Carotenoid Composition of a Leafy Vegetable in Relation to Some Agricultural Variables

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Cultivar differences, seasonal variations, and farming practices on the carotenoid composition of a leafy vegetable (kale) were studied. Although the samples were taken from commercial farms, sampling was planned so that the effect of each factor could be assessed. The carotenoids and the vitamin A value were significantly higher in the cultivar Tronchuda in the summer; the same tendency was observed in the winter, but the differences were not statistically significant. The β-carotene, lutein–violaxanthin, and total carotenoid levels, as well as the vitamin A value, were significantly higher in the winter than in the summer for cv. Manteiga kale. On the other hand, the neoxanthin content was significantly higher in the summer for the Tronchuda cultivar. All the constituent carotenoids were significantly higher in the samples from the "natural" farm as compared to those from the farm that used agrochemicals.

INTRODUCTION

Carotenoids accumulate in the chloroplasts of all green leaves, and the many plants analyzed, without exception, contain the same major carotenoids: β-carotene, lutein, violaxanthin, and neoxanthin (Goodwin and Britton, 1988). α-Carotene, β-cryptoxanthin, zeaxanthin, antheraxanthin, and lutein 5,6-epoxide are frequently present as minor constituents. In Brazilian leafy vegetables, α-cryptoxanthin was found instead of β-cryptoxanthin (Ramos and Rodriguez-Amaya, 1987; Mercadante and Rodriguez-Amaya, 1990). Although the relative ratios may also be similar, leaves vary markedly in their absolute quantitative composition.

Most of the studies on leafy vegetables have been performed to assess the vitamin A value. Thus, only β-carotene, occasionally also α-carotene, is quantified. The β-carotene contents, and consequently the vitamin A values, obtained from the analyses of more than one purchase or sample lot clearly show appreciable quantitative variation between samples of the same vegetable (Begum and Pereira, 1977; Bureau and Bushway, 1986; Ramos and Rodriguez-Amaya, 1987; Pepping et al., 1988; Heinonen et al., 1989). Cultivar differences, seasonal changes, stage of maturity, storage time and condition after harvest, etc. are held responsible for such lot-to-lot variations, but a comprehensive search of the literature shows that quantitative results to prove this assumption are lacking.

Bureau and Bushway (1986) determined the α-carotene, β-carotene, and β-cryptoxanthin levels of fruits and vegetables, including three leafy vegetables, obtained from five cities in the United States three times during a year. Although considerable between-lot variations could be noted, analysis of variance over all the foods was found not to reveal significant differences among either location or month of analysis. Klein and Perry (1982) reported varying vitamin A values for some vegetables, including cabbage, from six U.S. cities, but only one sample lot was analyzed for each geographic location. β-Carotene was found to vary appreciably in samples of 10 leafy vegetables collected at different times during the year from the same Brazilian city (Ramos and Rodriguez-Amaya, 1987).

Studies on the effect of herbicides and fungicides, undertaken in experimental plots, at first glance appear to be conflicting. A decrease (Sweeney and Marsh, 1971; Giannopolitis et al., 1989), an increase (Rouchaud et al., 1984), or no change (Sweeney and Marsh, 1971) in the carotenoid content had been claimed. Actually it apparently depends on the type of compound used, climate, and vegetable cultivar. It should also be noted that the herbicides used in the studies cited are not among those listed or cited as inhibitors of carotenoid biosynthesis (Fedtke, 1982a,b; Britton et al., 1989).

The present paper had a threefold objective: (1) verify compositional differences between the two kale cultivars commercialized in Brazil; (2) investigate seasonal changes of the two cultivars; and (3) verify possible differences in the composition of the same cultivar grown on a "natural" farm and on a farm that utilized agrochemicals. Instead of limiting the study to β-carotene only, the carotenoid composition, including carotenoids which would not be precursors of vitamin A, was determined in view of recent findings that attribute to these compounds physiological functions or actions other than that of provitamin A activity (Mathews-Roth, 1985; Olson, 1989).

