin A activity, corresponded to 10 International Unities (IU) of vitamin A or 1 Retinol Equivalent (RE). For α-carotene it was assumed that 12µg corresponded to 10 IU of vitamin A or 1 RE.

2.2.4 - Proximate analysis

The methods used comply to "Normas Analíticas do Instituto Adolfo Lutz" (1985) and AOAC (1995):

Moisture. The moisture was evaluated by gravimetry using an oven at 105°C.

Ash Content. The ash content was evaluated by gravimetry using an oven at 550°C.

Protein Content. The protein content was determined by using the micro Kjehldal method.

Fat Content. The fat content was determined by Soxhlet method.

Fiber Content. The total fiber was determined using the Total Dietary Fiber Kit from Sigma which uses the enzimatic method of Prosky *et al.* (1985), with some modifications.

Carbohydrate Content. The carbohydrate content was determined by difference.

2.2.5 - Statistical Analysis

The statistical analysis of data was done using Dixon's test for outliers, and analysis of variance using the SPSS for Windows software.

3 —RESULTS AND DISCUSSION

The two carotenes α and β-carotene were identified in both extracts of carrot leaves and roots, prepared as outlined in this paper. Their characteristics are listed in *Table 1* and their structures can be found in Isler (19) numbered 3 for β-carotene and 5 for α-carotene. Data of Rf values in *Table 1* were obtained in order to check the presence of certain functional groups.

TABLE 1. Characteristics of carotenes from carrots leaves and roots (*Daucus carota* L.).

Fraction	Identification	Absorption on petroleum ether (nm)	Rf values on TLC	Chemical reactions
1	alpha-carotene	419 442 471	0,99	trans + epox. –
2	beta-carotene	(425) 447 474	0,99	trans + epox. –

trans + = positive tet for all-trans isomers.
epox. negative test for epoxides.
TLC = Thin layer chromatography.

The main carotene was β-carotene either for carrots leaves or the roots. The quantitative results of carotene analysis obtained by Open Column Chromatography are presented in *Table 2* for carrots leaves and in *Table 3* for carrot roots.

TABLE 2. Concentration of carotenes (μg/g) from carrots leaves (*Daucus carota* L.) cultivars Brasília and Beta 3, raw, boiled and microwave cooked.

Cultivar	Thermal treatment	α-carotene				β-carotene			
		mean	SD	RSD%	n	mean	SD	RSD%	n
Brasília	raw	2.14	1.021	48	13	26.69	11.462	43	12
	boiled	1.58	1.086	69	11	25.95	16.691	64	11
	micro-waved	2.32	1.543	66	11	33.44	7.318	22	10
Beta 3	raw	7.99	5.398	68	12	19.64	8.912	45	12
	boiled	6.21	4.592	74	12	18.65	7.407	40	10
	micro-waved	7.78	4.380	56	11	20.58	7.717	37	11

SD = standard deviation.
RSD% = percent of relative standard deviation.
n = number of determinations.

Both α and β-carotene identified in carrots leaves and roots have provitamin A activity of 50% and 100% respectively (5).

All green tissues of higher plants generally contain the same major carotenoids: β-carotene, lutein, violaxanthin and neoxanthin and α-carotene can appear in small amounts (16). In this work, α-carotene was found in leaves of carrots in a measurable quantity. To confirm α-carotene identity, a standard was prepared from carrot roots, which was purified in a column of MgO : Hyflosupercel (1 : 2). The standard was co-chromatographed with the fraction "α-carotene" of leaves. In the column there was only one band and the absorbance spectrum was identical to the pure standard. Also the cis/trans isomerisation text indicated the isomer all-trans-α-carotene. The leaves extract was submitted to HPLC system to confirm the identification of the two carotenes, using (α- and β-carotene as external standards. The retention times and the absorption spectrum were similar to the standards. The HPLC chromatogram of carrots leaves extract is shown in *Figure 1*.

TABLE 3. Concentration of carotenes (μg/g) from carrots roots (*Daucus carota* L.) cultivars Brasília and Beta 3, raw, boiled and microwave cooked.

