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# Carotenoid composition of cooked green vegetables from restaurants

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**Abstract**

It is generally recognised that databases should be in terms of the food as eaten, but data on cooked foods are lacking. In this work, β-carotene, lutein, violaxanthin and neoxanthin in cooked green vegetables from restaurants were determined by HPLC. In general, there was no statistical difference in the carotenoid concentrations of vegetables taken from different restaurants. This result is surprising at first glance because of the many factors that can cause variation in the raw materials, aside from those brought about by the cooking conditions. However, the interplay of many factors might have masked individual effects. Moreover, taking the analytical sample from a large restaurant batch might have compensated the individual variations, giving more representative average concentrations. Between-lot variations of samples from the same restaurant were sometimes appreciable. Violaxanthin was the carotenoid most affected by cooking.


*Keywords:* Carotenoid; Analysis; Cooked vegetable; Restaurant food

## 1. Introduction

Carotenoids are among the phytochemicals most frequently cited as responsible for the reduction of the risk of developing degenerative diseases such as cancer, cardiovascular disease and macular degeneration. The provitamin A activity of some of these compounds, particularly β-carotene, has long been known. Thus, the need for reliable and more complete databases on the carotenoid concentrations of foods is recognised worldwide. Great efforts have been exerted to refine the analytical methods and insure that accurate data are obtained. However, carotenoid analysis is difficult and some errors persist in the literature. In addition, available data are wanting in two aspects: (1) most are restricted to β-carotene; and (2) most are on raw food. Information on carotenoids other than β-carotene and in terms of the food as eaten should be generated.

In recent years it has also been reported that the carotenoids of cooked/processed foods may have greater bioavailability than those of the raw commodities (Gärtner, Stahl, & Sies, 1997; Rock, Lovalvo, Emenhiser, Ruffin, Flatt, & Schwartz, 1998; Stahl & Sies, 1992). Thus, it is recommended that programs designed to alleviate vitamin A deficiency and/or to promote the health benefits of carotenoids should include means of enhancing bioavailability by cooking or processing (Olson, Parker, Reddy, Rodriguez-Amaya, Smitasiri, & Tsou, 1999).

In this paper, the concentrations of the principal carotenoids of cooked green vegetables from restaurants at or near the University of Campinas are presented. The reason for deciding on restaurant food, instead of simulating home cooking, was three-fold: (1) a good part of the university's population eat at restaurants daily; (2) data on restaurant foods are lacking; (3) restaurant foods permit a wider sampling while still representing typical Brazilian home recipes.

Cooked vegetables would have variations in their carotenoid composition brought about by varying cooking conditions (e.g. time and temperature), but also by compositional differences of the raw material, due to such factors as stage of maturity, cultivar, part of the plant utilised, climatic or seasonal effects, agricultural and post-harvest handling.

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## 2. Material and methods

### 2.1. Samples

The most commonly prepared and consumed cooked vegetables in Brazil were chosen as samples. Five 300-g samples of each vegetable were obtained from each of three restaurants (except for stir-fried samples of broccoli and endive, which were available only in two and one restaurant, respectively) at different times during the year, totalling 75 separate determinations. These cooked samples were prepared by the restaurants from lots of raw vegetables weighing approximately 4–6 kg, the time of cooking being 5–15 min for boiled broccoli, 10–20 min for stir-fried broccoli, 5 min for stir-fried endive, 10 min for stir-fried kale, 10–20 min for boiled green beans and 10–30 min for stir-fried beans. In one restaurant (restaurant A), stir-fried broccoli, kale and green beans were first boiled for 5 min before stir-frying for another 5 min. Analysis was carried out as soon as the sample was collected. Endive and kale were analysed only in the stir-fried form because they are not commonly consumed boiled in Brazil.

### 2.2. Carotenoid determination

The analytical method was based on a procedure established by Kimura and Rodriguez-Amaya (2002) for fresh vegetables, optimised and evaluated for cooked samples in a previous paper (de Sá & Rodriguez-Amaya, in press). Analysis of cooked vegetables has problems somewhat different from those of the fresh samples. Cooking softens the cell walls and makes extraction easier, but incorporation of oil and formation of degradation products during cooking may pose some analytical problems.

