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Carotenoid Analyses of Selected Raw and Cooked Foods Associated With a Lower Risk for Cancer

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We examined the carotenoid content of selected foods consistently found to be associated with a lower risk for various epithelial cancers in epidemiologic studies. Both raw and cooked samples of green, leafy vegetables and yellow or orange vegetables were quantitatively examined by high-performance liquid chromatography for individual carotenoid content. The results indicated that fresh, green, leafy vegetables were moderately high in beta carotene (0.5-14.6 mg/100 g) and very high in oxygenated carotenoids or xanthophylls, primarily lutein and its stereoisomers (2.3-63.0 g/100 g). The fresh, yellow or orange vegetables examined were very high in beta carotene (16.0-120.5 g/100 g) but had no detectable nonhydrocarbon carotenoids. Cooking differentially reduced the lutein content compared with the beta carotene content in green, leafy vegetables. These analyses suggest that consumption of carotenoids in addition to beta carotene may be associated with a lower risk for cancer. [J Natl Cancer Inst 82:282–285, 1990]

Epidemiologic studies on diet and cancer have found that the consumption of certain foods or classes of foods (e.g., green, leafy vegetables) is associated with a reduced risk of various epithelial cancers in humans (*1-23*). Most of the foods identified in these studies were considered to have high vitamin A activity (*3*). Some investigations have distinguished between intake of foods from animal sources containing preformed vitamin A and intake of foods from plant sources high in provitamin A activity (beta carotene) (*4*). Some studies have also attempted to compare the relative effects of raw vegetables with those of cooked vegetables (*5-8*). Recognition of the total carotenoid content of various foods associated with a lower risk of cancer has led to further clinical investigations, specifically focused on beta carotene (*2*) and facilitated by the commercial availability of this carotenoid.

New analytic techniques for better defining the carotenoid composition of various foods (*24,25*) provide an opportunity

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to help refine aspects of our understanding of nutrient composition relative to cancer risk in humans. Examining the effects of food preparation on carotenoid content and profile is also important to understanding the possible relations of foods and carotenoids to cancer risk.

This article presents data on the carotenoid content of foods associated with a lower risk for cancer. In addition, it presents some preliminary findings on the effects of food preparation on the carotenoid content of these foods as they may be consumed.

Materials and Methods

After extensively studying the epidemiologic literature, we selected 11 foods for quantitative analysis based on the results of human studies on diet and cancer and/or current diet–cancer hypotheses (*1–23,26,27*). These foods were divided into three broad categories for analysis: (*a*) green, leafy vegetables (broccoli, brussels sprouts, cabbage, kale, spinach); (*b*) yellow or orange vegetables and vegetable products (acorn squash, carrots, red palm oil, sweet potatoes); and (*c*) other foods (strawberries, tomatoes). Most of the green, leafy vegetables selected can also be classified as cruciferous vegetables.

Foods for analysis were purchased in single lots from samples of the U.S. food supply on the day of analysis, with the exception of red palm oil, which was obtained by scientific personnel from O.R.S.T.O.M. (Overseas Scientific Research Organization of the Republic of France) from the Central Market in Dakar, Senegal, West Africa, and shipped under carefully controlled conditions to the United States. Five units of each vegetable (1-pound bag) or vegetable product (1-quart bottle) were randomly selected and prepared for consumption. Every attempt was made to obtain analyses on the same foods that were reported in the epidemiologic literature. However, extensive sampling to assess variability in the nutritional productivity of different foods was not done. The food samples were prepared for analysis in much the same manner as food prepared for consumption. Edible parts of the vegetables were washed and drained, and large portions were reduced in size prior to homogenization (without added water) in a Cuisinart food processor. To investigate the effects of cooking, we cooked the vegetables in a microwave oven with a small amount of water for 6 minutes.

For the chemical analysis, reference samples of carotenoids were isolated from foods and purified by preparative thin-layer chromatography and high-performance liquid chromatography (HPLC). Details of extraction, isolation, and purification of carotenoids as well as the conditions of HPLC have been published elsewhere (*25*). Structural elucidation of carotenoids was based on mass and ultraviolet-visible absorption spectra of the individually isolated and purified components from various fruits and vegetables. In some cases, partial synthesis of carotenoids and chemical reactions of functional end groups provided further proof of the chemical structure of the carotenoids in question.