Cultivar	Thermal treatment	α-carotene				β-carotene			
		mean	SD	RSD%	n	mean	SD	RSD%	n
Brasília	raw	16.97	7.561	45	5	27.90	7.961	29	5
	boiled	11.76	7.773	66	5	21.92	10.021	46	5
	micro-waved	17.89	2.753	15	4	27.79	7.444	27	5
Beta 3	raw	9.81	1.404	14	3	17.61	5.812	33	4
	boiled	7.99	5.653	71	5	12.27	5.212	42	5
	micro-waved	4.52	1.145	25	3	16.61	5.801	35	4

SD = standard deviation.
RSD% = percent of relative standard deviation.
n = number of determinations.

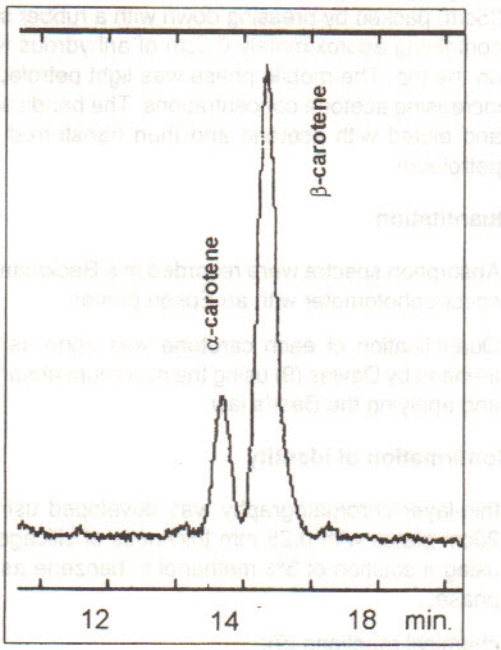

FIGURE 1. HPLC Chromatogram from extract of carrot leaves (*Daucus carota* L.).
HPLC conditions: ambient temperature with a C_{18} reversed phase column (Shimadzu CLC-ODS: 5μm, 4,6mm ID x 25cm). Detection at 454nm for 18 min. Mobile phase: isocratic system of a mixture of acetonitrile/dichloromethane/methanol (70:20:10) with a flow rate of 1.5mL999/min.

To perform the statistical analysis, we first used the Dixons's test to detect outlying values, and then, analysis of variance to test the difference between the two cultivars, the effect of thermal treatment and if there was any interaction between the kind of cultivar and thermal treatment.

There was no significant difference, at the level of 5% of significance, between the cooking treatments, either for α-carotene or for β-carotene, for leaves of carrots. Otherwise, the difference exists between the two cultivars as it can be seen in *Table 2* showing that Brasilia cultivar is richer in

β-carotene than Beta 3. The opposite occurs for α-carotene. For the roots (*Table 3*) there was no significant loss due cooking methods, but the cultivar Brasilia presented more α-carotene and β-carotene compared to Beta 3 cultivar.

Provitamin A value of both cultivars for leaves and roots were calculated using the data of *Table 2* and *3*, as recommended by NAS/NRC (25) and the results are shown in *Table 4*.

TABLE 4. Retinol equivalents (er)/100g for carrots leaves and roots (*Daucus carota* L.).

Cultivar	Thermal treatment	α-carotene				β-carotene			
		mean	SD	RSD%	n	mean	SD	RSD%	n
Brasília	raw	464.48	201.424	43	11	606.42	190.650	31	5
	boiled	445.64	281.267	63	11	463.30	227.226	49	5
	micro-waved	546.91	81.358	15	9	624.92	159.879	26	4
Beta 3	raw	393.85	148.931	38	12	400.67	81.317	20	2
	boiled	371.50	145.715	39	10	271.13	126.600	47	5
	micro-waved	407.88	126.345	31	11	306.03	114.341	37	3

SD = standard deviation.
RSD% = percent of relative standard deviation.
n = number of determinations.