MATERIALS AND METHODS

Materials. Two cultivars of kale (*Brassica oleracea* var. acephala) were analyzed: Manteiga and Tronchuda Portuguesa. Both cultivars have large and broad leaves, although the Tronchuda leaves are larger. The Manteiga leaves are green and smooth, while those of Tronchuda are bluish-green and ruffled at the margins.

Ten sample lots were analyzed individually for each cultivar in the summer (December–January) and in the winter (July), all samples being collected from the same farm which did not use agrochemicals (natural farm). For comparison, 10 samples of the cultivar Manteiga were harvested from a neighboring farm (thus subject to the same climatic condition) that utilized the herbicide glyphosate, the insecticide ethyl parathion, and a leaf fertilizer (containing nitrogen, phosphorus, and potassium). The experiment (from sample collections to analyses) lasted 3 weeks in the summer and 2 weeks in the winter. For each sample collection, an equal number of sample lots was taken for each variable.

For each sample, mature leaves were hand-picked at random from the farm (about 250 g) and taken to the laboratory for immediate analysis so that storage and handling effects were eliminated. The leaves were finely cut and mixed, and 4–5 g was taken for analysis.

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Table I. Variation of the Carotenoid Concentration (μg/g) and Vitamin A Value (RE/100 g) of Kale in Relation to Cultivar, Season, and Type of Farm[a]

	winter		summer		
carotenoid/vitamin A value	cv. Manteiga, natural farm	cv. Tronchuda, natural farm	cv. Manteiga, natural farm	cv. Tronchuda, natural farm	cv. Manteiga, farm using agrochemicals
β-carotene	54 ± 5	60 ± 14	44 ± 3	57 ± 8	38 ± 7
lutein + violaxanthin	111 ± 16	114 ± 10	84 ± 9	109 ± 10	71 ± 8
zeaxanthin	3 ± 2	2 ± 1	2 ± 1	2 ± 1	1 ± 1
neoxanthin	18 ± 7	19 ± 4	20 ± 3	26 ± 3	17 ± 2
total	187 ± 21	195 ± 24	149 ± 10	194 ± 19	127 ± 14
vitamin A value	906 ± 74	993 ± 229	731 ± 44	942 ± 128	627 ± 111

[a] Each value is the mean and standard deviation of 10 sample lots analyzed individually.

Table II. Statistical *P* Values (from ANOVA) for Comparison among Cultivar, Season, and Type of Farm

	cultivar comparison		seasonal comparison		
carotenoid/vitamin A value	winter	summer	cv. Manteiga	cv. Tronchuda	type of farm
β-carotene	0.2464	0.0001	0.0001	0.5494	0.0204
lutein + violaxanthin	0.5947	0.0001	0.0002	0.3010	0.0050
neoxanthin	0.6619	0.0001	0.5650	0.0001	0.0150
total	0.4362	0.0001	0.0001	0.9093	0.0007
vitamin A value	0.2680	0.0001	0.0001	0.5499	0.0204

The Brazilian kale was chosen among the leafy vegetables because it is available during both seasons and it does not form heads, making it easier to collect individual leaves of uniform maturity. Stage of maturity had already been shown to affect the carotenoid content of leaves appreciably (Ramos and Rodriguez-Amaya, 1987).

Carotenoid Determination. The analytical method was based on a previously reported procedure (Rodriguez et al., 1976) with some modifications to simplify and adapt it to the sample being analyzed. Briefly, it involved immersion of the sample in acetone for 10 min in the refrigerator, extraction with cold acetone in a Waring blender, filtration in a Büchner funnel (extraction and filtration being repeated until the residue became colorless), transfer to petroleum ether in a separatory funnel with the addition of water, washing, drying over anhydrous sodium sulfate, concentration in a rotary evaporator ($T < 35$ °C), and separation on a MgO/Hyflosupercel (1:2) column. Elution was accomplished with increasing concentrations of acetone in petroleum ether, β-carotene being eluted with 10–12%, lutein plus violaxanthin with 20–24%, zeaxanthin with 26%, and neoxanthin with 32% acetone in petroleum ether. The collected fractions were washed free of acetone, the visible absorption spectra were taken with a recording spectrophotometer, and the concentrations were calculated according to the method of Davies (1976). Confirmation of the identity of the carotenoids was based on the visible absorption spectra, chromatographic behavior on column and TLC, and specific group chemical reactions as described in detail previously (Mercadante and Rodriguez-Amaya, 1990). α-Carotene was not detected, and α-cryptoxanthin was found only in trace amounts. The efficiency of the extraction procedure and separation on the MgO/Hyflosupercel column had been demonstrated previously (Rodriguez-Amaya et al., 1988; Mercadante and Rodriguez-Amaya, 1989). The vitamin A values were calculated according to a NAS–NRC (1989) method.