Standards were isolated from leafy vegetables by open column chromatography (OCC) and samples were analysed by high-performance liquid chromatography (HPLC). Pre-chromatographic steps involved homogenising samples in a food processor, weighing subsamples (36–38 g for green beans and 2.5–4.0 g for the other vegetables), extracting with cold acetone, partitioning to petroleum ether, saponifying when necessary, concentrating in a rotary evaporator, drying under nitrogen, dissolving in HPLC grade acetone, filtering through a 0.22-μm PTFE filter and injecting immediately into the liquid chromatograph.

Saponification was necessary for stir-fried broccoli, endive and kale, which contained excess oil. This step had been shown to promote losses of carotenoids, especially lutein, violaxanthin and neoxanthin (Khachik, Beecher, & Whitaker, 1986; Kimura, Rodriguez-Amaya, & Godoy, 1990; Riso & Porrini, 1997), principal carotenoids of green vegetables. The magnitude of loss would depend on the concentration of KOH and the conditions during saponification. Thus, an alternative procedure was used in this study, involving removal of most of the oil by solidification through freezing and filtration, before saponification under mild conditions. Acetone extracts of the vegetables were left in a freezer for 2 h to solidify the oil and then filtered inside the freezer through a cold glass sintered funnel. No loss of β-carotene and lutein was observed during this 2 h of freezing in acetone (de Sá & Rodriguez-Amaya, in press). Partition to petroleum ether followed. Overnight saponification with 10% KOH in methanol (volume equal to that of the extract) at room temperature in the dark was then carried out. Butylated hydroxytoluene (0.1%) was added to the petroleum ether extract before saponification to prevent degradation of carotenoids.

Substantial loss of lutein and other xanthophylls could also occur during the washing subsequent to saponification (de Sá & Rodriguez-Amaya, in press). To avoid this loss, the following measures were taken: (a) after saponification, the petroleum ether layer, which was much easier to handle, was separated and washed separately from the methanolic phase; (b) acetone was mixed with the methanolic phase in the separatory funnel before transferring the carotenoids of this phase to additional petroleum ether to which ethyl ether (10%) had been added; (c) the transfer was done gradually, a small volume of the methanolic phase being added each time.

Saponification was also necessary for green bean samples to remove a chlorophyll degradation product that eluted close to β-carotene. Boiled green bean extracts were saponified for one hour to remove the chlorophyll degradation product, while stir-fried green bean extracts were saponified overnight to remove both the chlorophyll degradation product and part of the oil. The residual oil was then removed by keeping the carotenoids after redissolving in acetone in the freezer for 30 min, followed by filtration, before injection.

Identification of the carotenoids was based on the combined use of the retention times, co-chromatography with authentic carotenoids, the visible absorption spectra obtained spectrophometrically and by the photodiode array detector and chemical tests for xanthophylls. Chemical reactions such as acetylation with acetic anhydride of secondary hydroxy groups (as in lutein, violaxanthin and neoxanthin), methylation with acidified methanol of allylic secondary hydroxy groups (as in lutein) and epoxide-furanoid rearrangement of 5,6-epoxy groups (as in violaxanthin and neoxanthin), as described by Rodriguez-Amaya (1999), were carried out on carotenoid isolates obtained by open column chromatography.

### 2.3. HPLC conditions

HPLC conditions previously established for fresh leafy vegetables (Kimura & Rodriguez-Amaya, 2002)

were used. The analysis was carried out with a Waters separation module (model 2690) equipped with an autosampler, controlled by Millenium workstation (version 3.10). A $C_{18}$ monomeric column (Spherisorb S3 ODS2), 3 μm, 4.6×150 mm, was used and the mobile phase was composed of acetonitrile:methanol:ethyl acetate in a concave gradient, from 95:5:0 to 60:20:20 in 20 min, maintaining this proportion until the end of the run. The flow rate was 0.5 ml/min. Triethylamine (0.05%) was added to acetonitrile, as recommended by Hart and Scott (1995) to improve carotenoid recovery from the column. A UV–visible photodiode array detector (Waters model 996) was used, detection being set at the wavelengths of maximum absorption (max. plot).