Based on the statistical analysis, cultivar Brasilia is richer in provitamin A than Beta 3 either for leaves or roots (*Table 4*). Also there was no significant difference between the two ways of cooking. These two kinds of cooking were the mildest ways to cook the samples.

The Brazilian Food Composition Tables: ENDEF (14) and FRANCO (13) include the value of 1.100 Retinol Equivalents /100g for raw carrot roots. The Table of FRANCO (13) shows the value of 900 RE/100g for cooked roots. These values are higher than ours, probably because the provitamin A was calculated from total carotenoids content, not for the individuals carotenoids, as we did. From literature, the work which values are closer was the one from Almeida and Penteado (1) in which provitamin A value of Nantes cultivar for raw roots were 746 RE/100g and for the cooked samples 643 RE/100g.

In literature, many authors analyzed carrots carotenoids like LEE (22); BUSHWAY *et al.* (7); NAGRA and KHAN (24); TSAI *et al.* (28); HEINONEN *et al.* (18); HEINONEN (17); GRANADO *et al.* (15); CHEN *et al.* (8) and MANGELS *et al.*, (23). None of then analyzed the same cultivar we used and sometimes they used different methodologies. Regarding carrot leaves TAKAGI (29) in Japan, found the value of 743,5μg/10g for raw leaves and ERSHOW & WONG-CHEN (11) quoted for total carotene 2,00 mg/100g of edible portion. The Brasilian Composition Tables of ENDEF (14), as well as FRANCO (15), did not present the provitamin data of carrot leaves.

The proximate analysis results of carrots leaves are shown in *Table 5, and Table 6* for the roots. The Brazilian Composition Tables of ENDEF (14), as well as FRANCO (15), do not cary proximate analysis results of carrot leaves.

TABLE 5. Proximate analysis of carrot leaves (*Daucus carota* L.) cultivars Brasília and Beta 3, raw and cooked, expressed in % of fresh samples.

Cultivar	Thermal treatment	Moisture		Ash		Lipids		Proteins		Fiber		Carbohydrates
		M	SD	M	SD	M	SD	M	SD	M	SD	M
Brasília	raw	80.13	0.14	2.97	0.08	0.48	0.03	5.10	0.01	8.91	0.07	2.41
	boiled	91.80	0.88	0.58	0.01	0.40	0.10	2.10	0.09	3.60	0.18	1.52
	micro-waved	91.06	1.44	0.90	0.02	0.34	0.001	2.07	0.03	3.95	0.09	1.68
Beta 3	raw	83.1	0.45	2.75	0.07	0.47	0.07	5.13	0.09	6.90	0.52	1.64
	boiled	93.19	0.61	0.49	0.00	0.24	0.00	1.77	0.08	3.19	0.41	1.12
	micro-waved	91.98	1.23	0.64	0.03	0.40	0.01	1.98	0.07	3.36	0.10	1.64

M = mean SD = standard deviation. minimum of five analysis.

TABLE 6. Proximate analysis of carrot leaves (*Daucus carota* L.) cultivars Brasília and Beta 3, raw and cooked, expressed in % of fresh samples.

Cultivar	Thermal treatment	Moisture		Ash		Lipids		Proteins		Fiber		Carbohydrates
		M	SD	M	SD	M	SD	M	SD	M	SD	M
Brasília	raw	89.40	0.15	1.20	0.01	0.32	0.05	1.26	0.04	3.09	0.15	4.73
	boiled	94.80	0.18	0.41	0.07	0.14	0.00	0.51	0.01	2.41	0.11	1.73
	micro-waved	94.10	0.30	0.25	0.01	0.27	0.05	0.41	0.01	2.39	0.06	2.58
Beta 3	raw	90.48	0.59	1.09	0.01	0.16	0.08	1.01	0.09	2.85	0.03	4.41
	boiled	96.29	0.46	0.21	0.01	0.23	0.01	0.37	0.01	1.65	0.04	1.25
	micro-waved	95.19	0.21	0.28	0.00	0.16	0.02	0.36	0.01	2.33	0.06	1.68

M = mean SD = standard deviation. minimum of five analysis.

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