Saponification was not found necessary because the carotenoids of the unsaponified leafy vegetable were well separated on the column from the chlorophylls and from each other (the xanthophylls of the leaves being unesterified), except for lutein and violaxanthin, which eluted together and were thus quantified together. With its deletion, two time-consuming steps (saponification and the subsequent washing) were eliminated and quantitative losses of the carotenoids were avoided. Although β-carotene resists mild saponification, the xanthophylls lutein, violaxanthin, and neoxanthin are prone to degradation during this step (Khachik et al., 1986; Kimura et al., 1990). Lutein and violaxanthin could have been separated by rechromatography on an alumina column; however, the analysis time would have been prolonged and fewer sample lots would have been analyzed.

Statistical Analysis. Analyses of variance were performed, and the least significant difference in each set of data was determined by using the statistical analysis system (SAS–ANOVA) (SAS, 1987).

RESULTS AND DISCUSSION

Cultivar Differences. Statistical analysis of the results (columns 1 and 2, 3 and 4, Table I) showed that highly significant differences existed between the cultivars Manteiga and Tronchuda in the summer. β-Carotene, consequently the vitamin A value, lutein–violaxanthin, neoxanthin, and total carotenoid contents were significantly higher ($P < 0.0001$) in the Tronchuda cultivar. The same tendency was observed in the winter, but the differences were statistically insignificant. β-Carotene, vitamin A value, lutein–violanthin, neoxanthin, and total carotenoid content were higher in the Tronchuda kale at $P = 0.25$, 0.27, 0.60, and 0.44, respectively.

No other study comparing the carotenoid composition of different cultivars of the same leaf was found in the literature. Qualitative and quantitative differences among cultivars or varieties have been reported for many fruits such as oranges (Gross et al., 1972; Stewart, 1977), Japanese persimmons (Brossard and Mackinney, 1963), mangoes (Godoy and Rodriguez-Amaya, 1989), papaya (Kimura and Rodriguez-Amaya, 1987), and squashes and pumpkins (Arima and Rodriguez-Amaya, 1988) and for root crops such as carrot (Heinonen, 1990).

Seasonal Variation. As far as seasonal effects are concerned (columns 1 and 3, 2 and 4, Table I), the same trend was seen in the two cultivars: the β-carotene, vitamin A value, lutein–violaxanthin, and total carotenoid levels were higher in the winter, and neoxanthin was higher in the summer. However, the differences in β-carotene, vitamin A value, total carotenoid content, and lutein–violaxanthin were highly significant ($P < 0.0001$ for the first three and $P < 0.0002$ for the last case) only in the cultivar Manteiga. The corresponding differences in the cultivar Tronchuda were insignificant, P being equal to 0.55, 0.55, 0.91 and 0.30, respectively. With neoxanthin, on the other hand, the difference was highly significant ($P < 0.0001$) only in the Tronchuda kale, the difference in the other cultivar being at $P = 0.57$. The results appear compatible with greater destruction of leaf carotenoids on exposure to higher temperature and/or greater sunlight (Young and Britton, 1990) in the summer.

On the basis of lettuce purchased from retail food stores five times during 1 year and analyzed as pooled samples, Heinonen et al. (1989) also reported that the β-carotene and lutein levels were at their lowest in the summer. However, the samples had different origin (domestic in the summer and imported in the winter), no information was given as to the cultivar, storage time and condition after harvest, or stage of maturity, and the results were not statistically analyzed.