### 2.4. Statistical analysis

The results were submitted to analysis of variance and Tukey's test.

## 3. Results and discussion

As an example, the HPLC chromatogram of the carotenoids of boiled broccoli is shown in Fig. 1. The concentrations of β-carotene, lutein, violaxanthin and neoxanthin in cooked green vegetables obtained from restaurants are presented in Table 1.

Differences in carotenoid concentrations between restaurants were not found statistically significant in most of the vegetable samples analysed. This result is surprising at first glance because of the many factors that can cause variation in the raw materials, aside from variations brought about by the cooking conditions. However, it is possible that the interplay of so many factors might have masked the individual effects. Between-lot variation of samples from the same restaurant were sometimes appreciable as shown by the standard deviations. Moreover, taking the analytical sample from a large restaurant batch might have compensated the individual variations, giving more representative average concentrations.

Exceptions to the above observation were β-carotene in stir-fried broccoli and violaxanthin in stir-fried kale. In the case of β-carotene in stir-fried broccoli, usually prepared with varying amounts of stalk and small leaves found next to the flowerlets, the difference could be explained by the fact that restaurant E, the samples from which had higher β-carotene content, prepared this vegetable with more leaves than restaurant A. This difference could not be attributed to variation in preparation conditions because samples obtained from restaurant E always appeared more cooked than those from restaurant A, as seen through the texture and colour of the samples, and would have presented lower carotenoid content than those from restaurant A.

Violaxanthin had previously been reported as a very labile carotenoid during heat treatment (Khachik, Goli, Beecher, Holden, Lusby, Tenorio, & Barreira, 1992). The statistically lower concentration of violaxanthin in stir-fried kale samples obtained from restaurant B can be explained by the longer cooking time used in this restaurant.

It is also noteworthy that violaxanthin had lower levels than neoxanthin in all of the samples analysed. In raw green vegetables, violaxanthin usually surpasses neoxanthin. This observation reinforce the great lability of violaxanthin. In mango, violaxanthin, the principal

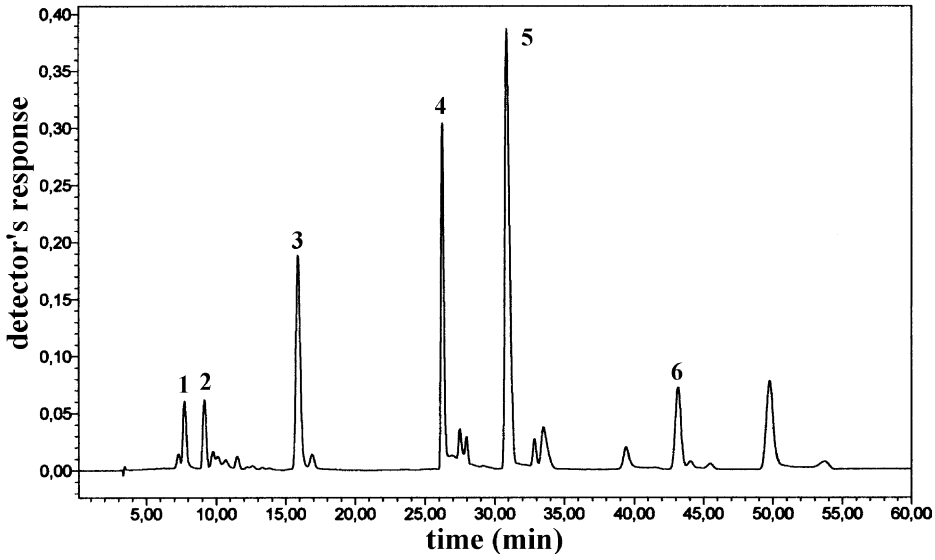


Fig. 1. HPLC chromatogram of the carotenoids of boiled broccoli. Chromatographic conditions are described in the text. Peak identification: 1, neoxanthin, 2, violaxanthin, 3, lutein, 4,5, chlorophylls, 6, *trans*-β-carotene. The other peaks are those of minor carotenoids and degradation products of chlorophylls and carotenoids.