Comparison of the HPLC retention times and ultraviolet-visible absorption spectra of unknown carotenoids with those of known reference samples was also done to identify the naturally occurring carotenoids. The abundant carotenoids of these vegetables and their corresponding HPLC peaks were subsequently identified by use of several spectroscopic techniques. Quantification was accomplished by determination of the areas under chromatographic peaks and calculation of the level of each component on the basis of standard curves generated with pure compounds.

Results

The green, leafy vegetables contain oxygenated carotenoids or xanthophylls (lutein, epoxy carotenoids), chlorophylls, and hydrocarbon carotenoids (beta carotene), whereas the other foods investigated contain predominantly hydrocarbon carotenoids (alpha carotene and beta carotene). The quantitative distributions of the abundant carotenoids in the foods that we examined are shown in table 1. All the green, leafy vegetables contained the same types of carotenoids but at varying concentrations. The most abundant carotenoids in the green, leafy vegetables were the oxygenated carotenoids, which made up 80%–90% of the total carotenoid content. The beta carotene content of these green, leafy vegetables made up 10%–20% of the total carotenoid content. Yellow or orange vegetables and red fruits and vegetables characteristically had high levels of hydrocarbon carotenoids and very low levels of oxygenated carotenoids (table 1).

A preliminary comparison between the chromatographic profiles of constituents in extracts from raw and cooked green vegetables (brussels sprouts, kale) revealed that 19%–57% of the xanthophylls (oxygenated carotenoids) in microwaved vegetables were destroyed. However, only 15% and 14% of the hydrocarbon carotenoids in cooked brussels sprouts and kale, respectively, were lost.

Among the oxygenated carotenoids (xanthophylls), lutein was the most stable compound and was more resistant to heat treatment than the other carotenoids (epoxy carotenoids) of this class. Acorn squash, however, had increased levels of all carotenoids after cooking (table 1). This observation can be explained by the disproportionate loss of water and other volatiles during cooking. When these data were presented on the basis of dry weight, oxygenated carotenoids were approximately 25% lower after cooking, whereas beta carotene was only 3% lower after cooking.

Discussion

Although high vitamin A activity is thought to be responsible for the lower cancer risk associated with some foods in epidemiologic studies, our analyses suggest that the oxygenated carotenoids (without vitamin A activity) may be important cancer-modifying components of these foods. Some hydrocarbon carotenoids (such as alpha carotene and beta carotene, but not lycopene) contribute to vitamin A activity, whereas most oxygenated carotenoids (e.g., lutein) do not. The analyses shown here, in conjunction with the epidemiologic data relating selected foods to cancer risk (*1–23,26,27*), suggest that carotenoids in addition to beta carotene (provitamin A) are potential cancer-modifying components of these foods.

Table 1. Carotenoid content of foods associated with reduction in cancer incidence rates

Vegetable or fruit	Carotenoid or carotenoid class (mg of carotenoid/100 g of edible food)*					
	Lutein†	Epoxy carotenoids‡	Alpha carotene†	Beta carotene†	Lycopene	Other hydrocarbon carotenoids§
Broccoli, raw¶	2.06	1.70	—	0.48	—	—
Brussels sprouts						
Raw¶	1.59	2.74	—	0.53	—	—
Cooked	1.29	1.17	—	0.45	—	—
Cabbage, raw¶	0.31	0.50	—	0.08	—	—
Carrots, canned**	—	—	2.80	4.76	—	0.70††
Kale						
Raw¶	39.6	23.4	—	14.6	—	—
Cooked	25.6	15.2	—	12.6	—	—
Palm oil, red**	—	—	66.9	120.5	20.0	—
Spinach, raw¶	15.9	13.8	—	6.71	—	—
Acorn squash						
Raw‡‡	0.38§§	0.11§§	—	0.22	—	—
Cooked	0.66§§	0.20§§	—	0.49	—	—
Strawberries	0.06	—	—	0.23	—	—
Sweet potatoes, canned**	—	—	—	16.0	—	—
Tomatoes, raw	—	—	—	0.50	6.70	— ¶¶