The carotenoid composition of fruits also appears to be subject to climatic conditions, but the effect seems contrary to that noted in leaves. In red-fleshed guavas, samples from the northeastern part of Brazil, where the climate is hot, all showed higher β-carotene levels than those from the state of São Paulo, where the climate is moderate (Padula and Rodriguez-Amaya, 1986). Papaya of the cultivar Formosa from the hot state of Bahia consistently presented higher concentrations of carotenoids, especially β-carotene, than those from São Paulo (Kimura and Rodriguez-Amaya, 1987). It is possible that the effect of sunlight on the carotenoids of fruits, being nonphotosynthetic tissues, is enhancement of the carotenoid synthesis (McCollum, 1954; Watada, 1976).

Type of Farm. All the constituent carotenoids were significantly higher ($P < 0.02$ for β-carotene and the vitamin A value; $P < 0.05$ for lutein–violaxanthin; $P < 0.015$ for neoxanthin; $P < 0.007$ for the total carotenoid content) in the samples of the kale Manteiga harvested from the natural farm as compared to those obtained from a neighboring farm that utilized agrochemicals, all samples being collected in the summer (columns 3 and 5, Table I). Considering the chemicals employed by the farm, this effect was probably due to residual herbicide (glyphosate) in the soil. Although inhibition of the shikimate pathway or aromatic amino acid biosynthesis is considered to be its primary molecular mode of action (Fedtke, 1982a,b; Coggin, 1990), glyphosate has been shown to cause significant reduction of the carotenoid content (Abu-Ismaileh and Jordan, 1978).

Interestingly, the present findings seem to explain the variation in kale (cultivar Manteiga) in a previous paper (Ramos and Rodriguez-Amaya, 1989). The β-carotene content of 10 sample lots purchased from different markets and groceries in Campinas at different times during the year varied from 23.6 to 59.0 μg/g, coinciding with the overall range of 23.2–61.7 μg/g (30 samples) for the kale Manteiga obtained in the present study. The previously reported mean of 35.1 μg/g is, however, much lower. When the individual data were considered, most of the previous results fell close to the values presently obtained for samples from the farm using agrochemicals (range 23.2–43.8 μg/g) with a few resembling those of the samples from the natural farm (range 40.7–61.7 μg/g). Thus, the earlier data appear to be truly representative since most of the farms in Campinas use agrochemicals with a few practicing natural farming.

The absence of significant differences in Bureau and Bushway's (1986) work may be due to the simultaneous interplay of several factors (cultivar, geographic location, season, stage of maturity, storage time and handling conditions), thus masking individual effects. The samples in our study were taken from commercial farms, to reflect what happens in practice, but sampling was planned so that the influence of each factor could be assessed.

The zeaxanthin content was also determined, and, contrary to the other carotenoids, it appeared to be higher in the Manteiga cultivar both in the summer and in the winter. Like the other carotenoids, its mean concentration was lower in the samples from the farm that used agrochemicals. Since it occurred at very low levels, the variation within each set of data being comparatively much greater, it was deemed meaningless to submit the data to statistical analysis.

Considering specifically β-carotene, which accounts almost exclusively for the vitamin A values of leafy vegetables, the results have practical implications. The study indicated that to alleviate vitamin A deficiency, which is still a serious problem in many developing countries, it is possible to choose provitamin A rich varieties not only of fruits or root crops but also of leafy vegetables. In the kale studied, the choice of cultivar is especially important in the summer when the difference is considerable, Tronchuda being the preferred cultivar. If the kale Manteiga, which is the most commercially grown cultivar in Brazil, is planted, the β-carotene content will be significantly higher in the winter. The application of agrochemicals could also be better controlled so that the carotenoid content of the cultivated plant would not be affected. The total carotenoid content followed the same trend as β-carotene; thus, the same practical considerations could be made in relation to the other physiological actions of carotenoids.

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Registry No. Vitamin A, 62624-08-2; β-carotene, 7235-40-7; lutein, 127-40-2; violaxanthin, 126-29-4; zeaxanthin, 144-68-3; neoxanthin, 14660-91-4.