Table 1
Concentrations of principal carotenoids in cooked green vegetables from restaurants[a,b]

| Food | Restaurant | Carotenoid concentration (μg/g) | | | |
|---|---|---|---|---|---|
| | | β-Carotene | Lutein | Violaxanthin | Neoxanthin |
| Broccoli, boiled | A | 18.9±5.7a | 34.6±8.3a | 6.8±1.5a | 8.3±2.7a |
| | B | 22.2±4.2a | 39.6±5.1a | 3.1±4.4a | 7.4±2.8a |
| | E | 15.7±3.5a | 31.1±5.2a | 6.0±2.1a | 6.7±1.9a |
| Broccoli, stir-fried | A | 11.4±3.7b | 27.6±2.7a | 5.0±2.0a | 7.4±1.5a |
| | E | 20.1±6.5a | 37.9±12.9a | 4.1±2.6a | 6.5±1.5a |
| Endive, stir-fried | C | 12.4±3.7 | 23.4±5.0 | 6.8±1.5 | 7.0±2.0 |
| Green bean, boiled | A | 1.3±0.2c | 2.2±0.5a | Trace | Trace |
| | B | 1.6±0.3b | 2.4±0.4a | Trace | Trace |
| | C | 2.0±0.3a | 2.9±0.7a | Trace | Trace |
| Green bean, stir-fried | A | 1.7±0.6a | 2.9±0.9a | Trace | Trace |
| | B | 1.8±0.6a | 2.8±0.5a | Trace | Trace |
| | C | 1.8±0.7a | 2.5±1.0a | Trace | Trace |
| Kale, stir-fried | A | 22.8±4.2a | 35.0±4.5a | 8.8±4.4a | 7.9±2.4a |
| | B | 22.4±4.2a | 28.6±4.9a | 2.8±1.6b | 4.9±2.1a |
| | D | 24.0±2.3a | 31.0±3.7a | 5.3±3.7ab | 6.3±2.7a |

[a] Each value is the mean and standard deviation of five different sample lots.
[b] For each vegetable sample, values in the same column bearing different letters are significantly different ($P < 0.05$).

carotenoid of commercial cultivars in Brazil, was not found in three brands of processed mango juice (Mercadante & Rodriguez-Amaya, 1998). Auroxanthin, a derivative of violaxanthin, was found instead.

Among the samples analysed, stir-fried kale and both boiled and stir-fried broccoli were rich sources of carotenoids. Cooked green beans had much lower carotenoids levels. The results confirm the predominance of lutein in green vegetables, as well as its good stability during cooking. Low levels of lutein are sometimes reported in green vegetables, but this could be due to losses during analysis, particularly in the saponification-washing step. The method used in this study had been shown to preserve lutein during analysis (de Sá & Rodriguez-Amaya, in press).

Zeaxanthin and *cis*-isomers of β-carotene were also detected but were not quantified because they were present at very low levels. There was no apparent increase in the *cis*-isomers during cooking.

Broccoli and green bean were analysed both boiled and stir-fried. Conditions during stir-frying might be more drastic than those of boiling, thus reducing the carotenoid levels. However, because of water loss, stir-frying may concentrate the carotenoids, giving higher carotenoid content per unit weight of vegetable. In boiling, water is absorbed by the food, causing dilution of the carotenoids. Degradation losses appeared to predominate in stir-fried broccoli from restaurant A since all carotenoids had higher concentration in the boiled than in the stir-fried samples. On the other hand, loss of water might have played a greater role in stir-fried broccoli from restaurant E because of its higher β-carotene and lutein concentrations than the boiled broccoli. The levels were so low in boiled and stir-fried green beans that this type of comparison could not be confidently made.

Few data on cooked food is found in the literature, but some can be compared with those obtained in this study (Table 2), although the results of other authors were obtained with samples prepared in the laboratory, simulating home cooking.

The β-carotene content in boiled broccoli was found to be 3.1 μg/g by Lessin, Catigani, and Schwartz (1997), 11.3 μg/g by Hart and Scott (1995) and 14.9 μg/g by Godoy and Rodriguez-Amaya (1998), the latter being a Brazilian data. The value obtained in this study (19.9 μg/g), agrees better with the latter data. The discrepancy with the other data, at least in part, can be due to varietal differences and cooking conditions.