*— = levels of carotenoid below detection limits of 1 μg/100 g of edible food.
†Includes all-*trans*- and *cis*-isomers.
‡Includes auroxanthin, luteoxanthin, lutein-5,6-epoxide, neochrome, neoxanthin, and violaxanthin.
§Includes zeta carotene, phytoene, and phytofluene.
¶Reference (*25*).
**Reference (*29*).
††Value represents zeta carotene.
‡‡Reference (*30*).
§§Portion of each carotenoid was present as fatty acid esters.
¶¶Other hydrocarbon carotenoids not quantified.

The green, leafy vegetables that have been associated with a lower risk for cancer (broccoli, brussels sprouts, cabbage, kale, lettuce, spinach) are very high in total carotenoids, moderately high in beta carotene, and very high in xanthophyll carotenoid constituents (primarily lutein and its stereoisomers). Vegetables that are uniquely high in beta carotene and low or totally lacking in other carotenoid constituents (carrots, sweet potatoes) were shown to be associated with a lower risk for cancer in earlier studies (*3,10,15*), although more recent investigations have not always confirmed this association (*12,27*). Our analysis also shows that red palm oil is extremely high in beta carotene and lacking in xanthophyll carotenoids. However, the suggestion that increased red palm oil consumption is associated with lower cancer rates in a Brazilian population (*1*) has not been confirmed to date (*22*).

Neither tomatoes nor strawberries are high in beta carotene or xanthophyll carotenoids. Tomatoes contain significant quantities of lycopene, which is a hydrocarbon carotenoid without provitamin A activity. Relative to the lower cancer risk associated with high consumption of strawberries in the Massachusetts study (*12*), it is possible that frequent, year-long consumption of this relatively expensive fruit in an elderly population in northeastern United States is more an index of socioeconomic status than of carotenoid intake in terms of cancer risk. Consumption of certain foods in certain populations, such as migrant groups (*5*), may act as an index of acculturation to the overall life-style of the host country (*28*) and thus be indirectly associated with cancer rates.

Additional suggestive evidence regarding carotenoids as active agents in foods comes from the preliminary data on cooked vegetables. Because heating destroys relatively more xanthophyll than hydrocarbon carotenoids, the findings of several studies of a greater or selective protective effect for raw as compared with cooked vegetables (*5-8*) may be compatible with effects of nonhydrocarbon carotenoids. Data on raw and cooked acorn squash confirm that xanthophyll carotenoids may be differentially lost with cooking, whereas hydrocarbon carotenoid content (beta carotene) is relatively retained (*31*).

These observations are consistent with the profile of carotenoids in human plasma after food consumption, where lutein and the hydrocarbon carotenoids are observed in significant quantities (*32-35*). In the normal population, a greater proportion of total blood carotenoid levels is due to the presence of lutein than to beta carotene (*34*). It is reasonable to assume that any suggested cancer preventive agent in foods would, after consumption, be required to appear in the blood to a significant extent to have an effect on cancer sites other than those of the gastrointestinal tract.

Many of these foods that are associated with a lower risk for cancer and are high in carotenoids may also contain biologically active, noncarotenoid constituents (*27,36*), such as vitamin C (*37*), selenium (*38*), plant phenols (*39*), or indoles (*40*). Many of these food constituents have also been proposed to reduce the risk of one or more epithelial cancers in humans through various mechanisms, including antioxidant potential to prevent free-radical formation (*36*). Whereas lycopene and most of the oxygenated carotenoids (xanthophylls) do not generally share provitamin A activity with alpha and beta carotene, beta carotene does share the free-radical quenching, antioxidant properties of all the carotenoids and of vitamin C (*37*) and selenium–vitamin E (*38*). In addition, many foods (e.g., beets, cauliflower, celery, cucumber, eggplant, onions, turnips), for which increased dietary consumption is associated with a lower risk for cancer, are totally lacking in detectable carotenoid content but are high in other vitamins and constituents (*23*). Further investigations, both laboratory and epidemiologic, are required to understand more completely the observed relations between the dietary consumption of selected foods and human cancer rates.

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