The concentration of β-carotene in cooked endive reported by Sweeney and March (1971) (9.6 μg/g), agrees with the concentration of β-carotene of 12.4 μg/g from this study.

As in the present paper, other authors demonstrated that boiled green bean is not a good source of carotenoids (Granado, Olmedilla, Blanco & Rojas-Hidalgo, 1992; Hart & Scott, 1995; Godoy & Rodriguez-Amaya, 1998), β-carotene concentration varies from 1.0 to 3.2 μg/g and lutein from 4.8 to 5.5 μg/g. An exception is the paper of Cruz-García, González-Castro, Oruña-Concha, López-Hernández, Simal-Lozano, and Simal-Gándara (1997), that reported much higher levels of these carotenoids in boiled green beans, the concentrations being 48.1–65.9 μg/g for β-carotene and 16.1–25.0 μg/g for lutein. This large difference cannot be easily explained by natural variability.

For stir-fried green bean, Godoy and Rodriguez-Amaya (1998) reported a value of 0.8 μg/g for β-carotene,

Table 2
Comparison of the carotenoid content of cooked vegetables with published data

| Vegetable/reference | Mode of preparation | Concentration (μg/g) | | | |
|---|---|---|---|---|---|
| | | β-Carotene | Lutein | Violaxanthin | Neoxanthin |
| ***Broccoli*** | | | | | |
| Hart and Scott (1995) | Boiled | 11.25 | 19.9 | nd[a] | nd |
| Lessin et al. (1997) | Boiled | 3.1 | nd | nd | nd |
| Godoy and Rodriguez-Amaya (1998) | Boiled | 14.9 | nd | nd | nd |
| This study[b] | Boiled | 18.9 | 35.1 | 5.3 | 7.6 |
| | Stir-fried | 15.7 | 32.8 | 4.5 | 6.9 |
| ***Endive*** | | | | | |
| Sweeney and March (1971) | Cooked | 9.6 | nd | nd | nd |
| This study | Stir-fried | 12.4 | 23.4 | 6.8 | 7.0 |
| ***Green bean*** | | | | | |
| Godoy and Rodriguez-Amaya (1998) | Boiled | 1.0 | nd | nd | nd |
| | Stir-fried | 0.8 | nd | nd | nd |
| Cruz-García et al. (1997) | Boiled | 48.1 | 16.1 | nd | nd |
| Granado et al. (1992) | Boiled | 2.4 | 4.8 | nd | nd |
| Hart and Scott (1995) | Boiled | 3.2 | 5.5 | nd | nd |
| This study[b] | Boiled | 1.6 | 2.5 | tr | tr |
| | Stir-fried | 1.8 | 2.7 | tr | tr |
| ***Kale*** | | | | | |
| Khachik et al. (1986) | Cooked | 126 | 235 | 7.7 | 27.6 |
| This study[b] | Stir-fried | 23.0 | 31.5 | 5.6 | 6.4 |

[a] nd, not determined.
[b] Overall means of the samples from the different restaurants.

less than half of the value found in this study (1.8 μg/g). The difference may be due to more drastic conditions in the laboratory-simulated stir-frying and/or use of younger beans in Godoy and Rodriguez-Amaya's work. In any case, the levels are low in both studies.

In the case of cooked kale, the concentration of β-carotene, lutein and neoxanthin of 23.0, 31.5 and 6.4 μg/g, respectively, do not agree with those reported by Khachik et al. (1986): 126 μg/g for β-carotene, 235 μg/g for lutein and 28 μg/g for neoxanthin. The β-carotene content in raw kale in Khachik et al.'s work (146 μg/g), also disagrees with previous Brazilian data for β-carotene in raw kale, 26 μg/g (Godoy & Rodriguez-Amaya, 1998) and 38 μg/g (Mercadante & Rodriguez-Amaya, 1991). This difference appears to be too large to be accounted for by varietal differences alone. The values reported for violaxanthin in the study of Khachik et al. and in the present study agree, being 7.7 and 5.6 μg/g, respectively.

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